MORPHOLOGICAL STUDIES ON THE DEVELOPMENT OF MELANOPHORES AND SCALES IN MALPIGMENTED *PARALICHTHYS OLIVACEUS*

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Abstract: In this study, integrated morphological observations were carried out on the occurrence and development of melanophores and scales in hatchery-reared normal and malpigmented Japanese flounder *Paralichthys olivaceus*. The results showed that starry larval melanophores occurred first on the skin of both sides of larvae. With the process of metamorphosis, on the ocular side (left side), adult melanophores occurred and replaced larval melanophores gradually, while on the blind side (right side), larval melanophores degenerated and no adult melanophores appeared. The blind side lost pigment gradually and became white in the end. Malpigmentation occurred at the post-metamorphosis stage. Albinism and ambicoloration formed almost at the same time. Due to unsuccessful replacement of adult melanophores by larval melanophores, the skin in ocular side of fish body lost pigment and formed white irregular patches. Albinism occurred. On the other hand, adult melanophores developed abnormally and replaced the larval melanophores and eventually formed dark patches on the blind side. It was called ambicoloration. At 30 days old, the malpigmentation became evident at the completion of metamorphosis. At about 60 days old, original cycloid scales occurred on both sides of larval bodies. The cycloid scales developed into ctenoid scales on the normal ocular side while remained original status on the normal blind side. Contrast to malpigmented individuals, it was observed that the albino ocular side was still covered by cycloid scales, but the cycloid scales developed into ctenoid scales on the ambicolored blind side. Furthermore, observations of the scales on different postions of recovering albino fish showed the development of scales from immature cycloid scales to mature ctenoid scales following the recovery of pigmentation. It is suggested that a close relationship existed between the occurrence of pigmentation and development of scales in Japanese flounder.

Key words: *Paralichthys olivaceus*; Malpigmentation; Melanophore; Scale; Development
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Flatfish naturally show a typical asymmetry of pigmentation in their body color. However, hatchery-reared flatfish often show a pigment disorder. Malpigmented fish are characterized by either a deficiency of pigment cells on portions of the ocular side (albinism) or excess pigmentation on the blind side (ambicoloration). As cultured products, their lower market price is due to their unpleasant appearance. This abnormality has been examined from various viewpoints. Seikai[1] described the transformation of pigment cells from larval melanophores to adult melanophores with the process of metamorphosis. He[2] also reported the occurrence time of scales and reported that the albino portion of skin was accompanied with scale abnormality in Japanese flounder. Further research on the morphological development of melanophores and scales in both sides of skin of normal and malpigmented Japanese flounder has not been carried out.

Many research reported that the shortage of nutriments (such as (n-3) PUFA and Vitamin A) and unsuitable living environment usually cause the malpigmenation in flatfish[3-7]. However, it was recently found that albinism would recover partly in hatchery-reared Japanese flounder when the nutrition and living environment were improved (Fig. 1). Ambicoloration was comparatively steady once it occurred on the blind side of fish. To date, few report is available to explain this phenomenon. In this study, the whole developmental processes of melanophores and scales in the skin of normal and malpigmented

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Japanese flounder are described. The recovery of albinism and morphological transformation of scales in recovering albino fish are discussed as well for further understanding of the mechanism of malpigmentation which occurred seriously in hatchery-reared flatfish.

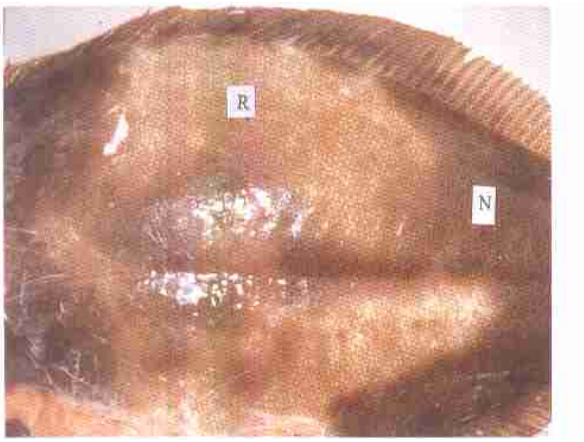

Fig. 1 Recovering skin on the ocular side of albino Japanese flounder. R: recovering skin; N: normal skin

1 Materials and methods

Fertilized eggs of Japanese flounder were hatched and reared in water volum(8m × 2m × 0. 8m) in Huaxin Marine Product Corporation in Weihai city, Shandong province. The salinity was 34 ‰ and the water temperature was maintained between 15—18℃. From 1 to 30 days after hatching, five individuals of larvae were sampled every 5 days, and from 30 to 80 days after hatching, five normal individuals and five malpigmented individuals were sampled respectively every 10 days. The specimens were stored in Bouin' s solution. The total length (TL), total height (TH, the widest part of body including the fins) and levels of albinism were measured.

Both sides of skin in each fixed fish (aged 1—80 days) were divested and fixed with 10% glycerol, and examined by light microscope. The numbers of melanophores per mm^2 on both sides of skin were counted. Photographs were taken to record the morphological development of melanophores and the early occurrence of scales was determined.

Young albino Japanese flounders (15. 0—18. 0cm in TL) in the recovery phase were collected. Scales were plucked from the albino, recovering and normal portions in the ocular side of the fish to investigate the morphological differences(Fig. 2) .

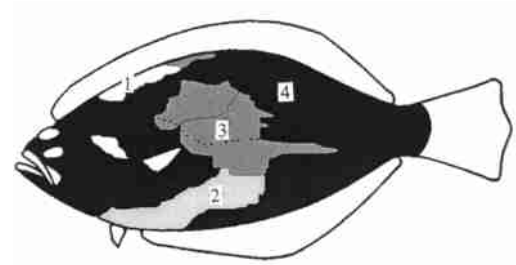

Fig. 2 Collecting positions of scale on the ocular side of recovering Japanese flounder

① albino skin; ②, ③ recovering skin; ④ normal skin

2 Results

2.1 Morphological development of melanophores in larval skin of Japanese flounder

Japanese flounder larvae (2. 6mm in average TL, and 0. 6mm in average TH) showed an approximate bisymmetry of integumentary pigmentation. A few starry yellow larval melanophores existed in the forepart of larval skin (Plate Ⅰ: 1) . These undeveloped larval melanophores had few dendrites and contained a low density of melanosomes. Their diameter was 5—8μm. With the development of larvae, the larval melanophores became larger and significantly darker, but their densities increased slowly (Fig. 3) . At the onset of metamorphosis, both sides of skin in 25 days old larvae were still covered with large matured larval melanophores in high density at about 24 cells/mm^2. These dispersed larval melanophores had numerous dendrites and the cytoplasm contained a high density of melanosomes(Plate Ⅰ: 2) .

The body color of larvae became asymmetrical during the metamorphosis. Larval melanophores on the skin of both sides degenerated and disappeared gradually. At the completion of metamorphosis, on the ocular side of 30 days old larvae, numerous adult melanophores with 30—50μm in diameter began to form. They replaced the larval melanophores quickly, so the density of melanophores increased dramatically(Plate Ⅰ: 3, 7, 9; Fig. 3) . However, on the blind side, the melanosomes in larval melanophores concentrated into inconspicuous punctate masses in the centre of cell and collapsed gradually. No adult melanophore appeared(Plate Ⅰ: 4, 8, 10) . So the density of melanophores decreased quickly on the blind side(Fig. 3) .

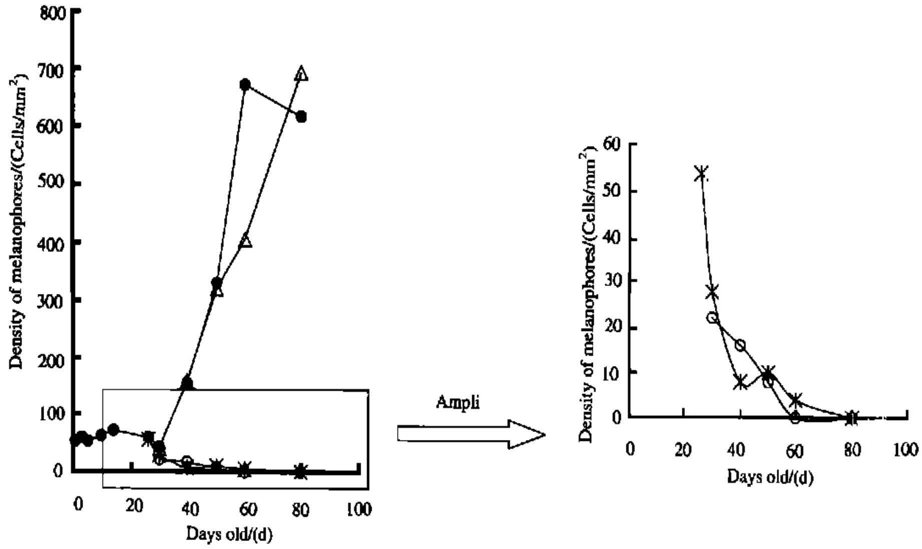

Fig. 3 The density change of melanophores on both sides of skin of normal and malpigmented Japanese flounder

● ocular side; △ pigmented blind side; * normal blind side; ○ albino ocular side

The albinism and ambicoloration occurred during the climax metamorphosis and became evident at about 30 days after hatching. On the albino ocular side of larvae (Plate Ⅰ: 5), the variation of melanophores showed a similar trend in density and morphology with those on the normal blind side (Plate Ⅰ: 4), and no adult melanophores appeared. The coloration of the ocular side disappeared significantly and albinism formed gradually. Instead of becoming white, the ambicolored blind side (Plate Ⅰ: 6) was covered with increasing numbers of adult melanophores just like the normal ocular side (Plate Ⅰ: 3). The albinism and ambicoloration would become serious when no control methods were used (Plate Ⅰ: 11, 12).

2.2 Occurrence of scales in hatchery-reared Japanese flounder

The cycloid scales formed first on both sides of skin of Japanese flounder juvenile at about 60 days old. These underdeveloped scales underlaid the epidermis, and no melanophores were found on their surface (Plate Ⅰ: 7, 8).

With the development of the juvenile, the scales became larger in size. The apical part of scales turned up and came out of the surface of skin on the ocular side of fish. Xanthophores, melanophores and a few spinous processes formed gradually on the apical part of scales. The cycloid scales began to transform into ctenoid scales (Plate Ⅰ: 9). But the blind side was always covered with cycloid scales and no melanophore was observed on the surface of scales throughout the life of Japanese flounder (Plate Ⅰ: 10). However, in malpigmented juveniles, the cycloid scales remained on the albino ocular side, and no melanophore occurred (Plate Ⅰ: 11). Many typical ctenoid scales with numerous melanophores and xanthophores were observed under the epidermis of the stained blind side (Plate Ⅰ: 12).

2.3 Transformation of cycloid scales to ctenoid scales in recovering albino Japanese flounder

After an investigation on the recaptured samples of cultured fish and released fish, A tendency was found that the albino skin partially recovered when the albino fish were fed with fresh diets and kept at low density or released into wild environments (Fig. 1).

Figure. 4, A-D, shows scales that were plucked from different parts of the ocular side skin in recovering adult fish, which were in different recovering stages (①②③④). These scales revealed a whole process of morphological changes of scales from cycloid scales to ctenoid scales with the recovery of albino skin. No melanophore and spinous process were observed on the scale collected from the albino skin in the ocular side of fish (Fig. 4-A). With the recovery of pigment, the density of melanophores and xanthophores increased associated with the occurrence of spinous process on the surface of scales (Fig. 4-B, C). Figure. 4-D shows typical ctenoid scales in normal ocular side

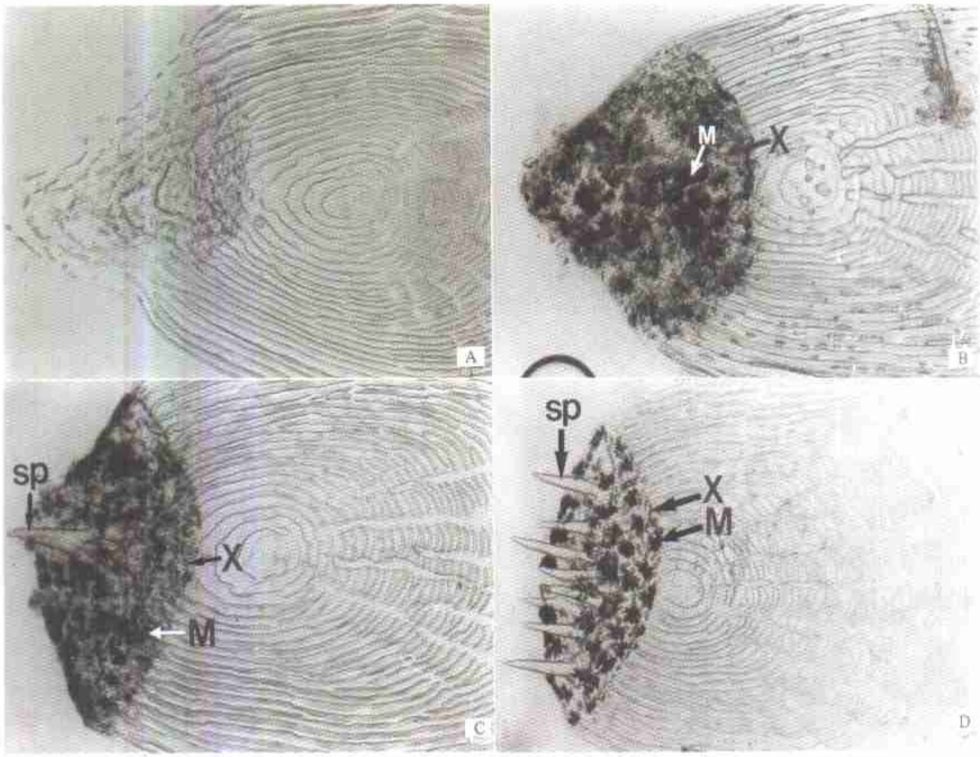

Fig. 4 Scales derived from the skin of ocular side in recovering albino Japanese flounder

A Cycloid scales in albino part of skin(Fig. 2, part ①); B Developing scales in recovering part of skin, with melanophores and xanthophores on the apical part (Fig. 2, part②); C Further developing scales in more recovering part of skin, with spinous process, melanophores and xanthophores on the apical part(Fig. 2, part ③); D Ctenoid scales in normal part of skin(Fig. 2, part ④)

M melanophore; X xanthophore; sp spinous process; Scale bar= 80μm

with a few spinous process, numerous melanophores and xanthophores.

3 Discussion

The present study shows that the development of pigmentation in Japanese flounder larvae occurs in two phases. First, the starry larval melanophore in the skin of hatchery larvae increased slowly in density and developed into larger mature larval melanophores. These mature larval melanophores contained a high density of melanosomes and a large number of dendrites through the completion of metamorphosis. Secondly, when metamorphosis was complete(about 30 days after hatching) , larval melanophores began to disappear gradually, and adult melanophores differentiated on the ocular side, but not on the blind side of fish. Malpigmentation seemed to result from the disruption of pigmentation in the second stage.

The albinism and ambicoloration occurred post-metamorphosis. Melanophores on the albino ocular side showed a similar developmental trend with those on the normal blind side, and density changes of melanophores on the stained blind side were similar to which occurred on the normal ocular side. The albinism and ambicoloration often occurred in the same malpigmented fish. Though previous studies tended to examine the albinism and ambicoloration as an isolated problem[8—10], this study suggests that albinism and ambicoloration, present on both sides of skin might have resulted from the same disruption of modulation of pigmentation caused by the external or internal factors. Moreover, the albinism of Japanese flounder could recover partly when the fish were fed with fresh diets and live in improved cultural environment similar to natural environment. These results lead us to presume that interior secretion modulation might play an important role in the occurrence of malpigmentation in Japanese flounders. This presumption agrees with previous results described by Seikai, et al[3, 10, 11]. They suggested that abnormal hormone secretions and modulation of neural system produced malpigmentation of flatfish. Further research could treat albinism and ambicoloration together, and focus on how stimuli from external or internal environments affects the section of hormones and transformation of signals by neu-tral system; and how these abnormalities disrupt normal development of pigmentation.

The present study also reveals that the more original and underdeveloped cycloid scales occurred first on both sides of larval skin. Gradually, whether on the ocular side or the blind side of normal and malpigmented fish, the cy-cloid scales can develop into ctenoid scales in pigmented skin (including normal ocular side and stained blind side) , but the cycloid scales in unpigmented skin(includ-ing albino ocular side and normal blind side) maintained their undeveloped status. This result agrees with those re-ported by Seikai[2]. A rational explanation for this phe-nomenon might be that the albinism could recover but the ambicoloration could not. When the living environments and nutrition were improved, the adult melanophores and xanthophores could reoccur on the albino skin and on the apical part of cycloid scale. The cycloib scales developed into ctenoid scales as well. Furthermore, once the adult melanophores and the ctenoid scales formed on the surface of skin, there was little chance to degenerate. Therefore it is reasonable to make the hypothesis that it is the same one or more kinds of substrate influences the synthesis of melanosomes, the differentiation of adult melanophores and the development of scales from cycloid scale to ctenoid scale. Further studies on the occurrence and morphological changes of scales are accessary to understand deeply the mechanism of malpigmentation.

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体色异常褐牙鲆皮肤色素及鳞片发育的形态学研究

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摘要: 本文观察比较了体色正常及体色异常褐牙鲆(*Paralichthys olivaceus*) 皮肤中黑色素胞和鳞片的发生及演变过程。结果显示仔鱼鱼体两侧皮肤中最先出现星状幼体型黑色素胞, 随着变态发育, 有眼侧皮肤中成体型黑色素胞逐渐替代幼体型黑色素胞; 而无眼侧皮肤中, 幼体型黑色素胞逐渐退化崩解, 成体型黑色素胞不出现, 无眼侧皮肤逐渐失去色素变为白色。体色异常现象出现于变态后期, 白化和黑化现象几乎同时发生。白化个体有眼侧皮肤中成体型黑色素胞不能正常替代幼体型黑色素胞, 逐渐失去色素形成白色斑块。黑化个体无眼侧皮肤中成体型黑色素胞则非正常地出现, 逐渐替代幼体黑色素胞形成黑斑。约 30 日龄变态完成时, 体色异常现象已经显著, 已能明显区分体色正常和异常个体。60 日龄左右, 幼鱼皮肤开始长出形态较为原始的圆鳞。体色正常个体有眼侧皮肤上

的圆鳞会逐渐发育成栉鳞,无眼侧则维持圆鳞。对比分析体色异常个体的鳞片形态,发现有眼侧白化部位的鳞片仍为圆鳞,而无眼侧黑化部位的鳞片则发育为栉鳞。同时,通过对体色正在恢复中的白化牙鲆的鳞片观察表明,伴随着白化部位色素的恢复,该部位的圆鳞会逐渐转变为栉鳞。由此推断色素的发生与鳞片的发育密切相关。

关键词: 褐牙鲆; 体色异常; 黑色素胞; 鳞片; 发育

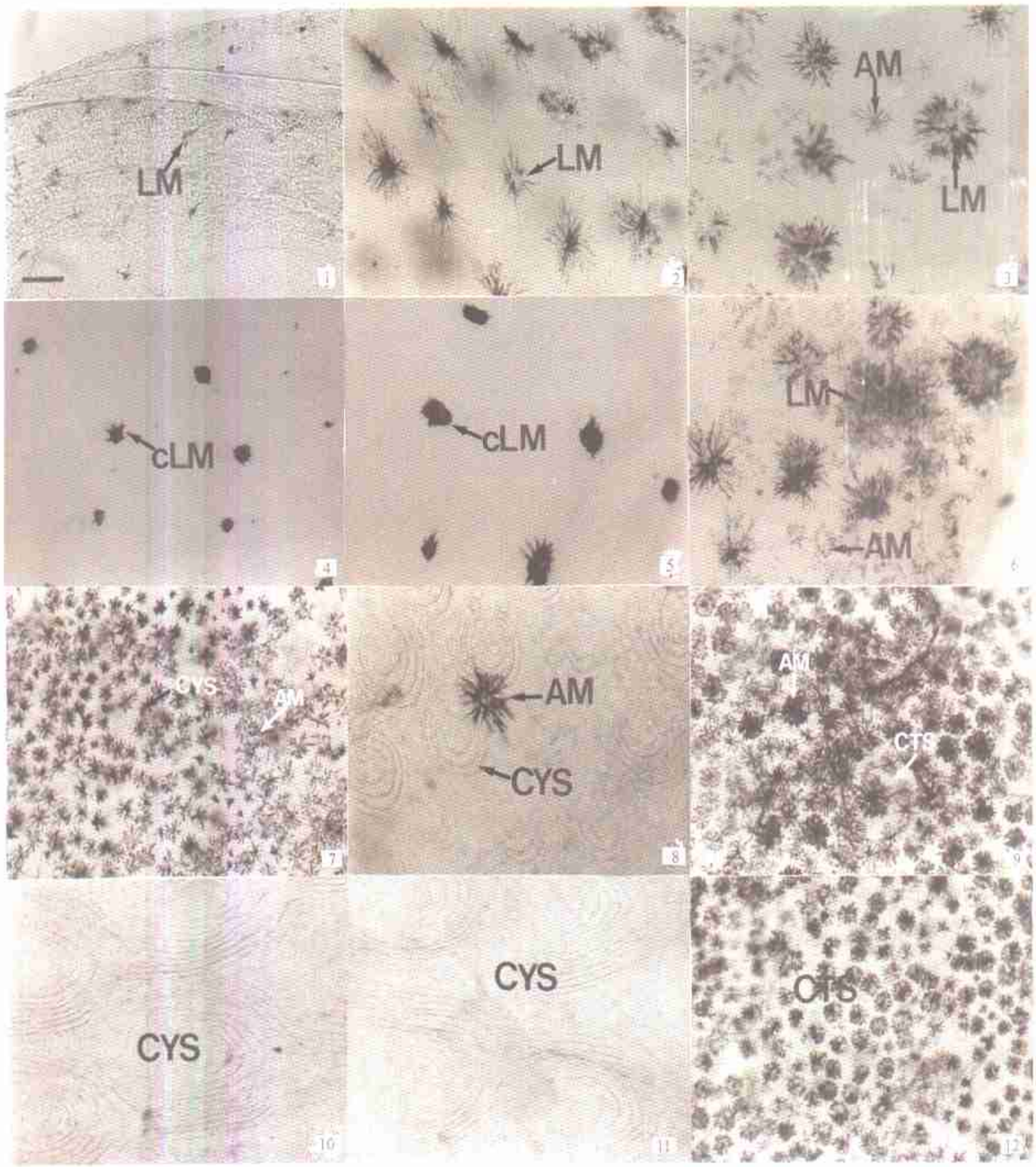

Plate Ⅰ Morphological development of melanophores in Japanese flounder larvae

1. Undeveloped starry larval melanophores in both sides of skin of 1 days old larvae, × 100; 2. Mature larval melanophores in both sides of skin of 25 days old larvae, × 100; 3. Normal ocular side skin of 30 days old larvae, containing developed mature larval melanophores and presented small adult melanophores, × 100; 4. Normal blind side skin of 30 days old larvae, larval melanophores concentrated into points, × 100; 5. Albino ocular side skin of 30 days old larvae, larval melanophores concentrated into points and no adult melanophore appear, × 100; 6. Ambicolored blind side skin of 30 days old larvae, developed melanophores and adult melanophore presented in it, × 100. 7. Adult melanophores and scales in normal ocular side skin of 60 days old larvae, × 100; 8. Normal blind side skin of 60 days old larvae, losing melanophores, scales occurred, × 100; 9. Normal ocular side skin of 80 days old larvae covered by adult melanophores and ctenoid scales, × 100; 10. Normal blind side skin of 80 days old larvae, losing all melanophores, still covered with cycloid scales, × 100; 11. Albino ocular side skin of 80 days old larvae, lost all melanophores, covered with cycloid scales, × 100; 12. Ambicolored blind side skin of 80 days old larvae covered with numerous adult melanophores and ctenoid scales, × 100. sLM: starry larval melanophore; LM: larval melanophore; cLM: concentrated larval melanophore; AM: adult melanophore. CYS: cycloid scale; CTS: ctenoid scale; Scale bar= 80μm