MORPHOLOGICAL STUDY OF THE SCALES OF *Latimeria menadoensis* POUYAUD *et al*

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Abstract

The scales of Latimeria menadoensis *has a variety of the shape of the scales from oval, rectangular, foot-print, elongated-pointed edge etc. The comparison in the portion of the exposed and embedded part of the total length of the scales of* Latimeria menadoensis *and* Latimeria chalumnae *at the approximately similar part of the body i.e. scale on the dorsal region and scales located on the region extending lateral posteriorly until caudal, indicated that this portion is different. In* L. chalumnae *the exposed part are one third and the embedded part are two third of the total length of the scale. The exposed part in the* L. menadoensis *are more than one third (average 35.9% of total length), while the embedded part are less than two third, but on the other part of the body i.e. dorsal lobe fin, the embedded part was 73.9% or approximately three fourth of the total length of the scale. The 175 loose scales were also examined and discussed.*

Some of the loose scales were examined under the scanning electron microscope (SEM) by using two kinds of preparations. It showed the apex region, the annular ridges, the radiating ridges and the denticles.

Key words: Coelacanth *Latimeria menadoensis*, morphology and structure of scales, microradiography, histological technique, exposed part, embeded part.

Introduction

The morphology and structure of the scales of adult *Latimeria chalumnae* Smith, 1953 was first described by Roux (1942) and then completed by Smith *et.al* (1972), Castanet *et al.* (1975), Giraud *et al.* 1978), Millot *et al.* (1978), and Meunier and Zylberberg (1998), while Miller (1979) and Smith (1979) described the morphology of the scales from its embryo. All of these authors examined the scales by using ground sections, microradiography and various histological techniques. The morphology and structure of the scales of the new Indonesian species, *L. menadoensis* Pouyaud *et al.* (1999), has not yet to be examined.

This paper reveals morphological features of scales which may become important characters for supporting the specific status of the Indonesian coelacanth. This study concurs in opinion with the claim of Holder *et al.* (1999) who stated that scale ornamentation was one of the important morphological characters that should be considered in the future examination. Forey (1998) also suggested that scales were the best characters available in investigating coelacanth systematic.

Material and Methods

A series of scales were plucked from ten places of the body of the specimen (the number of the specimen in the Bogor Museum is given) of *L. menadoensis*. The places

from which the scales were plucked were from the right side of the body from dorsal region above linea lateralis (DRs), from linea lateralis below anterior dorsal fin (Lls), dorsal fin lobe (DFLs), caudal peduncle region (CPDs), belly region (Bs),ventral region (Vs), ventral fin lobe (VFLs), anal region (As), anal fin lobe (AFLs)and caudal region (Cs) (Figure 1). Plucking was conducted as carefully as possible to avoid the damaging effect to the other scales. The scales were preserved in ethanol 70%. Each scale was photographed with a Nikon camera.

Two measurements were taken from the scales i.e the total length (TL) and the length of exposed part (EPL). Total length and length of exposed were measured using Mitutoyo digimatic caliper to the nearest mm. The measurements were taken from the longest part. Total length, which covers the embedded and the exposed part was measured from the anterior to the posterior edge of the scale. The exposed part was measured from the point of the exposed part to the posterior edge of the scale. The length of the embedded part was obtained by substracting the total length with the exposed part. Then the portion of each exposed and embedded part was the ratio of their contribution to the total length of the scale.

The measurements were also taken from a total of 175 loose scales. Based on the logaritmic classification the loose scales were classified into 8 class sizes (Nasution, 1981).

A number of scales were prepared for SEM. Two kinds of preparation were made for SEM. First, the scales were cleaned in sodium caccodylate buffer for two hours at 4°C. They were then agitated for a ultrasonic sonycation for 20 minutes, and fixed in 2.5% glutaraldehyde at 4°C for a night. Afterwards, the scales were soaked in 2% tannic acid solution at 4°C for 6 hours, and rinsed with sodium caccodylate buffer for 15 minutes at 4°C. This procedure were repeated four times, then samples were rinsed with 1% osmium tetraoxide in the same buffer at 4°C for 2-4 hours. The process was continued by rinsing the samples using distilled water for 15 minutes at 4°C. The scales were then dehydrated through a graded series of ethanol: 50%, 75%, 85% for 10 minutes at 4°C. Dehydrating process was continued using 94% and absolute ethanol at a room temperature. Afterwards, the samples were kept for two times in the t-butanol for 10 minutes, then frozen at - 3° or - 20°C. The frozen samples were put into the vacuum chamber of the freeze dryer for several hours where during this process the shape of the scales changed from a straight to a curl shape. The dried samples were then taken out from the chamber and glued onto a stub by using conductive past or both sides adhesive tape. Then the stub were put into a vacuum chamber of the ion coater and coated by gold coated. This first method was successful to examine the apex and the ridge of the scale, but the exposed part became fractured and curled. To overcome this drawback, the second method was used.

The second method emphasized to clear up the scales and prevent them from fractioning. Scales were cleared in 60% lactic acid for 3 days, then dehydrated with a

graded series of ethanol (70%, 80%, 90%, 95%) each for 15 minutes and then dehydrated with two series of 99% ethanol each 15 minutes. Afterwards, the samples were put into butyl alkohol for three times each 15 minutes. Then they were put into a small petridish with three microcover glasses which prevent from curling. Finally, the scales were kept at - 3° or - 20°C for 15 minutes. After this clearing process, the handling was similar to the first methods.

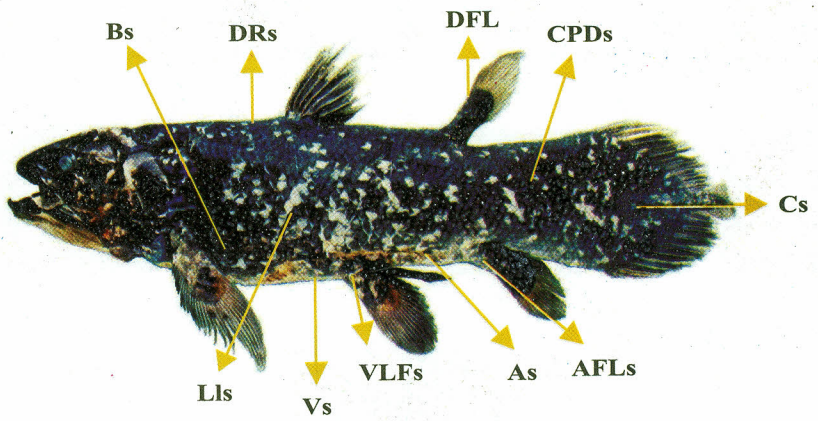

Figure 1. The ten areas on the body of *Latimeria menadoensis* where the scales were plucked.

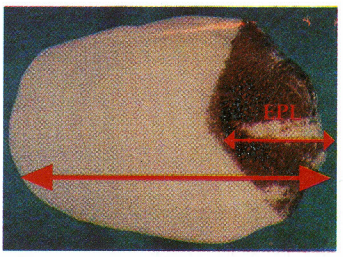

Figure 2. The shape and the measurements of the scales, total length (TL) and exposed part of the scale (EPL).

Results

The Shape

The shape of the scales of *L. menadoensis* differ significantly in some parts of the body (Fig. 3a-i). The group of the scales from the dorsal region (DRs), lateral line below dorsal fin (Lls), belly (Bs), caudal peduncle (CPDs), and caudal (Cs) are oval shaped (Fig. 3a-d). While, the scales from the others i.e ventral fin (VLFs), dorsal lobe fin (DLFs), anal

region (ARs) and anal lobe fin (ALFs) have a variety of shapes, even at the same part of the body, for instance there are 3 shapes of scale on ventral lobe fin (Fig. 3f), two shapes of scale on the anal (Fig. 3g) and three shapes of scale on the anal lobe fin (Fig. 3h). There is an unknown originated loose scale, which is oval with pointed edge (Fig. 3i).

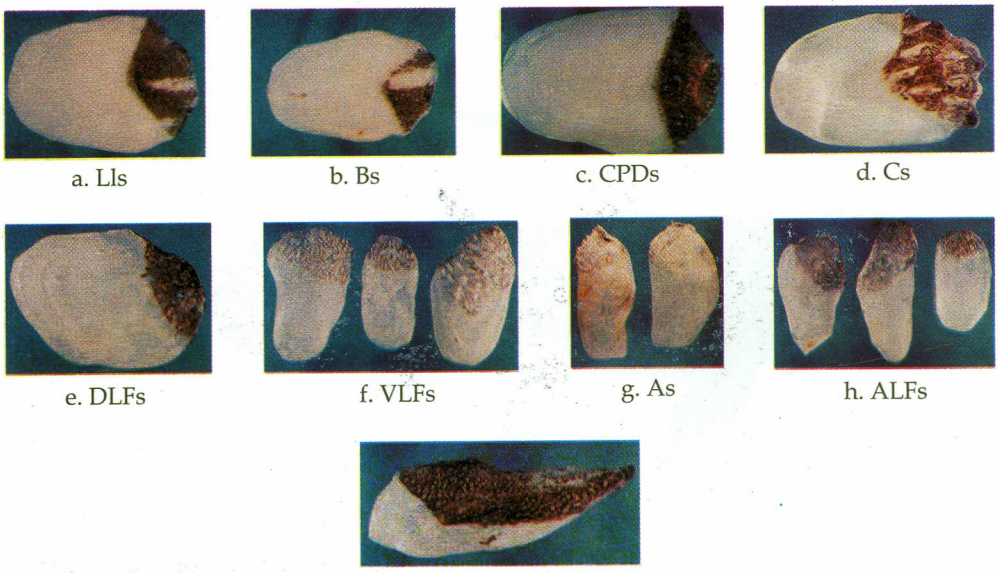

Figure 3. The shape of the scales of *L. menadoensis*; a. Lls, linea lateralis scale; b. Bs, belly scale; c. CPDs, caudal peduncle scale; d. Cs, caudal scale; e. DLFs, dorsal lobe fin scale; f. VLFs, ventral lobe fin scale; g. As, Anal scale; h. ALFs, anal lobe fin scale; i. Uls, loose scale from an unknown origin.

Size of scales

The size of the scales are also different in every part of body (Fig. 4). Excluding the longest scale (46.2 mm) from dorsal region (DR), there is a tendency of a decrease in size of scales from the anterior to the posterior part of the body. It was shown by the scale in which the precise location was known, size of the scales from linea lateralis (Ll) is 45.1 mm long, belly scale (B) is 42.5 mm long, until the caudal scale, which is the shortest scale with 8.9 mm long (Fig. 4).

The Embedded and Exposed Part of the Scales

The measurement of the embedded and exposed part of the scales are shown on the Figures 4 and 5. The portion of embedded and exposed part of the scales are different in every part of body. On the dorsal region (DRs), lateral line (Lls), belly (Bs) and caudal peduncle (CPDs), the exposed part (35.8%, 38.5%, 35.5% and 33.8% respectively) are about one third and the embedded part (64.1%, 61.4%, 64.5% and 66.2% respectively) are

about two thirds. On the ventral fin (VFs) and dorsal lobe fin (DLFs), the exposed part (29.0% and 26.0% respectively) are aproximately fourth and the embedded part (71.0% & 74.0% respectively) are aproximately three fourth. On the anal (As) and anal lobe fin (ALFs), the exposed part (39.9% & 41.4% respectively) are aproximately two fifths and the embedded part (60.1% & 58.6% respectively) are aproximately three fifth. However on the ventral lobe fin (VLFs) and caudal fin (Cs), the exposed part (48.4% & 46.3% respectively) and embedded part (51.6% & 53.7% respectively) are almost 1 : 1.

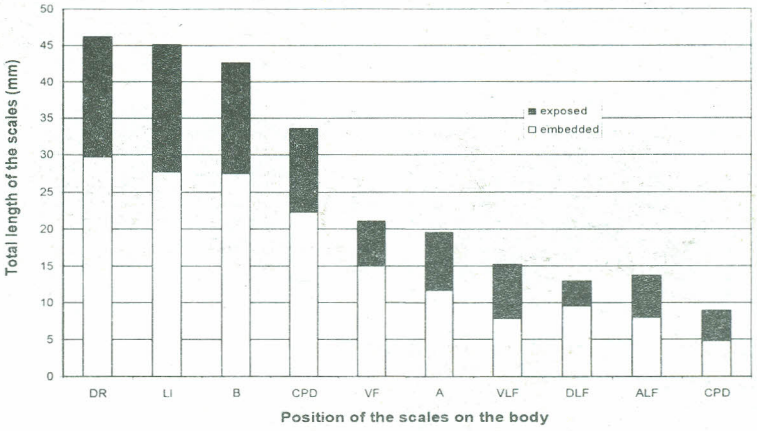

Figure 4. The measurements of the embedded and exposed part of the scales of *Latimeria menadoensis* Pouyaud *et al.*; DR, dorsal region below dorsal fin above lateral line scale; Ll, lateral line scale; B, belly scale; CPD, caudal peduncle scale; VF, ventral fin scale; A, anal scale; VLF, ventral lobe fin scale; DLF, dorsal lobe fin scale; ALF, anal lobe fin scale; C, caudal scale.

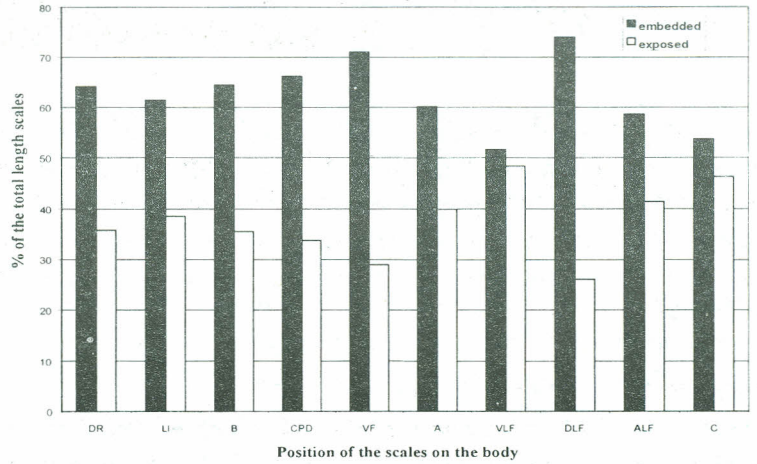

Figure. 5. Comparison of the portion of the exposed and embedded part of the scales of *Latimeria menadoensis*; DR, dorsal region below dorsal fin above lateral line; Ll, lateral line; B, belly; CPD, caudal peduncle; VF, ventral fin; A, anal; VLF, ventral lobe fin; DLF, dorsal lobe fin; ALF, anal lobe fin; C, caudal.

The size and shape of denticles of the scales of *L. menadoensis* are different in caudal peduncle, anal and caudal scales (Fig. 6a-d). The most conspicuous denticles occur in the caudal scales, which form long pointed horny projections (Fig. 6d). Some of the scales have a colony of a small denticles (Fig. 6c). Miller (1979) reported that this phenomena was likely to be new structures resulted from recolonizing denticles of post damaged scale.

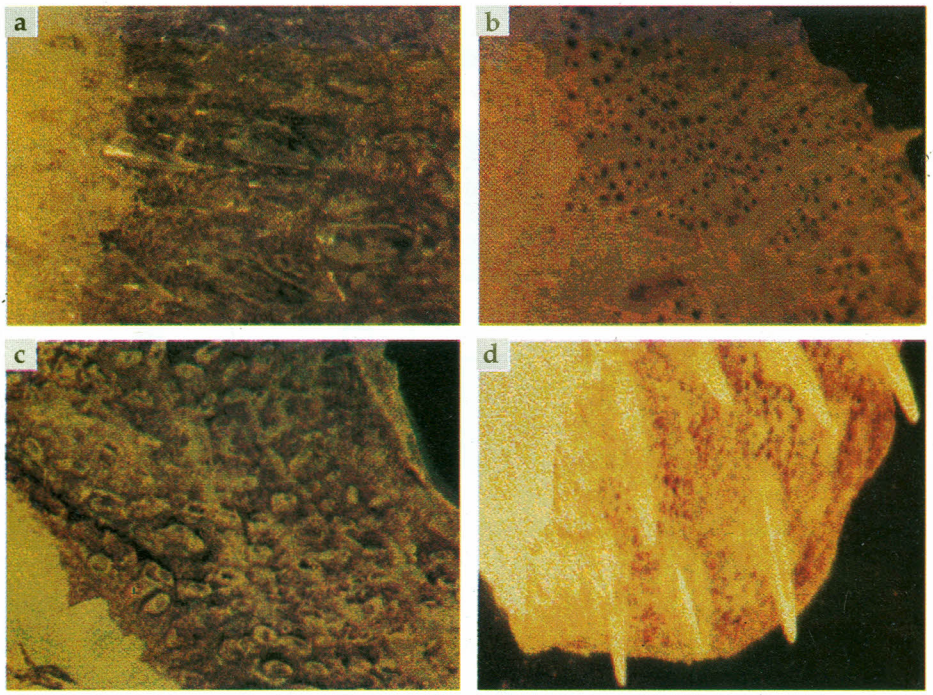

Figure 6. Structure and melanophore of the exposed portion of the: a, developed melanophores and transparent denticles an caudal peduncle scale; b, rare melanophores area on anal scale; c, the colony of small denticles that is likely to be new structures of the post damaged scale; d, caudal scale.

The exposed part of the scales of *L. menadoensis* has an ornamentation consisting of melanophores and denticles (Fig. 6a-d). The melanophores are more developed on the lateral part (Fig. 3a-e) than on the ventral part of the body (Fig. 3f-h); as well as the melanophores from caudal peduncle (Fig. 3c, Fig. 6a) are much developed than those on the anal part (Fig. 3g, h; Fig 6b). Denticles are transparent, the size and the exposed part of the denticles are different in the different part of the body. The small colony of denticles (Fig. 6c) were found on several scale. The most conspicious denticles are on the caudal scales (Fig. 6d).

The Loose Scales

There are eight class sizes of the total 175 loose scales (Fig. 7). The class size of 8.1-13.0 mm and 13.1-18.00 mm have two highest number of loose scale i.e 39 and 56 respectively. While the other class sizes consisted of less than 20 scales.

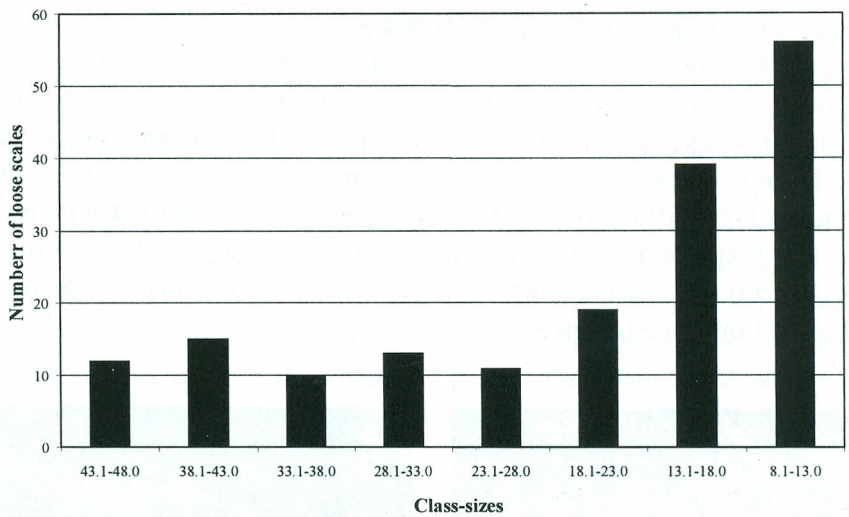

Figure 7. Number and class-sizes of loose scales of *Latimeria menadoensis*.

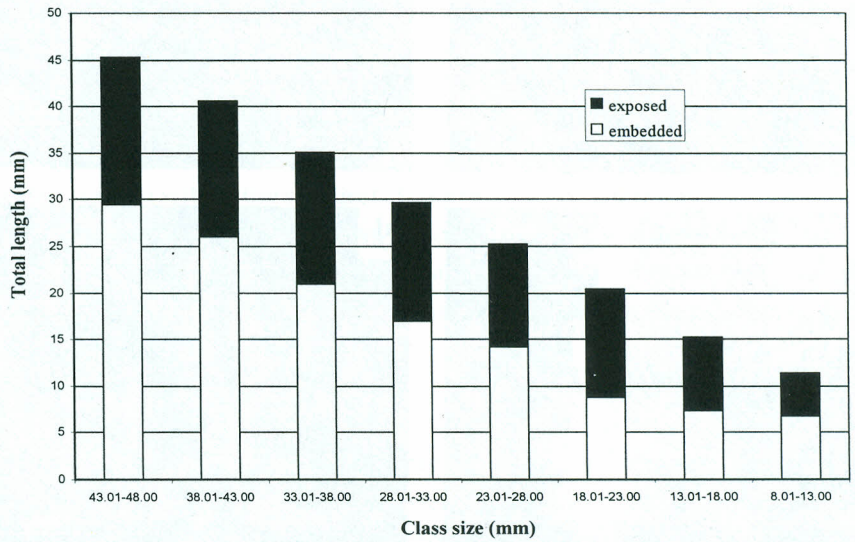

Figure 8. The exposed and embedded portion of the loose scales of *L. menadoensis* Pouyaud *et al.* 1999.

The exposed and embedded portion of the loose scales are shown on the Fig. 8. All scales of these eight class sizes have portion of the exposed part ranging from

about one third until more than a half. The class size in which the portion of exposed part was more than a half occurred on 18.0-23.0 mm and 13.0-18.0 mm. The presence of the portion of the exposed part that was more than one third to more than a half is similar with that on the origin unknown scales as shown in Figures 4 and 5.

The Scanning Electron Microscope (SEM) Image

The SEM on the structure of the scales showed that there is a thin amorphous film on the exposed part of the scales that stretches from the apex region to the edge of the posterior scales (Fig. 9a). The denticles project out from an oval ring of this film (Fig. 9b). The non coating scales showed the clear annular ridges and the radiating ridges (Fig. 9c). Figures 9 a-c were obtained using of the second method of preparation SEM. The first preparation method generated very clear image of the apex region of the scale (Fig. 9d). This method, however, gave rise to the homogenous layer of the exposed part of the scale fracture.

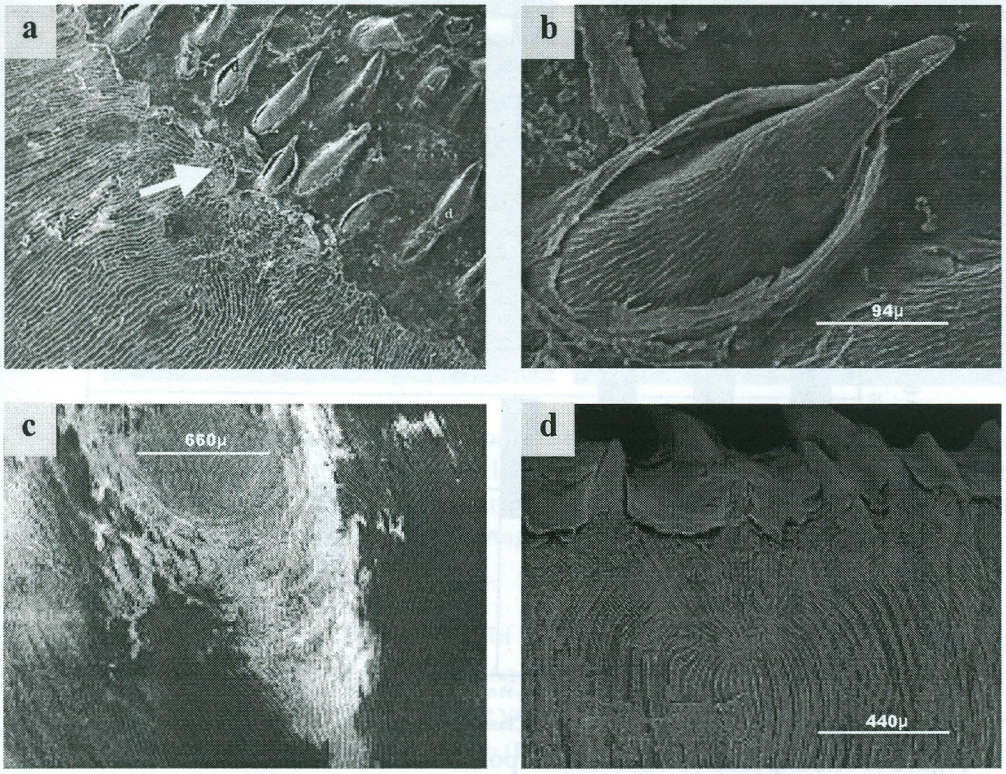

Figure 9. The structures of the scales of *L. menadoensis* viewed with scanning electron microscop; A thin amorphous film of the exposed part (a); The denticles appear from an oval ring of the film (b); The annular and radiating ridges of the embedded scales (c) and the apex region of the scales (d).

Discussion

The scales of *L. menadoensis* bear denticles and melanophores. There are annular ridges that are centered in the apical region and regular arrangement ridges on the surface of the scales. This gross morphology is similar to that of *L. chalumnae* scales as observed by Roux (1942); Smith *et. al.* (1972); Millot *et. al.* (1978).

The scales of *L. menadoensis* has a variety of shapes (Fig. 3) from oval, rectangular, foot print, elongated-pointed edge etc. However there were scales that have an oval shape i.e from lateral line, belly, caudal peduncle and caudal region. Whether the scale of *L. chalumnae* also varies as observed in *L. menadoensis* and whether the scale of *L. menadoensis* has variety of shape on its pectoral fin base as on *L. chalumnae* (Plate. Ib, Smith *et. al*, 1972) needs an examination. The ratio of the embedded part to the total length of scales is different in *L. menadoensis* and *L. chalumnae* at the approximately similar part of body i.e. scales on the dorsal region and on the region extending lateral posteriorly until caudal. The embedded part in *L. chalumnae* was two third (Smith *et. al*, 1972), while in *L. menadoensis* the embedded part was less than two third (the average 64.07%) and the greatest embedded part in *L. menadoensis* was 73.94% or almost three fourth (3/4) of the scale length, which occur in dorsal lobe fin scale.

In general, the size of the scales of *L. menadoensis* gradually decreases from the anterior to the posterior part of the body as it is shown in Figure 4; the more anteriorly the scale is located, the longer the scale is. In addition, in the more anterior part, the embedded part of the scale is greater than the exposed part. Therefore, the anterior scales are stronger because these scales stick more deeply to the dermis. In contrast, the more posteriorly the scale is located, the shorter the scale, and the portion of embedded and exposed part on the most posterior body such as scale on caudal fin the portion can approach 1 : 1 (Fig. 4). This explains why most of the loose scales, have a small size and are within the smallest class size (Fig. 7). Therefore the loose scales are likely to originate from the posterior region of body. The detacliment of the scales in *L. menadoensis* may have occurred during the handling process, fixation and preservation. The specimen was transferred container from one place to the other before putting into the permanent (aquarium) as currently done.

It is interesting to note that in the scales of *L. menadoensis* the melanophores are highly concentrated on the upper part of the body than from the ventral part (Fig. 3a-h; Fig. 6a-b), may be because the function of pigment skin is to prevent underlying tissues against amounts of harmful light as reported by Romer (1964) and Bloom & Fawcett (1975). While Fuji (in Hoar & Randall *ed.*, 1969) stated that the sides and ventral surfaces of fishes are often silvery and white. These surfaces are actually very good reflectors. The denticles of the scales of *L. menadoensis* are reported to have a unique of gold flecked appearance when it is still alive (Erdmann *et al*, 1998). The prismatic iridescence in fishes arise from iridocytes (Lagler *et al.*, 1962). The mirroring substance in iridocytes

is crystalline guanin. The guanin itself is a waste product of blood as an excretion (Lagler *et al.*, 1962). The gold flecked appears when the fish is still alive. After death, the denticles do not give the prismatic effect anymore and became transparent, as indicated on the caudal peduncle and anal scales (Fig. 6a, b, d).

Scale of *L. menadoensis* has a V forming line of three radiating ridges from the apical region (Fig. 9d). It was clearly shown from the first preparation methods of the SEM, but the homogeneous layer of the exposed part was into fractioned. The similar pattern of the apical region was also founded on the scales of the embryo of *L. chalumnae* (Miller, 1979). The ornamentation of the exposed part of this scale from the embryo is only rows of melanophores, the denticles however has not yet appeared.

We have concluded that scales on *L. menadoensis* various in shape and size. The shape of scales on the lateral of the body is oval, while scales on lobe fins have various shapes and usually small. The size of the scales gradually decreases from the anterior to the posterior part of the body. The oval shape of the scales on the lateral of the body is similar in *L. menadoensis* and *L. chalumnae*, but the proportion of the embedded part to the total length is different. The embedded part in *L. menadoensis* is less than two third of the total length (averages 64.1%), while in *L. chalumnae* the embedded part is two third (Smith *et al.*, 1972). This condition supports the separation of the Indonesia coelacanth (*L. menadoensis*) with the coelacanth from South African waters (*L. chalumnae*), but more study on *L. menadoensis* scales is needed to support this conclusion. Holder *et al.* (1999) indicated that scales are one of the important morphological characters that should be considered in future examination. The scales are the best characters available in investigating coelacanth systematics (Forey, 1998). There were many publications on *L. chalumnae* scales that could be compared with *L. menadoensis* scales, whenever additional specimens become available.

Acknowledgments

We gratefully acknowledge the support of Dr. Soetikno Wirjoatmodjo in doing this study, Dr. Arie Budiman, Dr. Siti Nuramaliati Prijono and Dr. Mark V. Erdmann (TNL Bunaken, Manado) for the support to attend the Coelacanth Mini Symposium in Bali. We thank Dr. Francois J. Meunier (Laboratoire d'Ichthyologie, MNHN, Paris) and Dr. Maurice Kottelat (Ichthyologist, Swiss) for the literatures and their kindness for reviewing the manuscript. Thanks are due to Dr. Darrell J. Siebert (NHM, London), Dr. Eugene K. Balon (Axelrod Institute of Ichthyology, Univ. of Guelph, Ontario, Canada), Dr. Rainer Froese (Fishbase, Philippine), Susan Jewett (NMNH, New York) and Rik Nulens (Belgie) for their generosity to provide the literatures. We also thank our friends: Endang Purwaningsih, L.E. Setyorini, Sri Hartini for their assistance during the SEM processes, Arief Supriatna for taking the photograph of the scales and Jumadi, Widodo, Ridwan and Hetty, for help typing the manuscript.

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Accepted January 2003