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Morphological Variations in Dorsal Scale Microstructure of Three *Butterflyfish* Species (*Chaetodontidae*) Revealed by Electron Microscopyin Support of Fishery Resources Conservation Efforts

Abdul Razak*
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang (UNP), Indonesia. *abdulrzk121@gmail.com

MulyonoSumitroBaskoro
Department of Fisheries Resources Utilization, Faculty of Fisheries and Marine Fisheries, IPB University, Indonesia
baskoro_mul@yahoo.com

Vauzia
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang (UNP), Indonesia
vauzia.ivo@gmail.com

Aprizon Putra
Research Center for Conservation of Marine and Inland Water Resources, National Research and Innovation Agency (BRIN),Indonesia
apri024@brin.go.id

ZetiHarriyati
Study Program of Medical Education, University of SyarifHidayatullah State Islamic (UINJK), Indonesia
zeti.h@uinjk.ac.id

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Abstract

Fish scales are an important structure used as a versatile research material. Research on fish scales from the point of view of ichthyological has unique characteristics for each fish species. This research aims to reveal the surface variations of the Dorsal Scale microstructure of three fish species from the *Chaetodontidae* family, namely *Chaetodon Auriga*, *Chaetodon kleinii*, and *Chelmonrostratus*in support of fishery resources conservation efforts. Research on reef fish families is still very minimal in Indonesia. On the other hand, this reef fish family is very massive in exploitation. This is the important reason for researching fish scales from the *Chaetodontidae* family. In the research on the microstructure of fish scales, the focus is more on the components of fish scales. The method used in this research involves extracting scales from *Chaetodon Auriga*, *Chaetodon kleinii*, and *Chelmonrostratus*. The results of this microstructural analysis using Transmission Electron Microscopy (TEM) and Scanning Electron Microscope (SEM) with a magnification of 65-15,000 times are evidence that can reveal differences between species, and genera of fish in the *Chaetodontidae* family. The results of this research indicate that three types of *Butterflyfish* from the *Chaetodontidae* family have different morphological forms (oval, rectangular, and elliptical), *ctenii*, lepiddont, and focus. In *Chelmonrostratus* no focus was found. This research concludes that the character of three types of *Butterflyfish* on the Dorsal Scale can show the characteristics of the scales which are evidence of identification for the taxonomic process of the *Chaetodontidae* family in addition to molecular.

Keywords:*Chaetodontidae*, Fish Scale Microstructure, Morphological Variations, Fishery Resources Conservation.

Introduction

The wealth of marine biological resources in the form of reef fish in Indonesia's tropical waters has not yet been fully disclosed. This is due to the lack of research on the aspects of reef fish life completely. The information available to date is not sufficient. Where the role of reef fish is very important for ecosystems and humans. Reef fish consists of three major groups, namely target fish, indicator fish, and other fish (Sahetapy et al, 2004). This statement is supported by Allen&Adrim (2003) who noted that the number of species from the *Chaetodontidae* family (the *Butterflyfish* family) has been known from all tropical and sub-tropical seas, with about 120 species belonging to 10 genera. An important and interesting potential resource for coral reef fish to research is the coral fish of the *Chaetodontidae* family (*Butterflyfish*). Reef fish are called *Butterflyfish* or in Indonesian, they are called *Butterflyfish* and Makassar people popularly call them *Butterflyfish*. These fish are colorful and varied in shape and graceful swimming styles (Johnson & Gill, 1998, Arbanto et al., 2020).

Reef fish from the *Chaethodontidae* family are referred to as indicator fish because they are natural guardians of coral reefs and their lives depend on the presence of coral reefs as food. If there is damage to coral reefs, the presence of these fish can be used as an indicator to assess and monitor the condition of coral reefs (Suasti et al., 2020; Pratama et al., 2021). Another aspect that we need to consider carefully and become a problem is the exploitation of the very massive *Butterflyfish* family as marine ornamental fish which are very popular with the public both at home and abroad. Therefore, research on reef fish is useful as basic data for managing and sustainable use of coral reef ecosystems and *Chaetodontidae* fish (Riansyah et al., 2018; Prarikeslan et al., 2019; Putra et al., 2023). In addition, information about the scales of the *Butterflyfish* family will be difficult to obtain if we do not research the scales of this *Butterflyfish*. This is an important issue and aspect of the *Butterflyfish* family scale research.Morphological scales have proven to be a useful tool in fish classification. This can be traced back to the time of Agassiz (1833–1843), he was the first to initiate and reveal the use of scales as a taxonomic tool for fish and divided fish scales into four categories according to the shape of the scales into fish, namely: *Placoidei*, *Ganoidei*, *Ctenoidei*, and *Cycloidei* (Bräger& Moritz, 2016). Fish scales are still minimally utilized in the field of fish classification in Indonesia. This is a problem that must be solved. In addition to its use or utilization in classification, morphological scales are an important tool in determining the food of predatory fish (*piscivorous*) or in paleontological analysis (Esmaeili&Gholami, 2011).Based on research by Razak (2014);Razak(2015) it was found that the attention of foreign scale researchers was minimal regarding the structure of lepiddon or spina on fish scales. This section is unique and specific to each type of fish. Research on fish scales is important and urgent because of the massive exploitation of reef fish from the *Chaetodontidae* family. Fish scales of this family have specific significance that can be used in the field of fish taxonomy, especially in the *Chaetodontidae* family. Scales are readily available, and preparation for image processing is very simple and this research did not need to kill the specimen (Ben Tamou et al., 2022).

The results of previous research that have been shown by Esmeili&Ghoulami (2011) as well as foreign researchers have been followed by the researchers themselves since 2013 until now that the detailed structure of the fish scale can help in studying the taxonomy and phylogeny of fish, age determination, the past environment experienced by fish. It also distinguishes between reared hatcheries and wild populations. Furthermore, migration, and fish pathology due to water pollution from water bodies were detected on fish scales, in addition to studying the age and growth of fish and determining the diet of predatory fish. Almost the same was done by Esmaeiliet al (2007) who conducted paleontological analyses and genetic studies related to the extraction of endangered fish species from Deoxyribo Nucleic Acid (DNA) from fish scales (Kumar et al., 2011).The problem with this research in the field of fish scales is that the availability of supporting tools is still not sufficient. From the results of the author's research over the last 5 years, there is a space that has not been filled, which has been studied in a focused manner, namely the denticular/spina component. This is related to the availability of

SEM or TEM tools which are expensive and require a magnification equal to or above 10000 times to be seen clearly.

The object of fish scales studied in this research is the species of *Butterflyfish* with the highest exploitation originating from PelabuhanRatu - West Java. In this research, the focus was revealed regarding the diversity of *ctenii* and Dorsal Scale lepidon of three types of *Butterflyfish* from the *Chaetodontidae* family, namely: *Chaetodon Auriga, Chetodonkleinii*, and *Chelmonrostratus.* Furthermore, spina or *ctenii* and lepiddont are components of fish scales in the Ctenoid type which are important parts and can be used as identification tools. This is by the research of Esmeili et al (2014) concluded the scales of the three *Mugilidae* fish which differ in terms of scale type, focal shape, circular shape, size, shape and arrangement of lepiddont as well as *ctenii* shape and size which can be attributed to their habitat and these characteristics can be used in fish identification. This research aims to reveal the surface variations of the Dorsal Scale microstructure of three fish species from the *Chaetodontidae* family, namely *Chaetodon Auriga*, *Chetodonkleinii,* and *Chelmonrostratus*in support of fishery resources conservation efforts.

The contribution of this research to science is 1) This research examines specific components of fish scales, namely *ctenii* and lepidon, as an identification tool. Referring to research by Esmeili et al (2014), who showed that these characteristics vary between species and can be linked to their habitat, this study deepens the understanding of how this variation can be used to more accurately identify *Butterflyfish* species; 2) By focusing on surface microstructural variations of the Dorsal Scales of three *Butterflyfish* species, this research fills a gap in the existing literature by providing new empirical data on scale microstructure. This is important for understanding the adaptation of species to their environment and for taxonomic identification purposes; 3) This research advances fish identification methodology by introducing detailed analysis of *ctenii* and lepidon on Dorsal Scales. This technique can be adopted by other researchers to identify other fish species, especially in the *Chaetodontidae* family, more accurately and efficiently; 4) Information obtained from this research can be used to support conservation efforts and management of fisheries resources. By understanding variations in scale microstructure, fisheries managers can better identify threatened species and develop conservation strategies specific to each species; 5) The results of this research add to the available taxonomic data regarding the *Chaetodontidae* family. These data can be stored in global taxonomic databases, enriching the information available to researchers worldwide and facilitating comparative studies between species; and 6) By linking variations in scale microstructure to each species' habitat, this research provides insight into how *Butterflyfish* species adapt to their environments. This helps in understanding the ecology of species and their interactions with the ecosystem. This research on the diversity of *ctenii* and lepidon on the Dorsal Scales of three *Butterflyfish* species not only provides new and in-depth data on scale microstructure but also develops a new identification method that can be widely applied. In doing so, this research advances knowledge in the field of fish taxonomy, supports conservation efforts, and helps better manage fisheries resources.

Methods

The research material for this scale is derived from the Dorsal Scale of *Chaetodon Auriga*, *Chaetodon kleinii,* and *Chelmonrostratus.* Other ingredients are alcohol and ethanol as preservatives and desiccants. The tool used is TEM at SepuluhNopember Institute of Technology (ITS) Surabaya and Scanning Electron Microscope (SEM) at Medan State University (UNIMED) Medan. Magnification is used from 36 times to 15000 times. The method used in the study for scale collection was the research of Esmaeli et al (2007). Scales were taken from the Dorsal underside and the lateral line as shown in Fig 1 below.

Figure 1. Shows where the scales on the dorsal side of the scales

The fish that became the object of this research were from the *Chaetodontidae* family. The species or types studied are *Chaetodon Auriga*, *Chaetodon kleinii*, and *Chelmonrostratus*.

- **Drying of *Butterflyfish* Scales**: The scales that have been taken are immediately cleaned by rinsing three times with distilled water, the scales are put into 5% Potassium Hydroxide (KOH) solution for a maximum of 10 minutes. The scales were then immersed in 30%, 50%, 70%, and 90% alcohol for one hour of dehydration each. Then, it is dried using filter paper to prevent shrinkage after 70% Ethanol, the scales are placed between the Microslides for two or three days. The dried fish scales were sent to ITS Surabaya to be observed using TEM and some were sent to UNIMED Medan to be observed with SEM (Esmaeli&Gholami, 2011).
- **Observation of Scales**: Observation of Dorsal Scale of *Chaetodon Auriga*, *Chaetodon kleinii*, and *Chelmonrostratus* using TEM at ITS Surabaya and SEM at UNIMED Medan, with a magnification of 65-15,000 times.

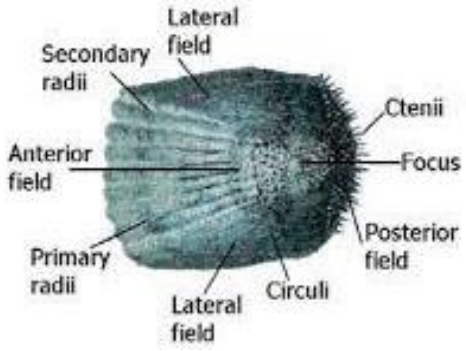
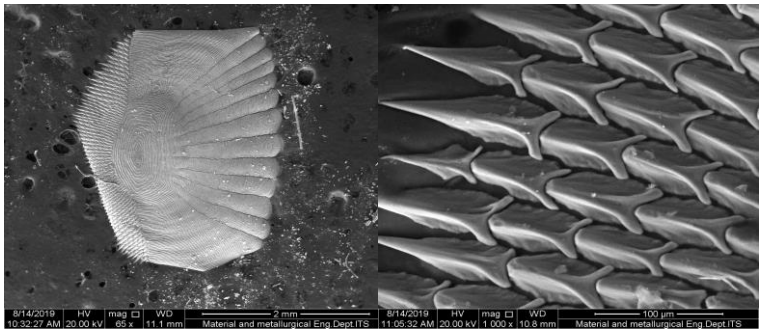


Figure 2. Terminology for Ctenoid Scale (following the use by Lagler, 1947).

Results

The results of observations of the Dorsal Scale of the *Auriga Butterflyfish* which were enlarged 65 times during SEM observations at UNIMED Medan. In Fig 3, the Dorsal Scale component has a complete section with 8 components starting from AI. The Dorsal Scale of the *Auriga Butterflyfish* are cordate (heart) and have a complete scale component as shown in (Roberts, 1993). Fig 3. The scales of this *Auriga Butterflyfish* are of Ctenoid type. This type of scale has *ctenii*. *Ctenii* there are two groups. Some are complex composed of several truncated spines and some are single *ctenii* called spines (Roberts, 1993). The scales of the three types of *Butterflyfish* studied generally have *ctenii*.Three important parts, namely the anterior, posterior, and the focus. This is the opinion of Esmailiet al (2007). According to Bräger& Moritz (2016), the scales of this *Auriga Butterflyfish* are cordate (like a heart). Foci are also found which are scales that develop during the growth process. The focus is divided into two parts, namely the head and tail (Esmaeili et al.,2007). According to Ganguly& Mookerjee (1947) the terms circle,

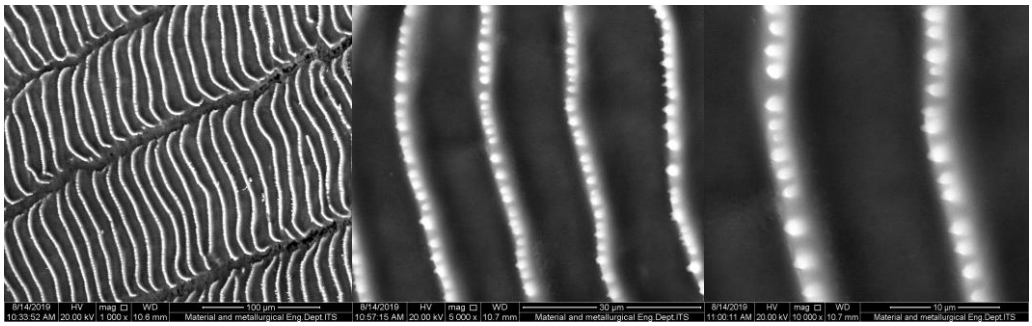
focus, radii, and *ctenii* are derived from explaining the terminology or term focus as a circular area of scales where the circles are arranged and centered. Circuli are curved lines on the outer surface of the scales, arranged converging or forming an incomplete circle. A radi is a sign, a rounded circle that extends from the edge towards the focus of the scale. Radii that experience bifurcation of a typical tooth-like structure are called denticles or lepidonts (Serban&Grigoras, 2018).More details can be seen in Fig 3.



(A)(B)

Figure 3. (A). Dorsal *Auriga Butterflyfish*scales; and (B) *Ctenii*Dorsal *Auriga Butterflyfish* Scales, scale 1:100 m.

Next up, are the *ctenii*, which are spine-like structures. According to Bräger& Moritz (2016), *ctenii* is not the same as spina. *Ctenii* are tooth structures that are ossified and separated in parts (see Fig 4C.) and/or attached to the parent scales. *Ctenii* is on the edge or sub-section. *Cteniia*re composed of several truncated spines, while the spines are single *ctenii* (Johnson, 1984). Fig 4 (A, B, and C) shows the lepiddont type of the *Auriga Butterflyfish*. *Auriga Butterflyfish* according to Ferrito et al (2003) has a lepiddont not aligned type as shown in Fig 4 (A and B). Furthermore, the *Auriga Butterflyfishcteniis* of a type called multi-rowed-nature because of the displacement or influence of physical factors such as body movements of the fish that cause the apex to connect with the basal component of the spine which moves wider upwards (Ganguly& Mookerjee, 1947). This can be seen in Fig (5. C) below.

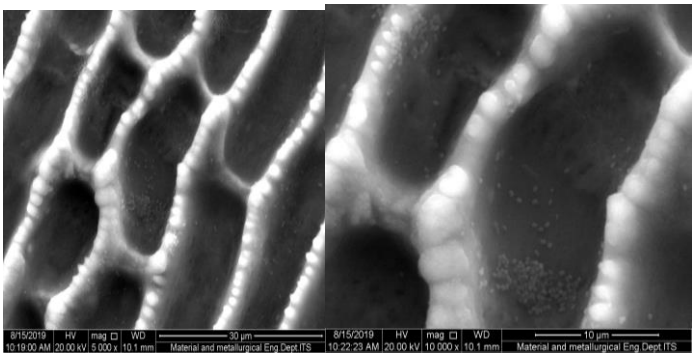


(A) (B) (C)

Figure 4. Lepdont*Auriga Butterflyfish* (A) Lepdont*Auriga Butterflyfish* with a magnification of 1000 times a scale of 1:30 m; (B) Lepdont*Auriga Butterflyfish* with a magnification of 5,000 times, Scale 1: 2mm; and (C) Lepdont a magnification of 10,000 times scale of 1:10 m

Variations of *Chocolate Butterflyfish*scales. The scales of the *Brown Butterflyfish* are shown in Fig 4 (A, B, and C). According to Bräger& Moritz (2016), the scales of this *Brown Butterflyfish* are cordate (like a heart). Foci are also found which are scales that develop during the growth process. The focus is divided into two parts, namely the head and tail (Esmaeili et al., 2007). According to Ganguly& Mookerjee (1947) the terms circuli, focus, radii, and *cteniia*re derived from Taylor (1914) (Ganguly& Mookerjee (1947). Taylor (1914); Ganguly& Mookerjee (1947) describes the terminology or the term focus as a circular area of scales where the circles are arranged and centered. Circuli are curved lines on the outer surface of the scales, arranged

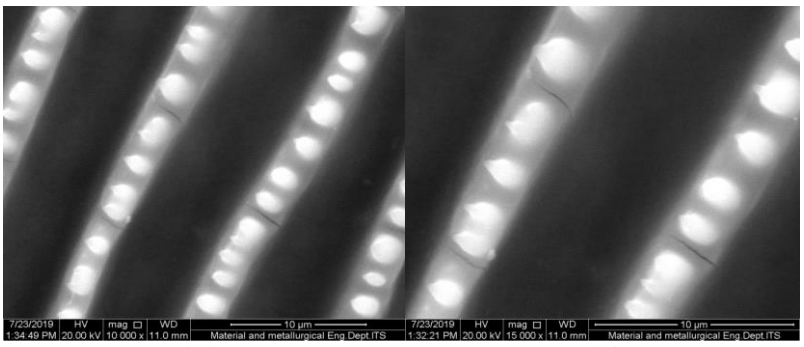
converging or forming an incomplete circle. A radi is a sign, a rounded circle that extends from the edge towards the focus of the scale. Furthermore, Bräger& Moritz (2016) explained about *ctenii*. *Ctenii* is not the same as spina. *Ctenii* are tooth structures that experience ossification and are separated in parts (see Fig 6 and/or attached to the parent scales (see Fig 5. C). *Ctenii* is located at the edge or sub-edge the of posterior field. Spina is a structure like teeth that is ossified and directly attached to the parent fish scales. The spine is located at the posterior edge or spreads to the posterior field (Bräger& Moritz, 2016). Circuli have septa or dividers. This distinguishes it from the *Auriga Butterflyfish* and the *MonyongButterflyfish*. According to Seifertet al (1998), the septa are Calcium Projection (CP).Next, Scales on *Fish Monkey* can be seen in Fig 6 (A and B). The scales of the *MonyongButterflyfish* show a rectangular shape. The shape of the rectangular scales without any focus. This is different from *Auriga Butterflyfish* and *Chocolate Butterflyfish*. Differences in the shape or morphology of these scales are often used for the analysis of population and species *diphriasis*.



(A) (B)

Figure 7. (A) Shows that the lepidont is aligned not (not in the middle of the radii (Ferrito et al., 2003). The presence or absence of denticles or lepidonts along with their shape, size, and distance between them can be used as an analysis of taxonomic criteria on fish species; and (B) LepdontDorsal Scale of *Brown Butterflyfish*; (B)Lepdont magnification 5000 times scale 1: 30 m CP

Lepidont or Dorsal Scale dentikula fish angelfish type *MonyongButterflyfishnot aligned* (rare) (Ferrito et al., 2003) as shown in Fig 8 below.



(A) (B)

Figure 8. Dorsal Scale of Lepidon*Butterflyfish* (A) 10,000 times magnification; and (B) *Ctenii* 15,000 times magnification.

Furthermore, variation from the Dorsal Scale of three types of fish angelfish is described in Table 1 below.

Table 1. Forms Dorsal Scale section three types of fish angelfish

Fish Spatula-Kapename	***CheatodonAuriga***	***Chelmonrostarus***	***Chaetodonkleinii***
Shape Scales	Pentagonal	FourTriangle	Ellipse
Shape *Ctenii*	✓	✓	✓
Shape Lepidont	Short /Taper	Short/Taper	Short / Taper
Distance Lepidont	Meetings	Rare	Meetings

Variations in the shape of scales are observed in *Chaetodon Auriga*, which is pentagonal, *Chelmonrostratus*, which is rectangular, and *Chaetodon kleinii*, which are elliptical. According to scale researchers, the utilization of electron microscope microstructure analysis proves to be a highly precise method for delineating differences and aiding in the identification of broader groups and species (Abraham et al., 1966; Bartulovic et al., 2011; Esmaeili, 2014; Kaur &Dua, 2004).The differences in microstructural features of scales, including circuli, radii, *ctenii*, and other characteristics such as focus and shape, are pivotal for taxonomic purposes, as demonstrated in Table 2 (Batts, 1964). Moreover, the intricate microstructure of scales significantly contributes to the identification process and research across various scale forms, aiding researchers in identifying fish up to the major group and species levels (Abraham et al., 1966; Bartulovic et al., 2011; Zahid et al., 2015).

Table 2. Focal characters of the Dorsal Scales of three types of *Butterflyfish*

Fish Spatula-Kapename	***CheatodonAuriga***	***Chelmonrostarus***	***Chaetodonkleinii***
Shape of focus	Oval	X	Ellips
Focus position	Ke-Posterior direction	X	To posterior
Focus	✓	X	✓
Ctenii	✓	✓	✓

Conclusions

This research highlights the detailed microstructure of *Butterflyfish* scales, with a particular focus on the Dorsal Scales of the *Auriga Butterflyfish*. Observations using an SEM reveal detailed features that are crucial for taxonomy and species identification, reinforcing the importance of electron microscope analysis in accurately describing species and understanding population dynamics. These findings contribute to a broader understanding of fish scale morphology and its implications for marine biology research and conservation efforts, providing valuable insight into the complexity of marine biodiversity that supports conservation efforts and management of fisheries resources. Through SEM observations, this research succeeded in identifying small-scale characteristics, such as shape, and composition, and special features such as circles, radii, and *ctenii*. Results from the Dorsal Scale of the *Auriga Butterflyfish* show a distinct characteristic, heart-shaped and classified as Ctenoid due to the presence of *ctenii*. These *ctenii* are further categorized into complex structures and single spines, which play an important role in scale morphology and taxonomic classification. Additionally, this study differentiates between *ctenii* and spina, highlighting differences in ossification and structure that contribute to scale morphological variation among *Butterflyfish* species. This variability serves as a valuable tool for population and species analysis, facilitating differentiation and proper identification through electron microscopic microstructural analysis. This research also discusses the diversity of scale shapes among *Butterflyfish* species, which range from pentagonal to rectangular and elliptical, emphasizing the importance of microstructural features for taxonomic purposes.

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