

Chorion microstructure for identifying five fish eggs of Apogonidae

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The chorion microstructure of the eggs of apogonids including *Apogon lateralis*, *A. aureus*, *A. nitidus*, *A. cookii* and *A. guamensis* was studied by scanning electron microscopy. In Apogonidae, egg chorion microstructures, especially those in the micropyle region, are useful characters for egg identification.

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The surface microstructure of external egg membranes has been used as a taxonomic character for species identification of fish eggs (Riehl, 1993). In previous studies, the outer surface of the chorion and the microstructure of the micropyle were the noteworthy features for egg identification and phylogenetic analyses in Serranidae, Sparidae and Mugilidae (Chen *et al.*, 1999; Li *et al.*, 2000). During fertilization a single sperm enters the micropyle, then the inner part of the micropylar canal becomes narrower and a plug-like blockage quickly forms on the micropyle to prevent polyspermy (Ohta & Iwamatsu, 1983; Hart, 1990). In fertilized eggs, the morphological changes in the micropyle render this feature difficult to use for species-specific egg identification. Most eggs collected from the wild have already been fertilized, therefore the characters of micropylar canals are unsuitable for diagnostic analyses.

Although the outer surface of the chorion generally does not show remarkable differences in microstructure between species of the same genus, it may be diagnostic for identifying genera, families or orders. For example, Myctophiformes have three-root pyramidal ornamentations and Synodontidae have hexagon reticulations on the chorion surface (Shao *et al.*, 2001). On the other hand, apogonid eggs have filaments that originate in the micropyle region

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(Shao *et al.*, 2001). The arrangement of these filaments and surface ridges in the micropyle region varies between species and may be an important feature for egg identification in Apogonidae.

Seventy-three species of apogonids are found along the coastal reefs of Taiwan (Shao & Chen, 1993). Apogonid males mouth brood the eggs for their protection. In this study, eggs taken from the mouth of male fishes were used to study the microstructure of the micropyle region, and characters that may be informative to species' identification are described.

Fertilized eggs of *Apogon lateralis* Valenciennes, 1832, *Apogon aureus* (Lacépède, 1802), *Apogon nitidus* (Smith, 1961) (= *A. holotaenia* Regan according to some authors; see Eschmeyer, 2006), *Apogon cookii* Macleay, 1881, and *Apogon guamensis* Valenciennes, 1832 were removed from the mouths of male fishes, collected from localities along the southern coast of Taiwan, as soon as the fishes were caught with nets. The fishes were released unharmed after the eggs were collected. The collected eggs were initially washed in fish saline buffer (0.14 M NaCl, 0.01 M KCl, 0.16 mM MgCl₂, 5 mM CaCl₂, 1.8 mM Na₂PO₄, 2 mM NaHCO₃, 5 mM glucose), then pre-fixed in 5% glutaraldehyde with 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4, and washed with phosphate buffer (0.1 M, pH 7.4). The eggs were post-fixed in 1% osmium tetroxide, then washed with buffer, and dehydrated in a graded series of ethanol. The samples in absolute ethanol were transferred into amyl acetate and dried in a critical point dryer with liquid carbon dioxide. The dried samples were mounted on stubs and sputter-coated with gold. The specimens were studied in a scanning electron microscope (SEM; S-2400, Hitachi) under an accelerating voltage of 15 kV.

Chorion characters including egg size, micropyle region diameter, number of ridges around the micropyle region, and the length of the longest and shortest ridges were measured using a SEM. Means and s.d. of the character values were calculated and analysed by using SAS software (Table I).

All examined species have spherical eggs and ridges arranged in a radial form around the micropyle. Filaments are attached at the outer end of the ridges. Morphometric measurements for each species are given in Table I, and the eggs are illustrated in Fig. 1. Projections were found in two rings outside the ridge in the micropylar region of *A. lateralis* [Fig. 1(e), (f)].

The morphometric measurements of the chorions of each of the five species were analysed using ANOVA and Scheffé's test. ANOVA was used to test the significant difference among the means of the chorion microstructures of the five species. The eggs were subsequently grouped according to microstructure features using Scheffé's test. The results of the statistical analyses are shown as box plots (Fig. 2).

Scheffé's test on egg size places the five species in three groups [Fig. 2(a)]. *Apogon cookii* eggs are the largest in size with a mean diameter of 557 µm, followed by *A. aureus* and *A. nitidus*, which are significantly larger than eggs of *A. lateralis* and *A. guamensis*. With light microscopy the egg size of *A. cookii* (c. 0.7 × 0.07 mm) is also the largest one compared with other apogonid eggs (c. 0.6 × 0.6 mm) (Shao *et al.*, 2001). The box plot for the size of the micropyle region, however, shows three different groups of species. *Apogon cookii* has the largest micropyle region followed by *A. guamensis*; the third group with

TABLE I. Microstructure characters of species of *Apogon* eggs. Values are means \pm s.d. (n = sample size)

	<i>A. guamensis</i> (n = 13)	<i>A. nitidus</i> (n = 15)	<i>A. aureus</i> (n = 10)	<i>A. cookii</i> (n = 10)	<i>A. lateralis</i> (n = 13)
Egg diameter (μm)	477.08 \pm 14.57	525.82 \pm 10.18	522.93 \pm 10.10	557.38 \pm 31.59	464.78 \pm 13.78
Micropyle region diameter (μm)	128.43 \pm 5.74	118.70 \pm 3.55	113.09 \pm 3.71	141.21 \pm 4.89	115.62 \pm 8.59
Ridge number	49 \pm 2	44 \pm 2	52 \pm 2	57 \pm 4	56 \pm 4
Length of longest ridge (μm)	50.02 \pm 1.68	45.84 \pm 3.51	37.28 \pm 1.35	61.60 \pm 2.40	23.65 \pm 1.60
Length of shortest ridge (μm)	11.99 \pm 0.88	14.46 \pm 1.73	8.95 \pm 1.28	13.88 \pm 1.97	17.46 \pm 1.18
Projection outside ridge	No	No	No	No	Yes

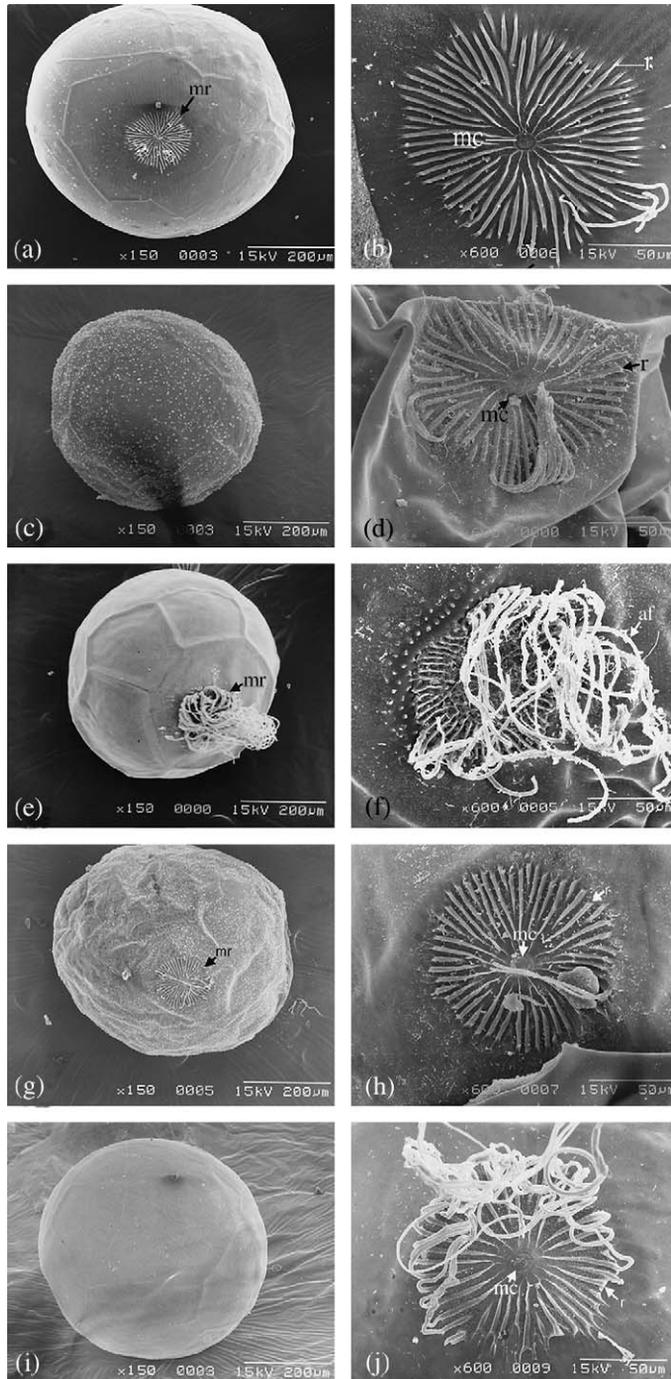


FIG. 1. Micrographs of species of *Apogon* eggs: (a) *A. cookii*, entire egg, (b) *A. cookii*, micropyle region, (c) *A. guamensis*, entire egg, (d) *A. guamensis*, micropyle region, (e) *A. lateralis*, entire egg, (f) *A. lateralis*, micropyle region, (g) *A. aureus*, entire egg, (h) *A. aureus*, micropyle region, (i) *A. nitidus*, entire egg and (j) *A. nitidus*, micropyle region. mc, micropylar canal; mr, micropyle region; p, projection; r, ridge.

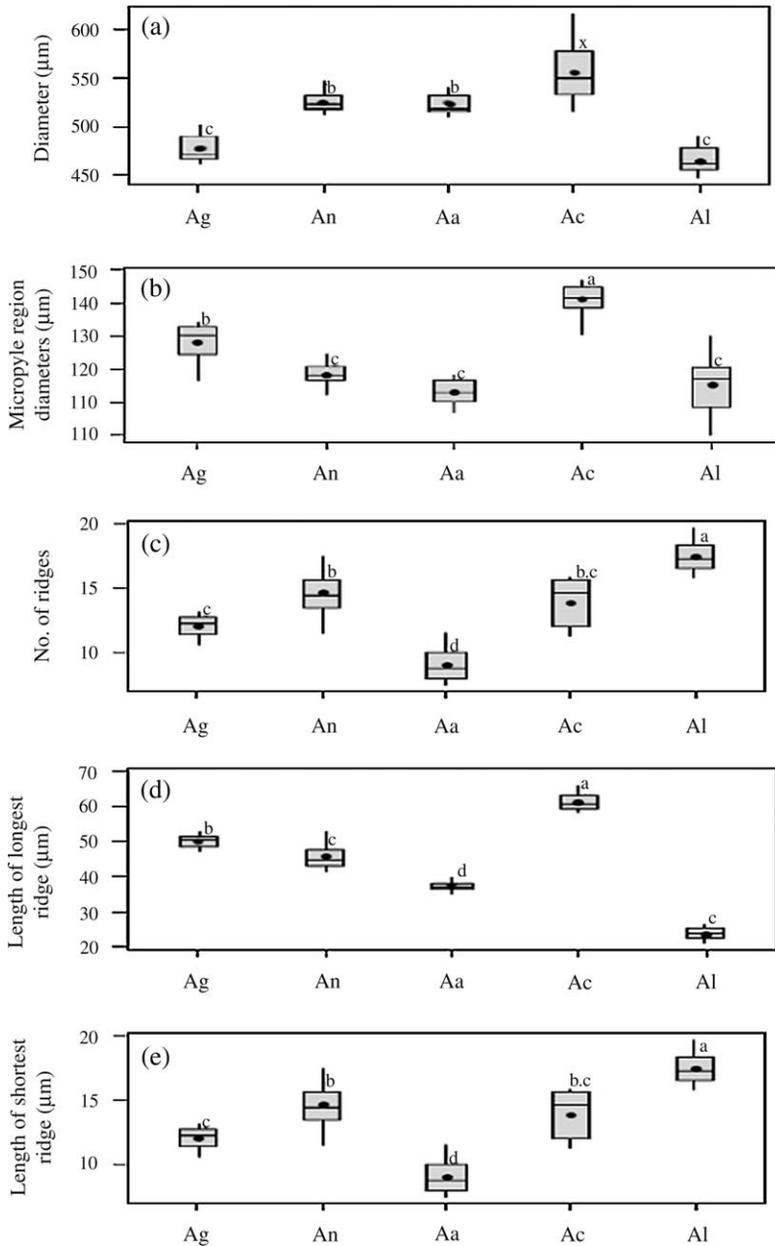


FIG. 2. Box plot of *Apogon* egg morphometrics and mersitics: (a) egg diameter, (b) micropyle region diameter, (c) number of ridges in micropyle region, (d) length of the longest ridge in the micropyle region and (e) length of the shortest ridge in the micropyle region. Aa, *A. aureus*; Ac, *A. cookii*; Ag, *A. guamensis*; Al, *A. lateralis*; An, *A. nitidus*. Inside boxes the small dots are means and the bars are medians. Means associated with the same lower case letter above bars are not significantly different by Scheffé's test ($P > 0.05$).

smallest micropyle includes *A. lateralis*, *A. aureus* and *A. nitidus* [Fig. 2(b)]. The number of ridges in the micropyle region can be used to distinguish *A. nitidus*, which has significantly fewer ridges than the other four species [Fig. 2(c)]. There is no significant difference in the number of ridges among *A. lateralis*, *A. cookii* and *A. aureus*, and between *A. aureus* and *A. guamensis*. The length of the longest ridge in the micropyle region is the most useful diagnostic character in *Apogon* eggs; each species is significantly different ($P < 0.05$) from all others for this character (Table I). The mean length of the longest ridge in the micropyle region is greatest in *A. cookii*, followed by *A. guamensis*, *A. nitidus*, *A. aureus* and *A. lateralis* [Table I and Fig. 2(d)]. The mean length of the shortest ridge in the micropyle region is also a relatively important diagnostic feature. *Apogon lateralis* has the greatest value for the mean length of the shortest ridge, and *A. aureus* has the smallest value; values for *A. cookii* and *A. nitidus*, and *A. guamensis* were not significantly different from each other ($P > 0.05$) [Fig. 2(e)].

The microstructure of micropyles in unfertilized eggs is an important character in fish egg identification (Amanze & Iyengar, 1990; Riehl & Appelbaum, 1991; Britz *et al.*, 1995; Chen *et al.*, 1999; Li *et al.*, 2000). The microstructure of micropyles and surface ornamentations, however, may change during fertilization (Hosokawa *et al.*, 1981; Groot & Alderdice, 1985; Linhart & Kudo, 1997). In apogonid eggs, the blockage of the micropylar canal occurs after fertilization (Shao *et al.*, 2001). Therefore, the microstructural features of the micropylar canal are not reliable for the identification of fertilized eggs but can be used for unfertilized eggs. Whereas the eggs used in this study were collected from wild-caught male fishes and were fertilized, it was not possible to study the micropylar canal features.

Thus, in *Apogon* egg identification the significant characters are egg size, the diameter of micropyle region, the number of ridges around the micropyle and the projections outside the ridges. A taxonomic key for identifying the five species of *Apogon* based on the characters in the micropyle region is as follows:

- 1a. Egg size $< 505 \mu\text{m}$ 2
- 1b. Egg size $> 505 \mu\text{m}$ 3
- 2a. Projections outside the ridges [Fig. 1(f)] *A. lateralis*
- 2b. No projections outside the ridges [Fig. 1(d)] .. *A. guamensis*
- 3a. Micropyle region diameter $< 130 \mu\text{m}$... 4
- 3b. Micropyle region diameter $> 130 \mu\text{m}$.. *A. cookii*
- 4a. Number of ridges < 50 *A. nitidus*
- 4b. Number of ridges > 50 *A. aureus*

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