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Studies on Cercariae from Kuwait Bay. IV. Description and Surface Topography of *Cercaria kuwaitae* IV sp. n. (Trematoda: Philophthalmidae)

J. Abdul-Salam and B.S. Sreelatha

Department of Zoology, University of Kuwait, P.O. Box 5969, Safat 13060, Kuwait

ABSTRACT. A philophthalmid cercaria, *Cercaria kuwaitae* IV sp. n., is described from the prosobranch gastropod, *Cerithidea cingulata* (Gmelin 1790) from Kuwait Bay. Details are presented on the morphology and behaviour of the cercariae. Scanning electron microscopy was used to study the surface features of the cercariae and daughter rediae. This is the first record of a philophthalmid cercaria from the Arabian Gulf region.

The prosobranch gastropod *Cerithidea cingulata* (Gmelin 1790) is prevalent in mudflats covering most of the intertidal region of Kuwait Bay (Clayton 1986). Cercariae representing different groups of digenetic trematodes have been reported in *Cerithidea* species from different geographical localities (Cable 1956, Ito 1957, Holliman 1961, Martin 1972, Yoshino 1975, Sousa 1983, Harada and Suguri 1989). Abdul-Salam and Al-Khedery (1992), in a survey of larval digeneans in some snails in Kuwait Bay, reported six species infecting *C. cingulata*. Subsequently, as part of a series on the larval stages of digenetic trematodes in molluscs in Kuwait Bay, Abdul-Salam and Sreelatha described two cyathocotylid (1993a, 1993b) and one opisthorchioid (1993c) cercariae from *C. cingulata* snails. This study presents both light (LM) and scanning electron microscopical (SEM) aspects of a new philophthalmid cercaria and the daughter rediae.

Materials and Methods

Cerithidea cingulata measuring 15-25 mm in shell length and being naturally infected with trematode larvae were collected from mudflats covering the southern shores of Kuwait Bay, approximately 10 km west of Kuwait City. The snails were individually isolated into 10 ml vials containing 4 ml of filtered sea water and left in the

sun for 1 h before they were examined under a stereoscopic microscope for emerged cercariae. Shedding and nonshedding snails were crushed, dissected and examined for developing larval stages. Observations on recovered larval stages were made on live, unstained or vitally stained (with 0.5% neutral red) specimens, as well as on specimens fixed in AFA and stained in acetocarmine. Larval morphometrics were taken from at least 10 specimens fixed in near boiling AFA. Drawings were made by aid of a camera lucida. Measurements are given in micrometers followed by averages in parentheses.

Living cercariae and daughter rediae were prepared for SEM by sequential fixation in 2% glutaraldehyde for 1 h and 1% osmium tetroxide for 10 min both in 0.1 M cacodylate buffer at pH 7.4. They were dehydrated in acetone, critically point dried, mounted on stubs and coated with gold-palladium. Specimens were examined in a Jeol JSM-840 scanning electron microscope.

The numerical system of designating species adapted by Cable (1956) was used for naming the new cercariae described.

Results

Cercaria kuwaitae IV sp. n.

(Figs. 1-16, Tables 1-2)

Description

Distome cercaria, suckers well developed; tail long, cylindrical (Fig. 1). Body oval to elongate depending on state of contraction, slightly indented at level of ventral sucker. Tegument devoid of spines. Parenchyma of body filled with cystogenous glands. Glands in immature cercariae disposed in four longitudinal rows. Tail filled with caudal bodies, with terminal invagination receiving ducts of four glands (Fig. 2). Cephalic glands extend posteriorly to mid lateral body region. Cephalic gland ducts running in compact bundles of seven on each side, opening at anterior edge of oral sucker. Oral sucker subterminal, slightly smaller than the medially located ventral sucker (Fig.1). Oral sucker leads to short prepharynx, followed by oval pharynx. Oesophagus very short, bifurcating into caeca extending to region of excretory bladder. Nuclei of genital primordium forms narrow cord extending from oesophagus to immediately in front of excretory bladder (Fig. 3). Excretory bladder thin-walled, ovoid, opens through caudal duct bifurcating to lateral openings at proximal tail region. Primary excretory ducts extend anteriorly to region between pharynx and oral sucker, then turn backwards to ventral sucker region, where they divide into anteriorly and posteriorly running secondary ducts. The flame cell formula is $2[(5 + 5 + 5) + (4 + 4 + 4)] = 54$. Cercaria body dimensions are shown in Table. 1.

The body and tail of the cercaria are capable of considerable extensions and contractions. Swimming is slow and occurs by undulation and contraction of the body and tail. The cercaria hangs from the surface film of water by an air bubble enclosed in the ventral sucker. On the bottom of the container the cercaria moves by flexing and creeping with the help of the suckers. Occasionally, the cercaria attaches itself to the glass surface by its invaginated tail-tip. Within 24 h after emergence, the cercaria encysts on the wall of the container or on the snail. Metacercarial cyst is oval, smooth above, attached to substratum by flat irregular base showing prominent lateral and posterior indentations (Fig. 4), measuring 260 -310 (285) in length and 240 - 280 (255) in width.

Rediae (Fig. 5): The cercariae develop inside pale yellow cylindrical rediae with tapered extremities; terminal oral opening and ambulatory buds (procusculi or lappets) located near about hind fourth of body. Oral opening leads into pharynx. Intestine sac-like, confined approximately to anterior third of body and contains bright orange coloured granules. Various developmental stages of cercarial germs are present in the rediae. Number of mature cercariae in each redia is 4-14 (9). Cercariae escape from the redia through a birth pore situated in the anterior region. Body dimensions of the redia are shown in Table 2.

Table 1. Body dimensions of cercariae of *Cercaria kuwaitae* IV sp. n.

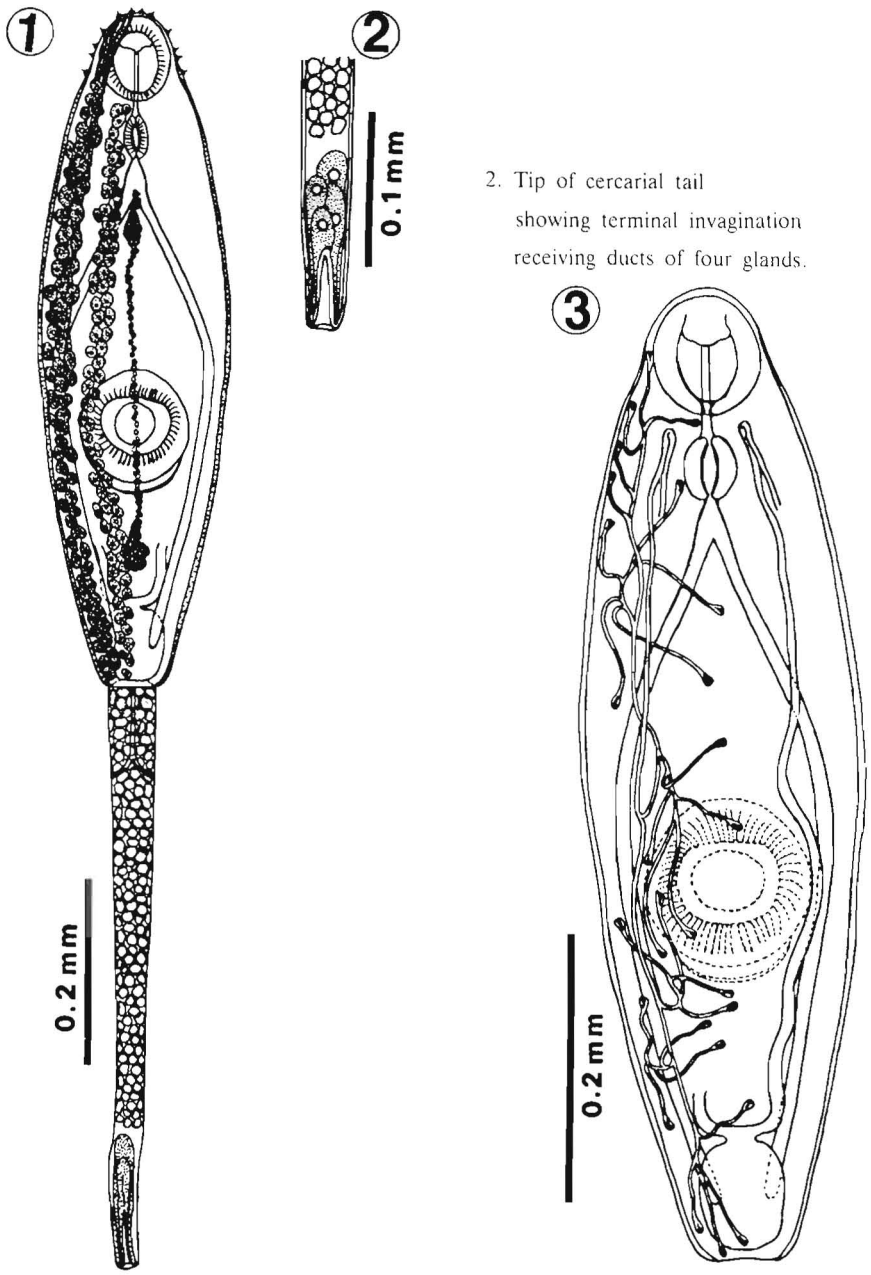
Body length	652.8-999.6 (789.5)
Body width	204.0-357.0 (272.3)
Distance of ventral Sucker from anterior end	260.0-440.0 (334.0)
Oral sucker	065.0-110.0 (087.5)
Pharynx length	035.0-057.5 (044.0)
Pharynx width	020.0-035.0 (026.3)
Ventral sucker	107.5-140.0 (125.8)
Oesophagus	025.0-047.5 (039.3)
Tail length	420.0-940.0 (709.0)
Tail width	052.5-112.5 (083.5)

Number examined = 10

Table 2. Body dimensions of the rediae within which the *Cercaria kuwaitae* IV sp. n. develops

Body length	1378.0-2132.0 (1604.2)
Body width	0338.0-0442.0 (0400.4)
Pharynx length	0045.0-0055.0 (0049.3)
Pharynx width	0030.0-0052.5 (0039.8)
Caeca length	0550.0-0800.0 (0708.0)

Number examined = 10

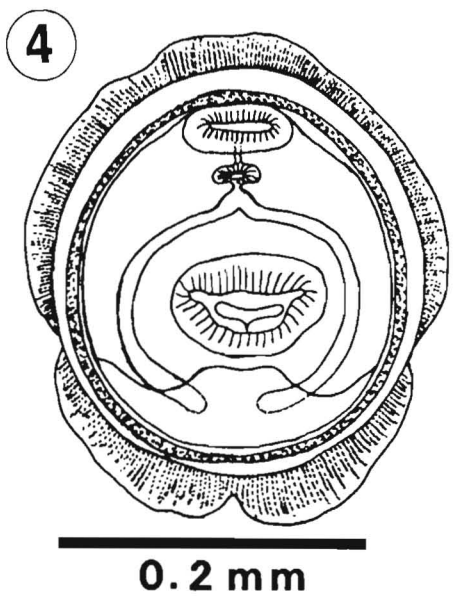


1. Entire ventral view of cercaria.

2. Tip of cercarial tail showing terminal invagination receiving ducts of four glands.

3. Cercarial excretory system.

Figs. 1-5 Drawings of *Cercaria Kuwaitae* IV sp. n.



4. Metacercarial cyst.

5. Mature daughter redia.

Surface topography

From SEM images, the body of the cercariae can be divided into two regions: the most anterior part, or the head, is rounded and clearly demarcated from the rest of the body (Fig. 6). The surface tegument of the head is spine-free, free of cystogenous material and displays concentrically arranged minute tubercles (Fig. 7), probably representing vestigial spines. Openings of cephalic glands on dorsal region of head (Fig. 7). The surface of the cercarial body, except the regions around the ventral sucker, is covered with a thick layer of cystogenous material deposited in cobblestone-like protuberances (Figs. 8,9). Two types of tegumentary papillae were identified: ciliated projecting ones from hemispherical bases (Figs. 7,10) and dome-shaped papillae (Figs. 7,9). The latter occur in rings around oral (Fig. 7) and ventral suckers (Fig. 9). Ciliated papillae on the body are arranged in vertical rows (Figs. 8,10), while on head being randomly distributed (Fig. 7). The tail is smooth, transversely annulated and ends with invaginated sucker-like structure (Figs. 6,11). Tegumentary papillae are scarce on tail. Metacercarial cyst convex with relatively smooth top and have a flattened base (Fig. 12).

SEM of rediae shows a cylindrical body with two posteriorly located spherical, ambulatory buds and an oral opening at the anterior tip (Figs. 13,14). The tegument is folded into transverse rings and covered with microvilli-like structures (Figs.14,15). The tegument around the oral opening is highly ridged, bears numerous ciliated papillae (Fig.15). Discoid processes are present at posterior tip of the body (Fig.16).

Type host: *Cerithidea cingulata* (Gmelin 1790)

Type locality: Shuwaikh, Kuwait Bay, Kuwait

Infection site: Hepatopancreas

Prevalence: 1.01% of 2358 snails

Specimens deposited: Helminth collection, Department of Zoology, University of Kuwait (Accession No. KUHC-C4).

Etymology: The specific name *kuwaitae* is after the name of the type locality: Kuwait Bay, Kuwait.

Discussion

Cercaria kuwaitae IV resembles in structure and behaviour, cercariae described by Cort (1915) and Sewell (1922) as megalurous type and later placed by Cable (1956) in the echinostomoid group. Echinostomoid cercariae are characterized by well-developed suckers, a glandular invagination at the caudal tip, and by their body being encased in a thick layer of cystogenous material. They are closely related to the cercariae of echinostomes except for the absence of a cephalic spined collar. Studies

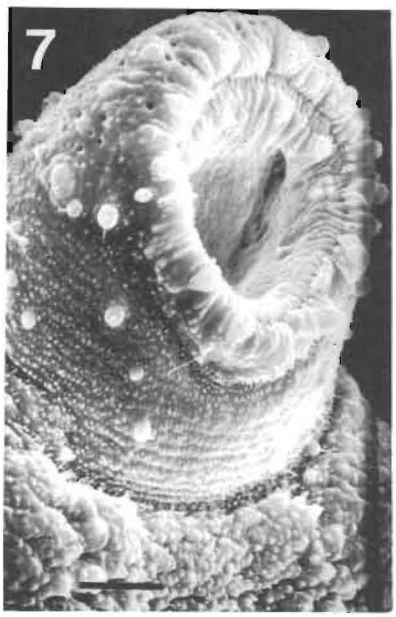
on the life history of echinostomoid cercariae have shown that they develop into philophthalmid adults of the genera *Cloacitrema, Parorchis* or *Philophthalmus* (Yamaguti 1975). *Cercaria kuwaitae* IV is distinguished from other philophthalmid cercariae (Sewell 1922, Cable 1956,Ching 1961, Holliman 1961, Howell and Bearup 1967, Velasquez 1969, Trimble and penner 1971, Fried and Grigo 1976, Mohandas 1979, Saxena 1984, Le Flore *et al.* 1985) by the large number of flame cells, but it closely resembles the cercariae of *Cloaciterma michiganensis* (Le Flore *et al.* 1985), of *C. narrabeenensis* (Howell and Bearup 1967) and of *C. philippinum* (Velasquez 1969), in the absence of tegumental spines, presence of a very short oesophagus and in the position of the genital primordium. The adult stage of the present species is unknown. However, it might be a parasite of birds that are abundant on the mudflat where the infected snails had been collected.

SEM micrographs of the cercariae complement the LM study by revealing small sized structures, particularly tegumental papillae. The dome-shaped and ciliated papillae found in the tegument of the cercariae are basically similar in structure and distribution to that of the majority of adults and larvae of digenetic trematodes (Smyth and Halton 1983). Furthermore, the distribution of the papillae on the body and around the suckers of the cercariae are similar to the cercarial chaetotaxy of *Philophthalmus* species determined by Albaret *et al.* (1988). The tegumental papillae are probably sensory organs that facilitate detection of a surface suitable for encystment. Based on LM studies, several authors (Goodchild 1943, Thomas 1958, Short and Cartrett 1973) have reported considerable variations in the distribution of papillae in the tegument of different types of cercariae and have assigned phylogenetic significance to such variations. The present study shows specific distribution of papillae on the suckers of *C.kuwaitae* IV. Moreover, SEM investigations have delineated the range of types of papillae. The distribution of the various types of papillae around suckers of philophthalmid cercariae may provide reliable diagnostic features, which may solve the taxonomic difficulties due to obstruction of the internal structures by the thick layer of cystogenous material. Microtopographical features, such as papillae, spines and tubercles, have also been considered in the taxonomy of some adult trematodes (Bakke and Lien 1978, Southgate *et al.* 1986).

The general fine structure of the tegument of the rediae of *C. kuwaitae* IV is similar to those reported for rediae of other digenetic trematodes (Rees 1971, 1980, Køie 1985, Fried and Awatramani 1992, Nollen 1992). Papillae and microvilli-like processes have been observed in the tegument of all rediae studied by SEM. The ciliated and dome-shaped papillae, being specially numerous in the anterior region of the rediae, have been associated with functions as chemo-or thigmoreceptors, presumably facilitating feeding and orientation during redial migration through snail tissues (Rees 1980). Furthermore microvilli are believed to increase the absorptive surface area (Hoskin 1975, Irwin *et al.* 1978). The discoid processes observed at the posterior tip of the rediae, appear to be unique in *C. kuwaitae* IV, and probably participate in locomotion.

6. Lateral view of cercaria showing rounded head, extended body and cylindrical tail. Bar = 100 μm.

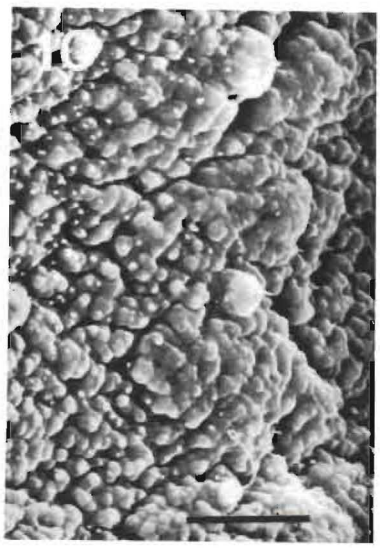
7. Ventrolateral aspect of cercarial head showing oral cavity and oral sucker surrounded by dome-shaped and ciliated papillae and cephalic gland pores on the dorsal rim. Note the tegument is encircled with fine tubercles. Bar = 10μm.



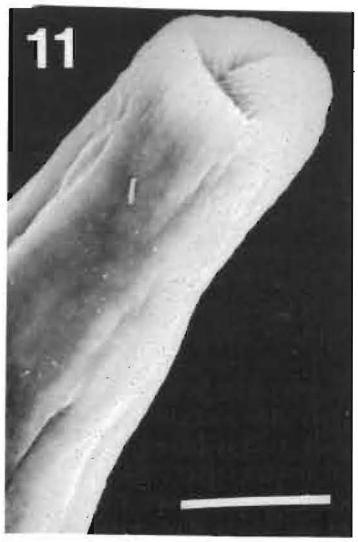
8. Ventral surface of cercarial body showing the area between oral and ventral suckers covered with cystogenous material, and distribution of papillae on the head and body. Bar = 10 μm.

Figs. 6-16 Scanning electron micrographs of *Cercaria kuwaitae* IV sp. n.

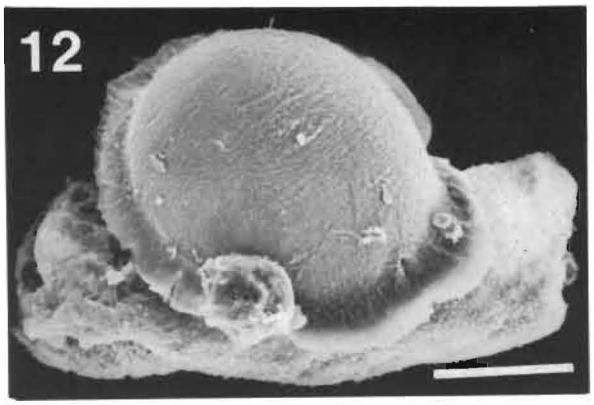
9. Midventral surface of cercarial body showing ventral sucker surrounded by dome-shaped papillae and thick layer of cystogenous material covering the tegument. Bar = 20 μm.



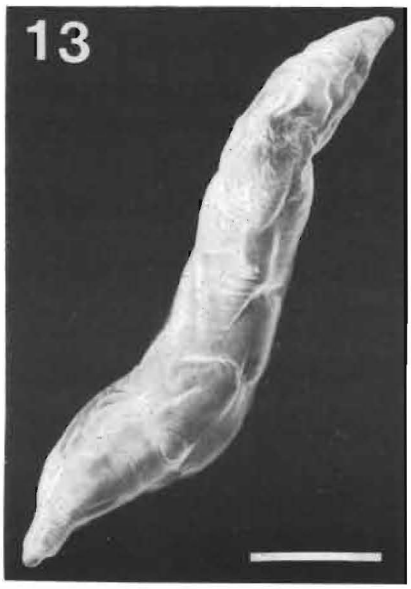
10. A high magnification of surface of cercarial body showing a row of ciliated papillae projecting from hemispherical bases among masses of cystogenous material. Bar = 10 μm.



11. Terminal region of cercarial tail showing sucker-like invagination at the distal end. Bar = 10 μm.



12. Metacercarial cyst with smooth round top and irregular base. Bar = 100 μm.



13. Daughter redia showing cylindrical body tapered at both ends and posteriorly located retracted ambulatory bud. Bar = 200 μm.

14. Anterior region of redia showing oral opening surrounded by papillae and ridges. Bar = 20 μm.

15. A high magnification of tegument of the anterior region of redia showing microvilli-like surface and ciliated papillae. Bar = 10 μm.

16. Posterior region of redia showing a retracted ambulatory bud and a discoid process at the tip. Bar = 40 μm.

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دراسات على مذنبات المثقوبات في جون الكويت
رابعاً: وصف وطوبغرافية *Cercaria kuwaitae* IV
نوع جديد، (ثنائية العائل: فيلوفثاليدي)

جاسم محمد عبدالسلام و بي. أس. سريلاثا

قسم علم الحيوان ـ جامعة الكويت ـ ص.ب: ٥٩٦٩ ـ الصفاة ١٣٠٦٠ ـ الكويت

تم وصف نوع جديد من المذنبات (cercariae) تنتمي إلى صنف المثقوبات (Trematoda) عائلة فيلوفثاليدي (Philophthalmidae) من القوقع *Cerithidea cingulata* (Gmelin 1790) من جون الكويت. إستخدم المجهر الضوئي والمجهر الألكتروني الماسح في دراسة الصفات التشريحية والطوبغرافية للمذنبة والريديا (redia) التي تنمو فيها. والدراسة هي جزء من سلسلة من الدراسات على مذنبات المثقوبات في جون الكويت، وتمثل أول سجل لمذنبة فيلوفثاليدية من قوقع في الخليج العربي.

تصاب القواقع من جنس *Cerithidea* في مناطق مختلفة من العالم، بأنواع مختلفة من يرقات المثقوبات. واتضح من الدراسة الحالية إصابة ١٪ من أصل ٢٣٥٨ قوقع من نوع *C. cingulata*، جمعت من المسطحات الطينية جنوب جون الكويت، بأطوار يرقية لمثقوبة فيلوفثاليدية تبين بعد دراستها إنها تمثل نوعاً جديداً أطلق عليها إسم *Cercaria kuwaitae* IV وتشارك هذه اليرقة أنواع الفيلوفثاليدي الأخرى في الصفات التركيبية والشكلية الأساسية لطور المذنبة المتمثلة بجسم بيضوي الشكل متصل بذنب طويل طرفه منغمد على شكل

ممص. ويتكون الفم من فتحة تقع في مقدمةالجسم محاطة بمحجم فمّي، ويلي الفم بلعوم عضلي يفتح إلى المريء الذي يتفرع إلى أعورين معويين، وثمة محجم بطني يتوسط الجسم بين فرعي الأمعاء. يتألف الجهاز الإبرازي من خلايا لهبية متصلة بأنيبيبات جامعة تؤدي إلى المثانة الموجودة في مؤخرةالجسم والتي تؤدي بدورها إلى فتحتين إبرازيتين عند بداية الذنب.

وهناك كذلك مجاميع من خلايا مولدة للأعضاء التناسلية تقع في وسط الجسم. وكما يحوي الجسم نوعين من الغدد الإفرازية؛ الغدد الرأسية في النصف الأمامي والغدد الكيسية المنتشرة تحت جدار الجسم. وتنمو هذه المذنبات في أكياس سبورية تعرف بالريديا تعيش بين أنسجة الغدة الكبدية ـ البنكرياسية للقوقع. وتتكون الريديا من جسم اسطواني الشكل مستدق الطرفين. تحمل الجهة الأمامية للريديا فتحة الولادة وفتحة فم تؤدي إلى بلعوم عضلي يفتح بدورة في امعاء قصيرة كيسية الشكل. وللريديا كذلك خلايا لهبية إبرازية وتجمعات من الخلايا الجرثومية التي تكون المذنبات. تخرج المذنبة من الريديا عن طريق فتحة الولادة إلى أنسجة القوقع ومن ثم إلى الماء من خلال جدار جسم القوقع حيث تسبح بمساعدة ذنبها، وفي غضون ٢٤ ساعة من وجودها في الماء تتكيس على سطح صلب بعد أن تفقد ذنبها لتتحول إلى طور مذنبة متكيسة (metacercaria). وتتميز المذنبة الجديدة بوجود عدد كبير من الخلايا اللهبية منتشرة على شكل ضفيرة نفريدية حسب المعادلة التالية:

٢ [(٥ +٥ +٥) + (٤ +٤ +٤)] = ٥٤. وبمقارنة المذنبة الجديدة بالمذنبات الفيلوفثاليدية المعروفة، تبين وجود تشابه كبير بينها وبين مذنبات تنتمي إلى جنس *Cloacitrema* من حيث خلو الجسم من الأشواك وقصر المريء وشكل تجمع الخلايا المولدة للأعضاء التناسلية.

وأظهرت نتائج فحص الأطوار اليرقية للنوع الجديد بواسطة المجهر الألكتروني الماسح تفاصيل طوبغرافية دقيقة أهمها وجود زوائد على شكل

حلمات وأهداب حول محاجم المذنبة وفتحة فم الريديا. ويحتمل أن تكون هـذه الزوائد مجسات تساعد اليرقات على تحسس محيطها داخل أنسجة القـوقع وأثنـاء السباحة قبل التكيس. هذا وقد تمت مناقشة إمكانية إستخدام هـذه الزوائـد في تصنيف اليرقات الفيلوفثاليدية.