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FINE STRUCTURE OF THE EGGSHELL OF *OMMATISSUS BINOTATUS* FIEBER (HOMOPTERA, AUCHENORRHYNCHA, TROPIDUCHIDAE)

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Abstract—The external morphology and fine structure of the eggshell of *Ommatissus binotatus* Fieber (Homoptera: Tropiduchidae) was investigated by light, scanning and transmission electron microscopy. The egg surface has 2 main regions: a specialized area and an unspecialized egg capsule. The specialized area is characterized by a large respiratory plate containing the operculum and a short respiratory horn. The latter consists of an external hollow tube and an internal cone-shaped projection hosting a micropylar canal. The eggshell has 4 layers: the vitelline envelope, a wax layer, the chorion and an outer mucous layer. The chorion has inner, intermediate and outer parts. The functions of the different parts of the eggshell are discussed. Characters useful to define the eggs and the oviposition habit in the family Tropiduchidae were provided. The size and morphology of the egg, plate, respiratory horn and operculum are suggested as useful characters for ootaxonomic analysis.

Index descriptors (in addition to those in the title): Egg; ootaxonomy; respiratory plate; operculum.

INTRODUCTION

The taxonomic and phylogenetic importance of eggshell structure in pterygote insects has been demonstrated in various orders at different levels (see Hinton, 1981 for a review). For each family of the Homoptera Auchenorrhyncha, Cobben (1965) provided characters useful to define the egg morphology and the ovoposition habits, emphasizing evolutionary trends and their phylogenetic value. In the Tropiduchidae family, these characters included eggs with an operculum, a respiratory horn containing the micropylar canal, an inner porous layer and oviposition in plant tissue.

However, the eggs of the tropiduchids are still largely unresearched. Little information is available on the external morphology of the eggs of most species and no detailed studies of the fine structure of the eggshell have been done. Alfieri (1934) illustrated the eggs, larval instars IV–V and adults of *Ommatissus lybicus* Bergevin. Hussain (1963) and Gharib (1966) studied the biology of *O. libycus* and published general data on the eggs, nymphs and adults of this species. Fletcher (1979, 1981) described and illustrated the eggs, nymphal instars and adults of *Kallitambinia australis* Muir. Guglielmino (1997) studied the biology and described and illustrated the egg and post-embryonic development of *Ommatissus binotatus*.

Here we report a study of the external morphology and fine structure of the eggshell of *O. binotatus* with the aim of providing data useful for taxonomic and phylogenetic studies.

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MATERIALS AND METHODS

Leaves of *Chamaerops humilis* L. containing eggs of *O. binotatus* were collected in the Vendicari Natural Reserve (Siracusa, Italy) and brought to the laboratory. Dissection in Ringer's solution to remove the eggs was done about 24–48 h after egg-laying. The eggs were processed for light and electron microscope examination.

For scanning electron microscopy (SEM), the eggs were fixed for 3 h in 4% paraformaldehyde–5% glutaraldehyde buffered with sodium cacodylate (0.1 M and pH 7.3), rinsed in the same buffer overnight and post-fixed for 1 h in 1% osmium tetroxide buffered with 0.1 M sodium cacodylate. They were then dehydrated in a graded ethanol series, dried in a Balzers Union CPD 020, sputter-coated with gold in a Balzers MED 010 unit and observed with a JEOL JSM 5200 scanning electron microscope.

For light and transmission electron microscopy (TEM), the eggs were fixed and dehydrated as above, then embedded in Epon resin. Semi-thin and ultra-thin sections were cut with an LKB Nova ultramicrotome. The semi-thin sections were stained with toluidine blue and observed with a Zeiss Axiophot microscope. The ultra-thin sections were stained with uranyl acetate and lead citrate and observed with a JEOL JEM 1200 EX II transmission electron microscope.

RESULTS

The females of *O. binotatus* make holes about 700 μm deep and 300 μm wide with their ovopositor in leaves of the host plant (*C. humilis*) (Fig. 1A–C). The eggs are embedded in the holes with only the tip of the cephalic egg pole exposed (Fig. 1A–C). The eggs are laid in rows along the dehiscent lines on the lower surface of the leaf lamina (Fig. 1A). The material removed from the holes is piled up outside the cavity (Fig. 1A–D).

The egg of *O. binotatus* measures 720–800 μm in length and 280–320 μm in width. It is ellipsoidal in shape with a pointed anterior pole and a rounded posterior pole; the fore side is slightly concave, the opposite side convex.

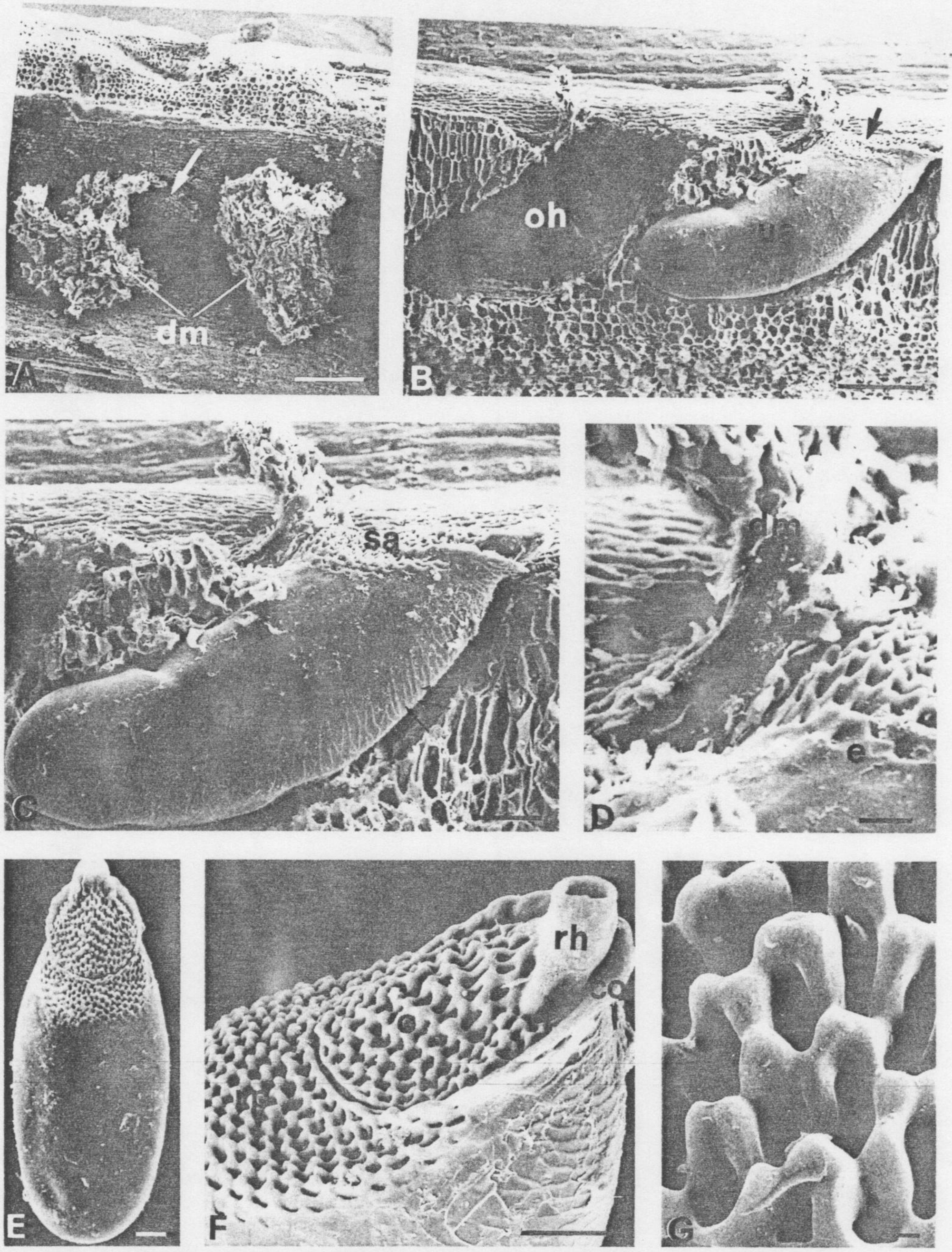

Fig. 1. SEM micrographs of eggs of *Ommatissus binotatus* Fieber. (A) Eggs laid in row on leaf of *Chamaerops humilis*, showing specialized area (arrow) exposed to external environment, and discarded material (dm) of host plant. Bar = 250 μm. (B) Longitudinal section of *C. humilis* leaf with embedded eggs. Oviposition hole (oh), specialized area (arrow), and unspecialized area (ua). Bar = 200 μm. (C) Egg as (B) at higher magnification. Specialized area (sa), unspecialized area (ua) with smooth chorion, and polygonal chorionic pattern on dorsal side (arrows). Bar = 100 μm. (D) Discarded material (dm) of host plant at high magnification. Egg (e). Bar = 25 μm. (E) Whole egg extracted from leaf. Bar = 50 μm. (F) Specialized area showing respiratory plate (rp), operculum (o), respiratory horn (rh) and collar (co). Bar = 50 μm. (G) Detail of external cavities in specialized area. Bar = 5 μm.

The cephalic pole has a flat, obliquely disposed surface that bears 3 specialized structures: the subcircular plate, operculum, and respiratory horn (Fig. 1E, F).

SEM observations showed different features of the chorion in the various regions of the egg. Outside the specialized area, the chorion surface is smooth, except on the dorsal side where short and unequal ridges define polygonal areas. These ridges are more numerous at the anterior pole and gradually decrease and disappear towards the posterior pole (Fig. 1B, C). The specialized area is characterized by a complex network of thicker chorionic ridges defining a system of communicating cavities open to the external environment (Figs 1E–2A).

The operculum is circular with a diameter of about 120 μm and bears the respiratory horn in apical position. A clear boundary separating the operculum from the surrounding egg surface facilitates larval eclosion (Fig. 2B). The egg surface in the area of the operculum is flat at the anterior margin and raised to form a hardy collar at the lateral and posterior margins. The collar seems to

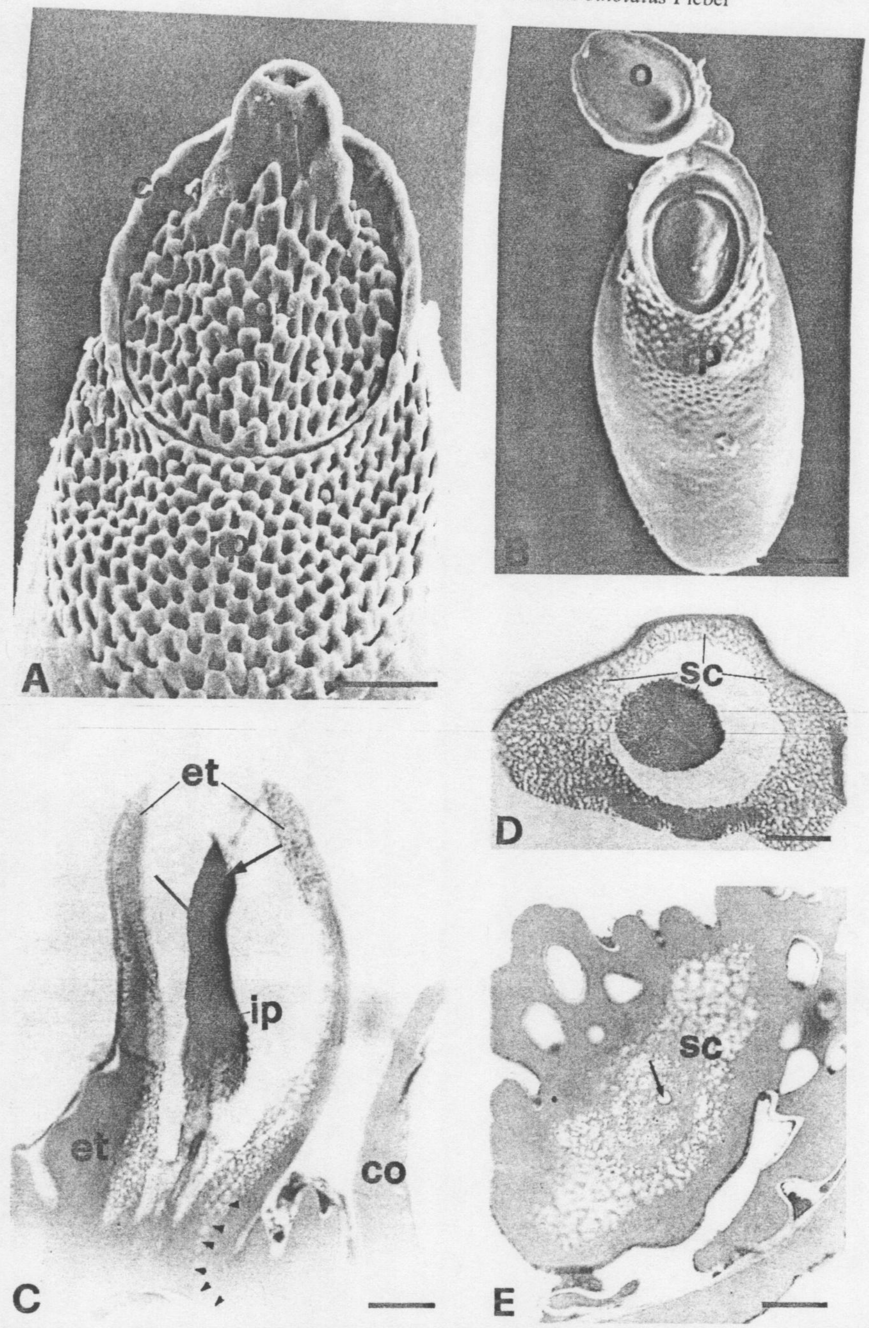

Fig. 2. (A and B) SEM micrograph of egg with closed (A), and open (B) operculum (o). Respiratory plate (rp), respiratory horn (rh) and collar (co). (A) Bar = 50 μm, (B) Bar = 100 μm. (C) Semi-thin section of respiratory horn showing external tube (et) surrounded by collar (co), and internal projection (ip) hosting micropylar canal (arrows). The spongy chorion of these regions communicates with the intermediate chorion of unspecialized area (arrowheads). Bar = 10 μm. (D and F) Cross-sections through distal (D) and basal portion (E) of respiratory horn. Spongy chorion (sc), micropylar canal (arrow). Bar = 10 μm.

protect the operculum and the respiratory horn (Figs 1E, F, 2A).

The respiratory horn is short (about 70 μm long) and narrows gradually towards its apex (Figs 1E, 2A). Semi-thin sections showed that the respiratory horn consists of a hollow tube containing a cone-shaped projection hosting the micropylar canal (Fig. 2C, D). The chorion of the external tube is compact on the outside and porous (spongy) inside. The chorion of the inward projection is porous on the basal side and more compact on the apical side (Fig. 2C, D). Basally, the spongy chorion of internal projection and the external respiratory tube fuses (Fig. 2C, E). The respiratory horn is elliptical in transverse section with the micropylar cone in eccentric position (Fig. 2D, E).

The eggshell varies in thickness in the different regions of the egg (Fig. 3A). It is thicker in the specialized area, ranging from 6 to 25 μm (Fig. 3A, B), against about 1.7 μm in the unspecialized area (Fig. 3A, E, F). However, in both areas the eggshell always has 4 layers: the vitelline envelope, a wax layer, the chorion and an outer mucous layer (Fig. 3B–D).

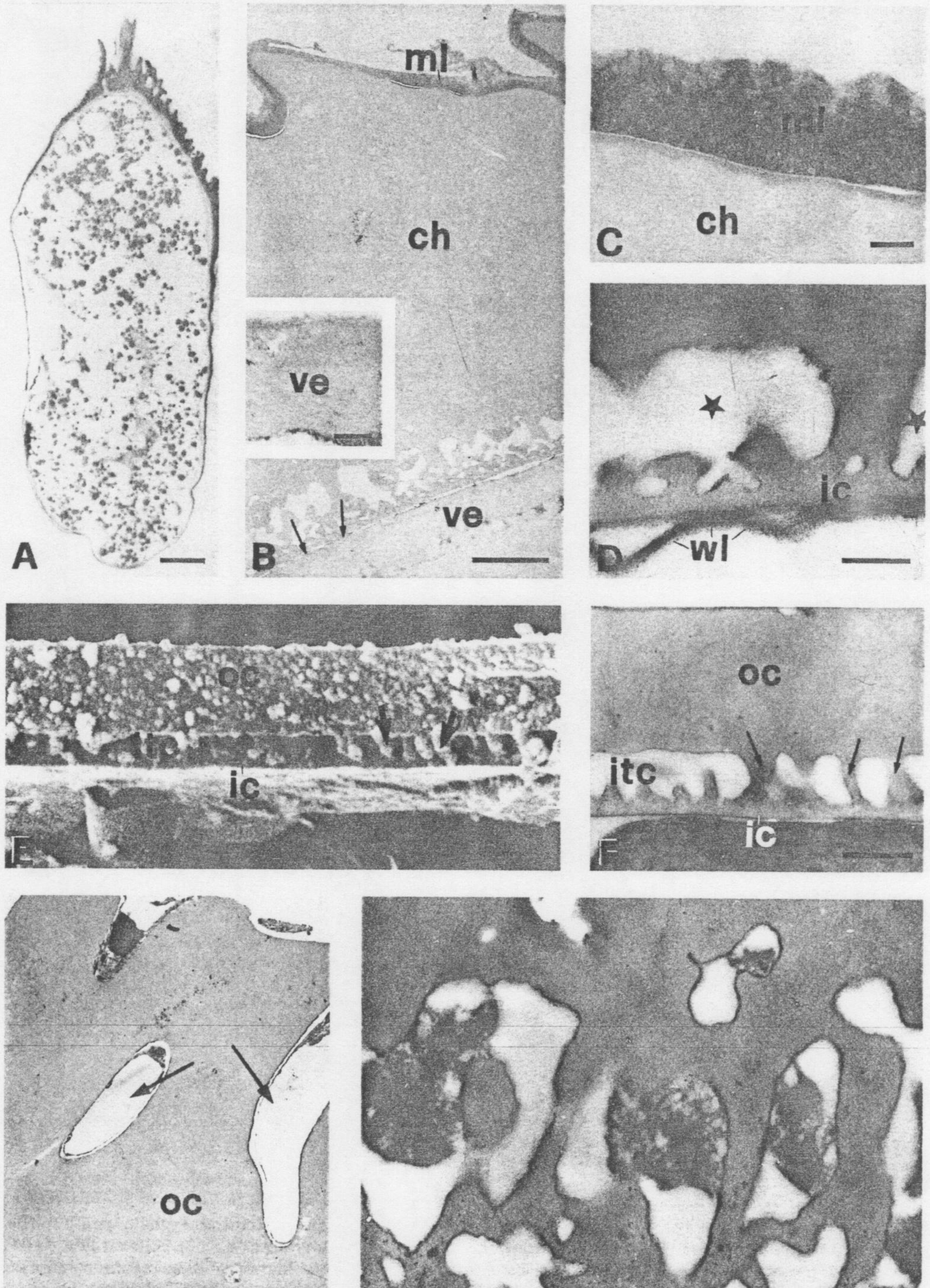

Fig. 3. (A) Subsagittal section of whole egg showing thickness of eggshell in different regions. Bar = 50 μm. (B) Subsagittal section through specialized area showing vitelline envelope (ve), wax layer (arrows), chorion (ch) and mucous layer (ml). Inset of vitelline envelope at higher magnification. (B) Bar = 2 μm; inset bar = 250 nm. (C) Mucous layer (ml) of (B) at higher magnification. Chorion (ch). Bar = 200 nm. (D) Subsagittal section through unspecialized area with wax layer (wl), inner (ic) and intermediate chorion with cavities (stars). Bar = 200 nm. (E and F) SEM (E) and TEM (F) micrographs of chorion in unspecialized area showing inner chorion (ic), intermediate chorion (itc) with chorionic pillars (arrows) defining regular cavities, and outer chorion (oc). (E) Bar = 1 μm, (F) Bar = 500 nm. (G) Subsagittal section of chorion in specialized area showing intermediate chorion with irregular system of cavities (stars), outer chorion (oc) with large cavities (arrows) communicating with environment. Bar = 2 μm. (H) Irregular cavities of intermediate chorion at higher magnification. Bar = 500 nm.

The vitelline envelope, a multilayered structure, has the same thickness (about 0.7 μm) throughout the egg (Fig. 3B, inset). It is separated from the chorion by a thin, very electrondense layer, which seem to be a wax layer (Fig. 3D).

The chorion has a uniform electrondense structure in which inner, intermediate and outer parts can be distinguished (Fig. 3B, E–H). The compact inner part is thinner (about 90 nm) and in close contact with the wax layer. The intermediate part is characterized by cavities extending between the inner and outer parts. In the unspecialized area, the intermediate part consists of chorionic pillars (about 360 nm in height) defining a system of regular cavities (Fig. 3E, F). In the specialized area, this part is thicker and more articulated with many irregular cavities (Fig. 3G, H) in continuity with the spongy chorion of the respiratory horn (Fig. 2C).

The thicker outer part of the chorion has a compact structure, which gives the eggshell considerable consistency. In the specialized area, it is characterized by a system of cavities larger than those of the intermediate part and corresponding to the system observed by SEM described above (Figs 1E–2A, 3G).

A thin layer of viscous, electrondense material coats the outside of the eggshell (Fig. 3C).

DISCUSSION

Data in the literature and our observations show that the eggs of *Ommatissus binotatus* and other known Tropiduchidae species (Alfieri, 1934; Hussain, 1963; Gharib, 1966; Fletcher, 1979, 1981; Guglielmino, 1997) are adapted to be inserted into living plant tissue and have the same basic plan. They are ovoidal, slightly curved, with a plate, an operculum and a short respiratory horn hosting the micropylar canal at the anterior pole. The latter represent the only exposed area of the egg.

The general structure of the eggs of *O. binotatus*, *O. lybicus* and *K. australis* confirms the characters that Cobben (1965) considered typical of this family. On the basis of the egg morphology, the Tropiduchidae seems to be a homogeneous family. Oviposition habits are also similar in this family. From morphological and functional points of view, Tropiduchidae have fulgoroid- and piercing-type ovopositor, respectively (Bourgoin, 1993). This organ is used to dig holes in living plant tissue, in which eggs are laid in rows.

In the eggs of *O. binotatus*, the external chorionic system of large cavities of the plate and the spongy chorion of the respiratory horn communicate with the cavity system of the intermediate chorion and the external environment. This communication guarantees the oxygen supply and gas exchange between the developing embryo and the environment. Furthermore, the location of the operculum in this area facilitates eclosion of the larva.

Although a relatively large area of the egg surface is exposed to the atmosphere, desiccation could be avoided by the layer similar to the wax layer underlying the chorion. In addition, the viscous layer on the egg probably regulates the humidity, as suggested by Hinton (1961) for the material covering the egg of *Nepa cinerea* and by Cobben (1968) for the suprachorionic layer of Saldidae eggs. According to the nomenclature of Ludwig (1874), this eggshell layer should be considered a tertiary envelope. It is secreted by the cells of the female genital tract. All the other layers are secondary envelopes secreted by the follicle cells. The rest of the egg surface is compact, sturdy and well-embedded in the substrate; it anchors the egg to the substrate and protects the embryo against physical damage.

From the taxonomic point of view, the 3 tropiduchid species with known eggs belong to 2 different tribes. *O. binotatus* and *O. lybicus* belong to Trypetimorphini, and *K. australis* to Tambiniini. These species are monophagous and live on *Chamaerops humilis*, *Phoenix dactylifera* and *Aegiceras corniculatum*, respectively.

The lack of data on the eggshell of these species prevents ootaxonomic analysis. Although the eggs of these species have similar features, we believe that such morphological characters as size and morphology of the egg, plate, respiratory horn and operculum could be useful for a species-specific identification.

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