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***Paragonimus gondwanensis* n. sp. (Digenea, Paragonimidae), parasite of mammals (humans and carnivores) in Cameroon**

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Abstract

Paragonimus gondwanensis n. sp. (hitherto undescribed) is reported from the lungs of naturally infected cat (*Felis catus* Linnaeus, 1758) and civet [*Civettictis civetta* (Schreber, 1776)] from Cameroon. Its description is based on the morphology of adult worms but also, on the dimension of fresh, living eggs released from naturally and experimentally infected cats and hospitalized patients (in the metratem of worms, emitted in the beginning of prepatent period and laid by living worm), length of prepatent period and duration of eggs incubation. The presence of this species in human patients is confirmed.

Key words: *Paragonimus gondwanensis* n. sp., Cameroon, paragonimiasis.

Introduction

The paragonimid species are known as an agent of paragonimiasis, the zoonotic parasite disease of humans and some mammals, reported from many subtropical and, especially, tropical countries. This disease is stated in 12 countries of Africa.

Paragonimiasis is a chronic condition, persisting sometimes for many years. Pathological effects are due to the presence of adult worms and their eggs in the lungs and other tissues of hosts, to the movement of parasites through tissues, and to their toxic and allergenic metabolites. The symptoms of paragonimiasis, such as bronchial rales and haemoptysis associated with paroxysms of coughing, are sometimes similar to those of tuberculosis, leading to misdiagnosis.

All paragonimid species have the complex life cycle, including two intermediate hosts (first: snail, second: crustacean) and several definitive hosts (mammals: humans, carnivores, rodents etc.). Adult worms lay eggs which are released outside in sputum or stools. In water, eggs develop in swimming miracidia which can penetrate molluscs, multiply and evolve until cercarial stages, which can infect Crustaceans. Humans and other mammals become infected when they eat raw or undercooked crustaceans harbouring metacercariae, or ingest uncooked meat of paratenic hosts such as pigs (Blair *et al.* 1999a).

The first report on paragonimiasis in Africa was published by Onorato (1920). All other reports since then concerned the intertropical countries of West Africa, around the Gulf of Guinea, with exception of one case of South Africa (McCallum 1975). Those countries are: Cameroon, Nigeria, Congo, Democratic Republic of Congo, Gambia, Liberia, Ivory Coast, Gabon, Equatorial Guinea and Benin (Aka *et al.* 2008). However, two of those countries were mainly affected by this disease: Nigeria (1778 cases studied) and Cameroon (454 cases). In other mentioned countries a total of 63 infected patients were examined and reported by Van Hoof (1933) – Congo; Bowesman (1960) – Gambia; Darras *et al.* (1960) – Democratic Republic of Congo; Coulibaly *et al.* (1975) – Ivory Coast; Petavy *et al.* (1981) – Gabon; Sachs & Voelker (1982) – Liberia and Equatorial Guinea; Sachs *et al.* (1983) – Gabon; Bossé (1984) – Ivory Coast; Sachs *et al.* (1986) – Liberia; Sachs (1987) – Liberia; Simarro *et al.* (1991) – Equatorial Guinea; Vuong *et al.* (1996) – Gabon; Aka *et al.* (1999) – Benin.

Some authors did not identify the species of causative agent, but mentioned “*Paragonimus* sp.” or “paragonimiasis” only (Nwokolo 1964; 1972; Guemgne Simo 1994, etc.). The authors who carried out the studies of this disease in Nigeria and Cameroon identified two or even three species.

The most frequent species found in Nigeria was *Paragonimus uterobilateralis* Voelker & Vogel, 1965: Nwokolo (1974), Oelerich & Nwokolo (1974), Onuigbo & Nwako (1974), Udonsi (1989), Arene (1998), Ibanga & Eyo (2001). The species *Paragonimus africanus* Voelker & Vogel, 1965 was mentioned just once: Voelker & Nwokolo (1973).

When Cameroon is concerned, two species were described by Voelker & Vogel (1965) from the material studied in that country: *P. africanus* and *P. uterobilateralis*. The authors report *P. africanus* as the most frequent species (Timsit 1978; Ripert *et al.* 1981; Kum & Nchinda 1982; Sam-Abbenyi 1985; Moyou-Somo & Tagni-Zukam 2003; Moyou-Somo *et al.* 2003; Voelker & Sachs 1977).

Some authors (Zahra 1952; Vogel & Crewe 1965) reported the findings of *Paragonimus westermani* Kerbert, 1878; these findings were based on the identification only by measuring formalin-fixed eggs and have never been confirmed later. The presence of *P. uterobilateralis* was only suspected in Cameroon (Sam-Abbenyi 1985). Bayssade-Dufour *et al.* (1994) suggested, after specific identification by multivariate analysis of paragonimid eggs, the presence of four species of *Paragonimus* in Cameroon: two already known (*P. africanus*, *P. uterobilateralis*) and two undescribed ones. The later study of paragonimid eggs from 22 West-African strains, conducted by Cabaret *et al.* (1999), confirmed the previous findings.

Our investigations aimed to describe these two new species of Paragonimidae from Cameroon and to study their complete life cycle. In this paper, we will describe new species of *Paragonimus*; in the next successive publications, another new species and the life cycle of both of them will be presented.

Material and Methods

In order to describe a new species we used our original material and unpublished data and the material deposited by Cabaret *et al.* (1999) and by Ripert *et al.* (1981) in the Collection of MNHN Paris.

Research on the intermediate hosts

Collection of snails (first intermediate host) and crabs from the family Potamonautidae, such as *Sudanonautes africanus*, *S. granulatus* and *S. pelli* (second intermediate hosts) and their dissection, in order to find paragonimid metacercariae cysts, were realized in the creeks of South Province (S) and South West Province (SW) of Cameroon. In South Province, the collection of crabs took place at Bikipwae, near Ebolowa (Minsolo Creek) at 2° 59' N and 11° 17' E; altitude 636 m.a.s.l. In South West Province, at Bulutu, near Kumba (Mejeck Creek) the search is realized at 4° 38' 25'' N and 9° 26' 21'' E; altitude 2457 m.a.s.l. (Fig. 1)

Registration numbers for final hosts

Humans:

1. 158 TM-1017 (patient from South West Province)
2. 143 TM (patient from South West Province)

Cats:

1. 186 TM (from South Province)
2. 187 TM (from South Province)

3. 101 TM (from South West Province)
4. 102 TM (from South West Province)
5. 155 TM (from South West Province)
6. 157 TM (from South West Province)
7. 160 TM (from South West Province)

Civet:

1. 894 TA (from South Province)



Figure 1. Studied area in Cameroon: ★ – sampling sites.

Registration numbers for adult parasites

1. 894 TA₁ (from South Province)
2. 187 TM₃ (from South Province)
3. 187 TM₅ (from South Province)
4. 157 TM₄ (from South West Province)

Studied eggs are marked as:

158 TM-1017_n, 143 TM_n, 187 TM_n (where n=batches of eggs from different hosts)

Experimental infection of laboratory animals (cats and rodents)

The metacercariae, extracted from naturally infected crab *Sudanonautes africanus* (Milne Edwards, 1869), were used for experimental infection of cat (*Felis catus* Linnaeus, 1758), *Rattus rattus* (Linnaeus, 1758), *Mus musculus* Linnaeus, 1758 and *Acomys* sp.

South Province: the first cat, registered as 186 TM, was infected with 19 metacercariae from Bikpwa strain (14 with 2 cyst walls, 2 with 1 wall and 3 without wall); the second cat, 187 TM, with 34 metacercariae, from the same strain (28 with 2 cyst walls, 6 with 1 wall). The cats 186 TM and 187 TM were sacrificed 334 and 85 days after metacercarial meal, respectively. Both experiments were realized in Veterinary School of Maisons-Alfort in Paris.

South West Province: Two other cats, 101 TM and 102 TM, were infected in Faculty of Medicine Paris V with Bulutu strain metacercariae. The cat, registered as 101 TM, was infected with 15 metacercariae with 1 cyst wall; the cat 102 TM, with 26 metacercariae with 2 cyst walls. They have been sacrificed 136 and 362 days after metacercarial meal, respectively.

Other infections, with Bulutu strain metacercariae, were realized in Center of Tropical Medicine of Kumba, Cameroon, on 3 cats: 155 TM, 157 TM and 160 TM. The cats received unprecised number of metacercariae, without data on number of walls. These cats were sacrificed after confirmed emissions of paragonimid eggs in stools.

The cats were anesthetized with Imalgen or Penthotal, and then dissected by routine procedure. The adult parasites and eggs were isolated from the lungs.

Three individuals of *Mus musculus* were infected with 8, 16 and 10 metacercariae, respectively. Seven individuals of *Rattus rattus* were infected with 4, 7, 2, 4, 7, 20 and 8 metacercariae and five individuals of *Acomys* sp. with 14 metacercariae (2 with a cyst and 12 excysted). The individuals of *M. musculus* were sacrificed between 112 and 136 days after the metacercarial meal. Three individuals of *R. rattus* were sacrificed after 30 days, two individuals after 156 days and remaining two after 83 or 115 days. The five individuals of *Acomys* were sacrificed after 172 days.

The rodents were anesthetized with CO₂ inhalation, and then dissected by routine procedure.

Naturally infected hosts

South West Province: one cat, registered as 156 TM, native of Kumba, naturally infected, releasing Paragonimidae eggs in stools, was examined, too. Only one adult worm remained for identification at the Faculty of Medicine Paris V.

The sputum of several hospitalized children, suffering from paragonimiasis, was studied and the paragonimid eggs were stated. The 35 eggs from the sputum of two boys from Béchaty (registered as 143 TM and 158 TM-1017), where measured (143 TM_n and 158 TM-1017_n).

South Province: the specimen of one adult paragonimid species from *Civettictis civetta*, deposited by Ripert *et al.* (1981) in the collection of MNHN Paris (registered as 894 TA₁), was also used in description of new species.

Study methods for eggs

Five parameters were selected:

1. length of prepatent period: the cats 186 TM and 187 TM were monitored,
2. size of living eggs emitted at the beginning of the prepatent period (from 187 TM₃, and 187 TM₅),
3. size of eggs laid by a living worms from cat (187 TM_n) and humans (143 TM_n and 158 TM-1017_n),
4. size of eggs in the metraterm (terminal portion of the uterus) of worms (187 TM₃ and 187 TM₅),
5. duration of incubation, until hatching of eggs (from feces: 101 TM and 102 TM; from tracheal mucus: 186 TM and 187 TM_n).

South West Province. One parameter was considered: the size of eggs in the metraterm of worms (187 TM_{3,5} and 143 TM₁).

Study methods for adult worms

Three studied worms from South Province (894 TA₁, 187 TM₃, 187 TM₅) and one from South West Province (157 TM₄), were flattened between slide and coverslip and fixed in formaldehyde. Then, they were stained in toto by Chlorhydric Carmin, mounted in Canada balsam and drawn with camera lucida.

Results

Family Paragonimidae Dollfus, 1939
[Syn. Euparagoniminae Chen, 1963]

Genus *Paragonimus* Braun, 1899
[Syn. *Polysarcus* Looss, 1899
Euparagonimus Chen, 1962
Pagumogonimus Chen, 1963
Rodentigonimus Chen, 1963
Megagonimus Chen, 1963]

***Paragonimus gondwanensis* n. sp.**

Material studied: Four adult worms from naturally or experimentally infected hosts. The type specimens were deposited in National History Museum of Paris, France.

Registration number: holotype 187 TM₃, 187 TM₅; paratype 894 TA₁, 157 TM₄.

Etymology: The specific name *P. gondwanensis* come from Gondwana, the name of ancient continent uniting Africa and South America.

First intermediate host (snail): unknown.

Second intermediate host (crustacean): *Sudanonautes africanus* (A. Milne-Edwards, 1869).

Definitive hosts: *Civettictis civetta* (natural); *Homo sapiens* (probably natural); *Felis catus* (natural and experimental) and *Canis familiaris* Linnaeus, 1758 (experimentally infected in Ivory Coast: Bossé 1984; Bayssade-Dufour *et al.* 1994; Cabaret *et al.* 1999). Experimental infection of rodents was unsuccessful.

Site: lungs.

Type locality: Bikpwae, in South Province of Cameroon: 2°59' N, 11°17' E, altitude: 636 m.a.s.l.

Other localities: Bulutu, Béchati, in South-West province of Cameroon. Bulutu is settled at 4°38'25'' N, 9°26'21'' E; altitude 2457 m.a.s.l; Ouagalilié, Lakota, Ivory Coast settled at 6°2'0'' N, 5°47'0'' W.

Description of the adult worms

They came from the lungs of final hosts, naturally or experimentally infected. New species is described after 4 adult ovigerous worms: 894 TA₁, 187 TM₃, 187 TM₅ and 157 TM₄.

The bodies of these worms (from fixed, stained and mounted specimens, more or less flattened), measure 7 x 4,5 mm for 894 TA₁, 7,5 x 3,7 mm for 187 TM_{3,5}, 7 x 3 mm for 157 TM₄. All present a plump elongate body, with spined tegument. Oral sucker ventro-terminal, ventral sucker in the 1/3 of the body. Oral sucker diameter/ventral sucker diameter ratio (OS/VS): OS/VS>1 in adults; OS/VS=1 in young specimen; OS/VS<1 in metacercaria. Digestive apparatus with a mouth in oral sucker, pharynx well developed, oesophagus short, undulating caeca large, reaching the hindbody. The genital apparatus with a lobed ovary

opposite to uterus; uterus convoluted, ovary and uterus pretesticular, seminal receptacle small, profuse dendritic vitellaria, extending dorso-laterally, from the forebody to the hindbody, two transverse vitelloducts ending in a median rounded bag, metraterm opening in hermaphrodite pore, immediately posterior to the acetabulum, lobed testes in hindbody, characteristics of the Paragonimidae.

All four worms had a short excretory bladder, reaching the lower part of acetabulum. Ovary with five deep lobes. Mehlis gland small. Seminal receptacle small. Two large testes star-shaped with 4-6 deep lobes with basis in form of “corolla”. Thin spermiducts, reaching a crook curved seminal vesicle, devoid of cirrus sac, near a short pars prostatica, lying on the left side, between the uterus and the acetabulum. Uterus overlapping, on the left side, the anterior part of testis and transversal part of vitelloducts. The Laurer’s canal was not conspicuous.

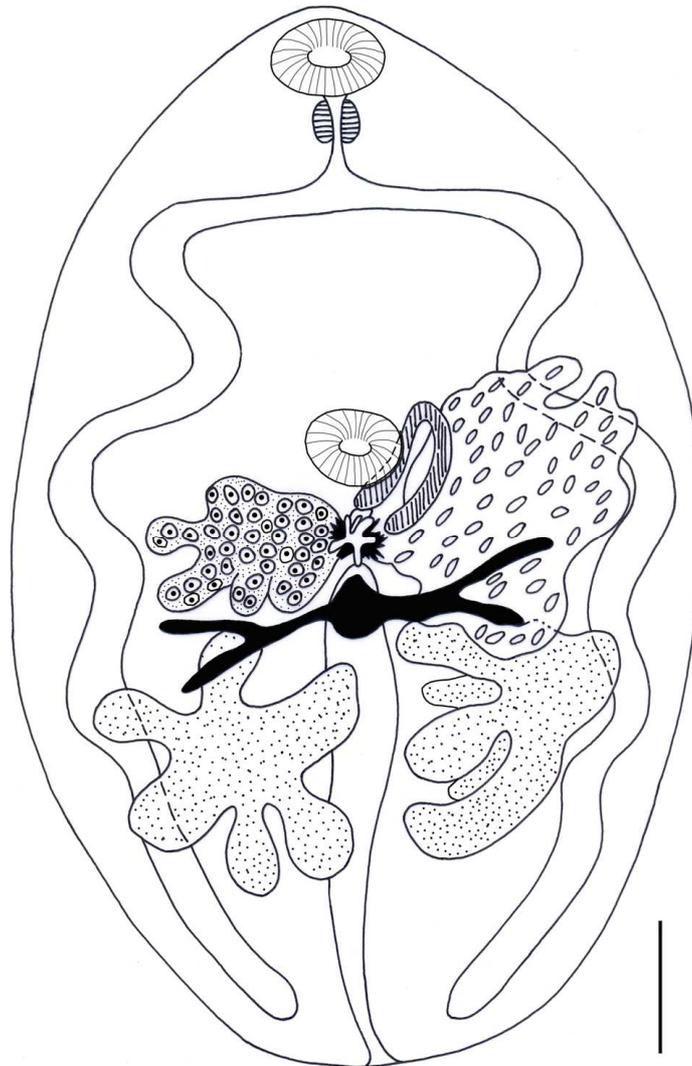


Figure 2. *Paragonimus gondwanensis* n. sp., scale bar 1mm (vitellaria not presented).

Measurement of eggs

The metraterm eggs measure in length 78 (71-82) and in width 43 (35-51) μm , based on 10 specimens.

First living laid eggs measure 83.67 (± 2.59) x 47.89 (± 1.25) μm (30 specimens), 8-10% larger and wider than corresponding metraterm eggs.

Eggs from paragonimian children. 35 eggs from the sputum of hospitalized boy from Bechati measured 85.2 (± 2.08) in length and 48.6 (± 1.07) μm in width.

The duration of incubation (35 eggs monitored), at 22 °C, was 55-56 days, with a very long period of *morula* stage.

Prepatent period (period between infection with a parasite and appearance of eggs in feces) was 66 days.

Discussion

The paragonimid species with short excretory bladder seem to be very rare. Chen (1962) described, in China, the new species with that type of bladder (*Euparagonimus cenocopiosus*) and created new genus *Euparagonimus*; one year later (1963), Chen proposed new subfamily Euparagoniminae. Chen & Ho (1965) gave more details on adults, metacercaria and egg size of above mentioned species. The second species of the genus, *E. hongzesiensis*, was described by Hu *et al.* (1990) in China. Unfortunately, the authors did not give enough details or drawings. Miyazaki & Hendricks (1975) reported the finding, in Panama, of a very rare metacercaria with short bladder belonging, probably, to the genus *Euparagonimus* (the species was never described).

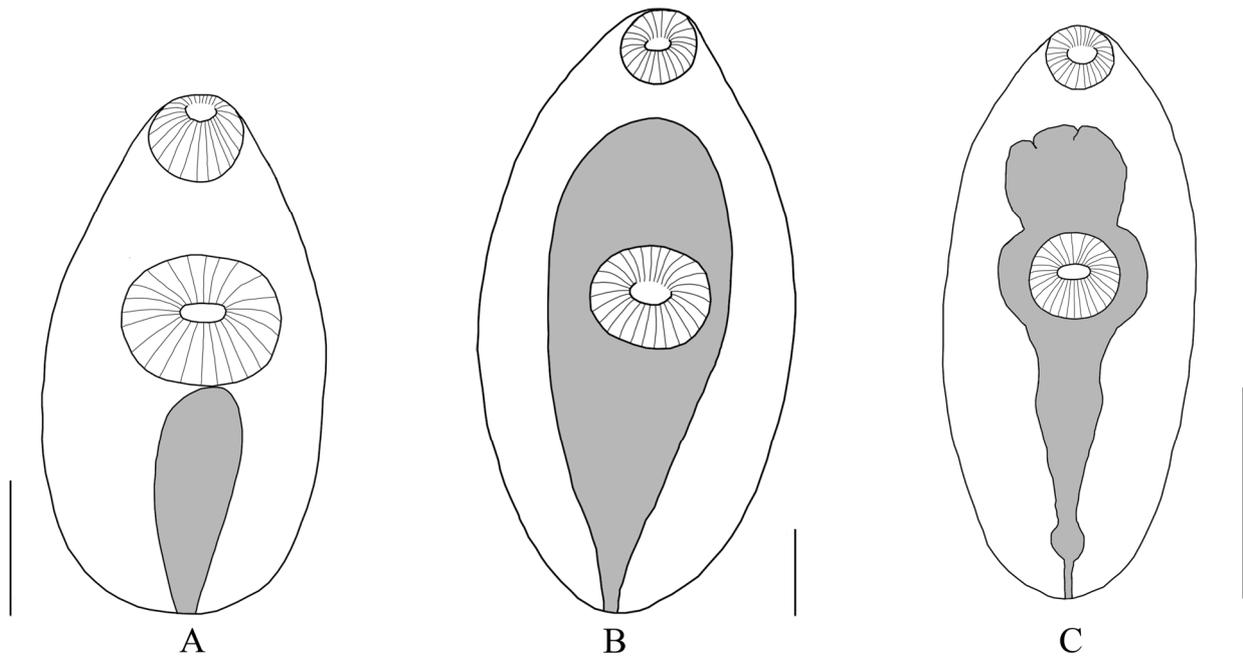


Figure 3. Metacercariae of different paragonimid species: A - *P. gondwanensis* n. sp. (scale bar 100 μ m), after Cabaret *et al.* (1999), modified; B - *P. africanus* (scale bar 100 μ m), after Cabaret *et al.* (1999), modified; and C - *P. uterobilateralis* (scale bar 300 μ m), after Voelker & Sachs (1985), modified.

Kurochkin (1987) recognized *E. cenocopiosus* as a valid species, and Blair *et al.* (1999a) recognized both of described species (*E. cenocopiosus* and *E. hongzesiensis*). Later the same year, on the basis of DNA sequence data obtained by his group of authors and morphometric analysis of 16 paragonimid species (Zhan *et al.* 1997), Blair *et al.* (1999b) placed *E. cenocopiosus* within the genus *Paragonimus* and concluded “it seems that this species should not be placed in a distinct genus”. Their conclusion was confirmed by Blair (2008): “the former genus (*Euparagonimus*) must fall as a synonym and the subfamily Euparagoniminae Chen, 1963 is not recognized”.

So, the present new species, *Paragonimus gondwanensis* n. sp., differs from all other *Paragonimus* species (with exception of *P. cenocopiosus*) in the length of excretory bladder (Fig. 3). This species is the third paragonimid species described from Africa.

This species can be distinguished from *Paragonimus cenocopiosus* Chen, 1962 as follows:

- by form and number of testicular lobes: 4–6 in new species, and 5–6 main lobes + 0–3 lobules = 5–9 lobes or lobules in *P. cenocopiosus* (Figs. 4A, 4D),
- by form and number of ovary lobes: 5–6 in new species, and 10 (5x2) lobes in *P. cenocopiosus* (Figs. 5A, 5D),
- oral sucker diameter/ventral sucker diameter ratio (OS/VS): 0.5 in metacercaria and 1.18–1.52 in adults of *P. gondwanensis*; in metacercaria and adults of *P. cenocopiosus* this ratio is ~1 (Table 1),
- by size of eggs (Table 1).

Table 1. Comparative measurements of adult African paragonimids and their eggs (OS—oral sucker; VS—ventral sucker; mean values in brackets).

Parameters	<i>P. gondwanensis</i> n.sp.	<i>P. africanus</i>	<i>P. uterobilateralis</i>	<i>P. cenocopiosus</i>
Length (mm)	7–7.5	16.7	5.2–7.0	5.78
Width (mm)	3–4.5	10.0	2.8–3.8	2.65
OS (mm)	0.82–1.08	1.39	0.63–0.68	0.62
VS (mm)	0.69–0.71	0.88	0.56–0.73	0.59
Eggs (length) μm	83.67–85.2 (84.4)	65.1–113.2 (92.8)	62.3–73.6 (68.5)	68–95 (81.5)
Eggs (width) μm	47.89–48.6 (48.2)	36.8–52.3 (49.7)	34.0–50.9 (42.7)	39–55 (47.0)

P. gondwanensis n. sp. differs from previously described *P. africanus* by:

- the body size (Table 1),
- the shape and length of the excretory bladder (Figs. 3A, 3B),
- the size of suckers and OS/VS ratio (Table 1),
- the size and shape of testes and ovaries (Figs. 4A, 4B, 5A, 5B),

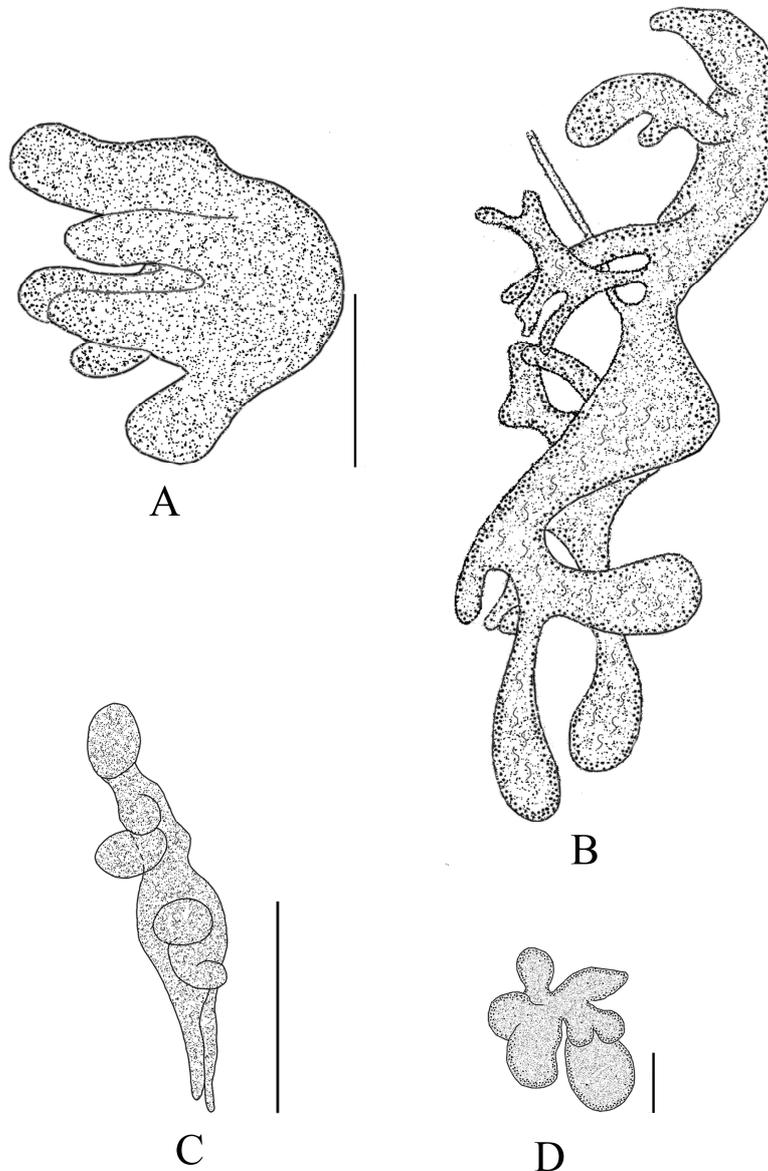


Figure 4. Testes of different paragonimid species: A - *P. gondwanensis* n. sp. (scale bar 1 mm), B - *P. africanus* (scale bar 2.5 mm), after Voelker & Vogel (1965), modified; C - *P. uterobilateralis* (scale bar 1 mm), after Voelker & Vogel (1965), modified; D - *P. cenocopiosus* (scale bar 0.5 mm), after Chen (1965), modified.

- the uterus overlapping the left testis (Fig. 2)
- the size of eggs (Table 1).

New species differs from *P. uterobilateralis* by:

- the body size (Table 1)
- the size of oral sucker and OS/VS ratio (Table 1)
- the shape and size of excretory bladder (Figs. 3A, 3C)
- the shape and size of testes and ovaries (Figs. 4A, 4C, 5A, 5C)
- the uterus overlapping the left testis (Fig. 2)
- the size of eggs (Table 1).

P. uterobilateralis differs from all African paragonimids by an uterus extending into both halves of the body.

The authors believe that the question of genus *Europaragonimus* is still open and the merit further investigation and eventual recognition.

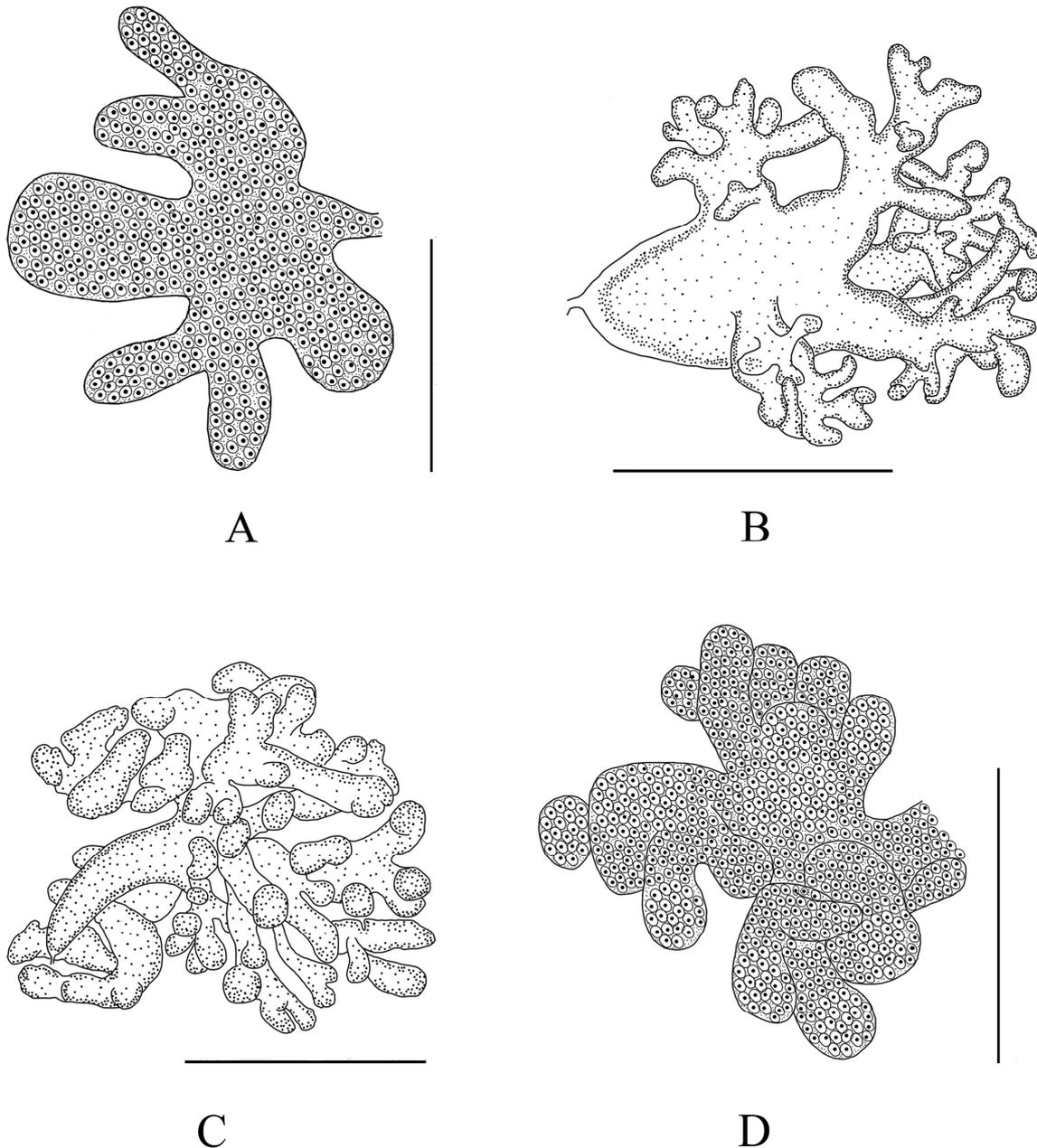


Figure 5. Ovaries of different paragonimid species: A - *P. gondwanensis* n. sp. (scale bar 0.5 mm), B - *P. africanus* (scale bar 1 mm), after Voelker & Vogel (1965), modified; C - *P. uterobilateralis* (scale bar 0.5 mm), after Voelker & Vogel (1965), modified; D - *P. cenocopiosus* (scale bar 0.5 mm) after Chen (1965), modified.

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