

Pygidiopsis ardeae n. sp. (Digenea: Heterophyidae: Pygidiopsinae) in the grey heron *Ardea cinerea* L. from Denmark

Marianne Køie

Marine Biological Laboratory, University of Copenhagen, DK-3000 Helsingør, Denmark

Accepted for publication 6th June 1989

290

Abstract

A new species, *Pygidiopsis ardeae* (Heterophyidae, Pygidiopsinae), is described, using both light and stereoscan electron microscopy, from the grey heron *Ardea cinerea* L. (Ardeidae) from Denmark. The species has previously (as *P. genata*) been recorded in dogs fed on plaice and flounders from Danish waters. It developed experimentally in domestic chickens and pigeons. No species of *Pygidiopsis* Looss, 1907 has previously been described from birds in northern Europe. *P. ardeae* is very similar to the type species, *P. genata* Looss, 1907, from Egypt, but the two species have different life-cycles. *P. ardeae* differs from *P. piclaumoreli* Dollfus & Capron, 1958 from Senegal in its size and the disposition of vitelline follicles. *P. plana* (Linton, 1928) Price, 1933, from a green heron from the eastern USA, is larger than *P. ardeae* and has a wide, sac-like prepharynx.

Introduction

During experimental life-cycle studies of fish digeneans, metacercariae taken from brackish-water fish developed into *Pygidiopsis* sp. in experimentally infected domestic chickens and pigeons. The specimens obtained from the experimental infections differed from most previously described species of *Pygidiopsis* Looss, 1907, and, in cases in which the adults were similar, their larval developmental stages were different. Specimens identical to those obtained in the experimentally infected hosts were found naturally in the grey heron, *Ardea cinerea* L.

The life-cycle of the present species has been worked out experimentally (Køie, 1990).

Materials and methods

The grey herons, *Ardea cinerea* L., were shot at Eskildsø in the Roskilde Fjord (the Isefjord complex), Zealand, Denmark, in August and September

1988. The live digeneans removed from the intestine were fixed unflattened in FAA, Bouin's fluid or Berland's fluid and stored in 70% ethanol. Specimens were stained in carmalum, with or without counterstaining in eosin, and mounted in DPX or cleared unstained in lactophenol and mounted in glycerine jelly. Worms fixed in Bouin's fluid were embedded in Epon and serially sectioned (3 µm) (transversely, sagittally and horizontally) and stained in toluidine blue. Measurements (in micrometres) are of specimens fixed unflattened in FAA and mounted in DPX. Other specimens were fixed in glutaraldehyde for scanning electron microscope studies (see Køie, 1987).

The present specimens were compared with the following material:

(1) *Pygidiopsis genata* Looss, 1907 from experimentally infected hamsters from Egypt. Loan from Dr L.M. Boulos, Egypt. *P. genata* from *Milvus migrans* from Cairo and experimentally reared specimens in ducklings from Egypt. Loan from British Museum (Natural History).

(2) *Pygidiopsis plana* (Linton, 1928) Price, 1933. Holotype (USNM Helm. Coll. No. 7940) from *Butorides virescens* from Woods Hole, Mass., USA.

(3) *Pygidiopsis piclaumoreli* Dollfus & Capron, 1958 from *Sterna hirundo* L. from Rufisque, Senegal. Loan from Muséum National d'Histoire Naturelle, Paris.

(4) Various specimens of *Pygidiopsis* sp. from piscivorous birds from Europe. Loan from British Museum (Natural History).

Family Heterophyidae (Leiper, 1909) Odhner, 1914
Subfamily Pygidiopsinae Yamaguti, 1958
Genus *Pygidiopsis* Looss, 1907

***Pygidiopsis ardeae* n. sp. (Figs 1-3)**

Host: Ardea cinerea L. (Ardeidae).

Locality: Roskilde Fjord, Zealand, Denmark.

Site in host: Intestine.

Specimens deposited: Zoological Museum, Copenhagen, holotype and paratypes. Paratypes BM(NH) Reg. No. 1989.3.17.1-2 and USNM Helm. Coll. No. 80756.

Description

Measurements of *P. ardeae* and related species are included in Table I. Description based upon 9 gravid, stained specimens (holotype and 8 paratypes), 6 serially sectioned specimens and SEM studies of 20 gravid specimens.

Body pyriform, tapering towards anterior end, bluntly rounded posteriorly, broadest at about posterior third with conspicuous ventral cavity. Forebody dorso-ventrally flattened; hindbody less so. Body with regularly arranged scale-like spines decreasing in size posteriorly, except for small pre-oral unspined area and 2 elongate, unspined areas ventro-laterally in hindbody. Oral spines deeply embedded in oral sucker in 2 rows; anterior row with 16 approximately 4 μm long scale-like circumoral spines; posterior row restricted to 4 slightly smaller dorsal spines.

Pigment granules scattered between oral sucker

and level just posterior to intestinal bifurcation. Numerous unicellular glands open onto body-surface throughout but especially antero-ventrally.

Oral sucker ventrally subterminal or almost terminal; oval or globular. Length of prepharynx and oesophagus depending on position of pharynx, which varies between lumen of oral sucker and just anterior to intestinal bifurcation. In resting position prepharynx and oesophagus approximately equal in length. Wall of prepharynx provided with an arched fold (with a narrow split ventrally) at junction with pharynx. Arched fold usually anteriorly orientated. Pharynx oval to globular. Wall of oesophagus with longitudinal thickenings. Intestinal bifurcation about two-thirds of distance from oral sucker to ventral sucker. Caeca terminate blindly close to anterior margin of ovary; extremities tend to curve medially and dorsally. Ventral sucker globular to transversely oval.

Excretory vesicle Y- to T-shaped; reaches anteriorly to mid-ovarian level; stem funnel-shaped, with thickenings close to terminal pore; arms broad, dorsal and ventral to testes.

Numerous gland-cells ventro-laterally in hindbody, beneath unspined area of body and anterior to vitellarium.

Testes symmetrical, unlobed, transversely oval, near posterior end of body, separate or contiguous. Seminal vesicle immediately post-acetabular, sinistro-dorsal, bipartite, voluminous. Ejaculatory duct runs anteriorly sinistral to ventral sucker; opening into genital sac between gonotyl and ventral sucker.

Ovary entire, spherical to subspherical, anterior to or antero-median to right testis. Seminal receptacle globular or transversely oval; often voluminous; median; dorsal to and overlapping anterior parts of testes and posterior part of ovary. Laurer's canal present. Vitellarium composed of 5-10 transversely oval follicles, contiguous or overlapping, in 2 lateral fields which extend from close to posterior extremity to about mid-ovarian level. Common vitelline ducts unite immediately posterior to ovary near mid-line to form small vitelline reservoir. Uterine coils in mature specimens occupy space between testes and ventral sucker,

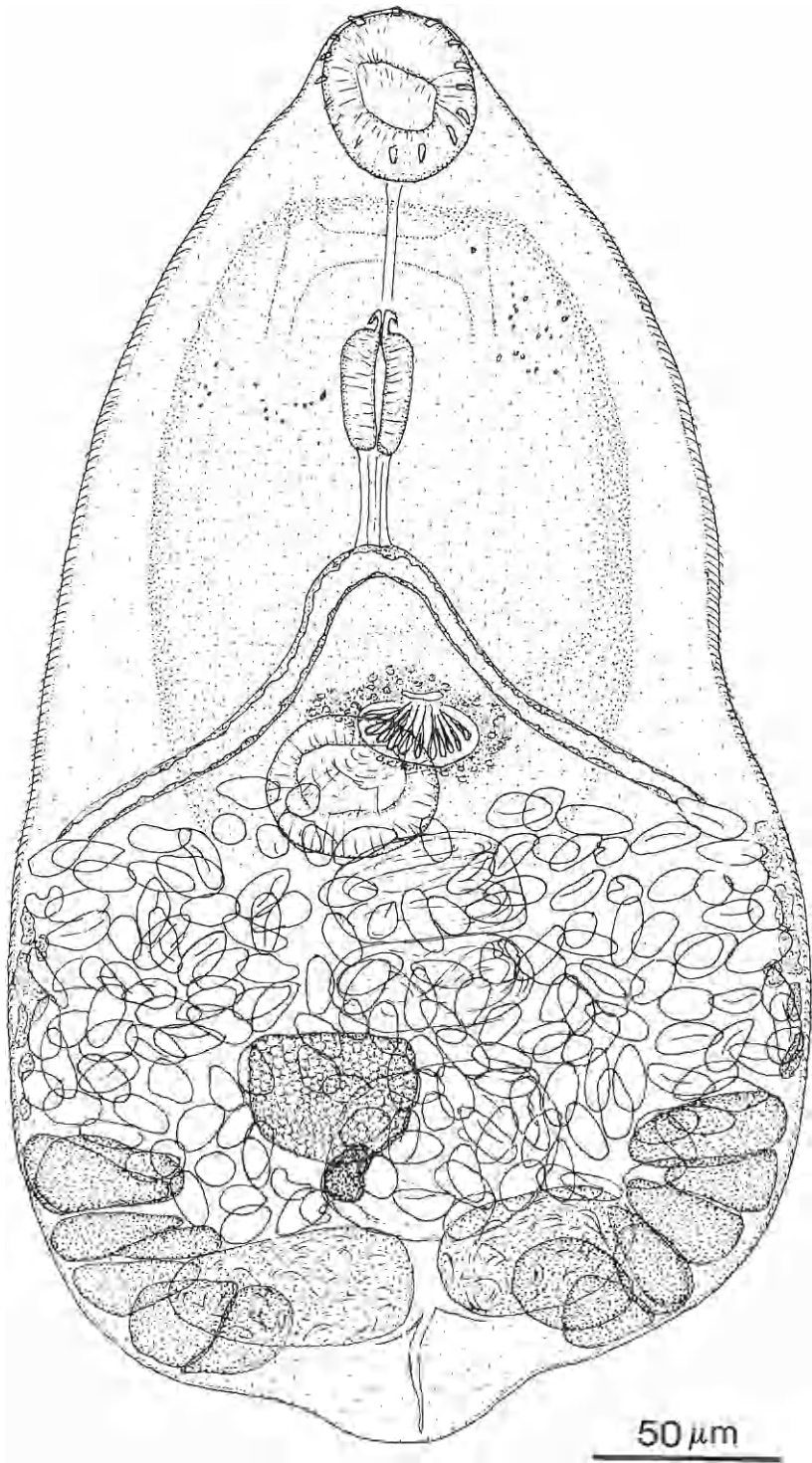


Fig. 1. *Pygidiopsis ardeae* n. sp., ventral view.

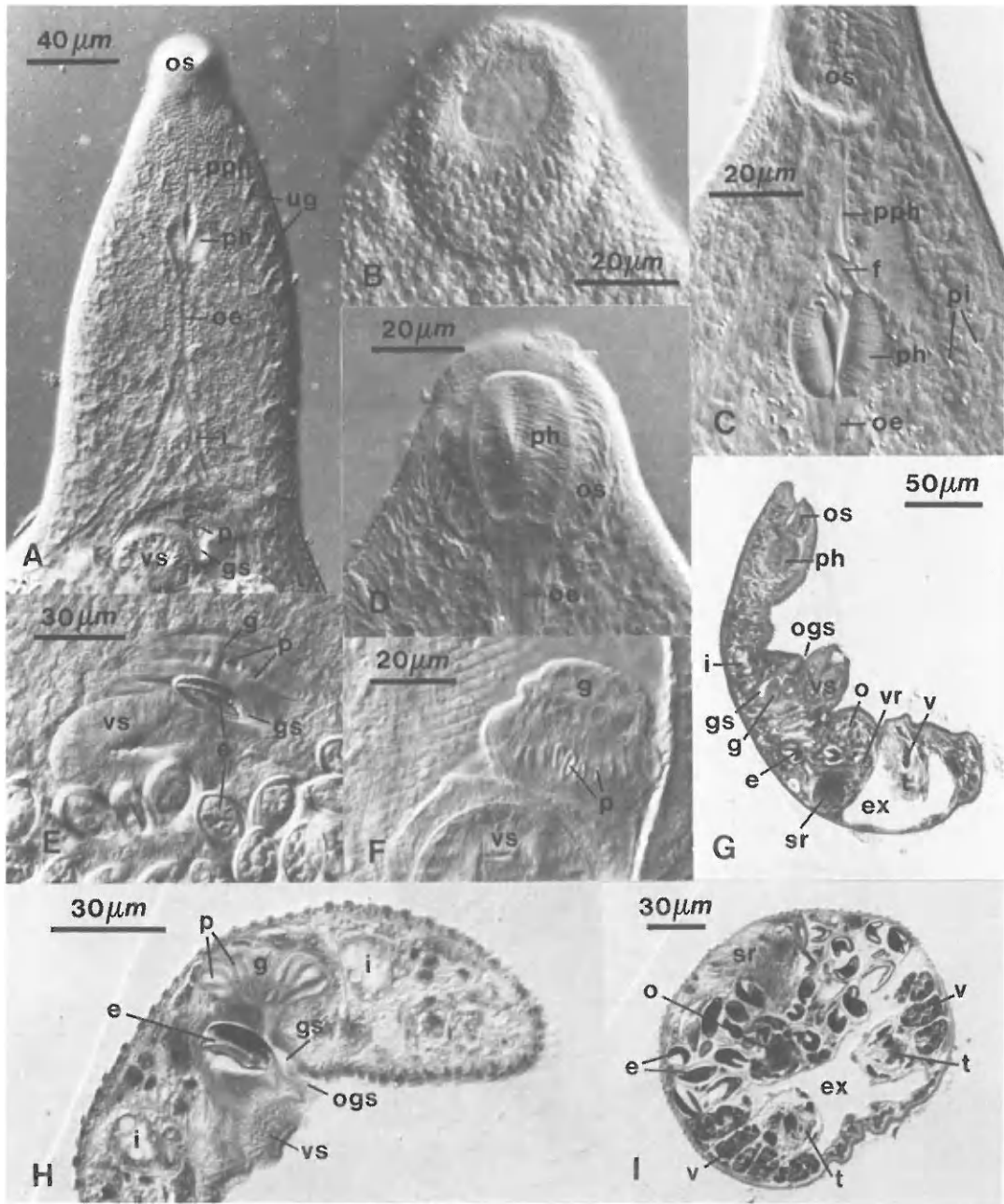


Fig. 2. *Pygidiopsis ardeae* n. sp. Whole-mounts (A–F) fixed in Berland’s fluid and shown in ventral view. A, D, C and F mounted in DPX, B and E mounted in glycerine jelly. A. Forebody. B. Oral sucker with circumoral spines. C. Anterior extremity with pharynx in usual position. D. Specimen with pharynx in lumen of oral sucker. E. Specimen with gonotyl in genital sac. F. Specimen with protruded gonotyl. G. Sagittal section of whole worm. H. Transverse section through region of gonotyl. I. Transverse section through posterior region of same specimen as H. Abbreviations: e, egg; ex, excretory vesicle; f, fold in prepharynx; g, gonotyl; gs, genital sac; i, intestinal caecum; o, ovary; oe, oesophagus; ogs, opening of genital sac; p, pocket in gonotyl; ph, pharynx; pi, pigment granules; pph, prepharynx; sr, seminal receptacle; t, testis; ug, unicellular subtegumental gland; v, vitelline follicle; vr, vitelline reservoir; vs, ventral sucker.

overlapping caeca laterally. Uterus enters right margin of genital sac. Genital pore immediately antero-sinistral to ventral sucker. Gonotyl, a thick muscular pad, eversible, sinistral to anterior margin of ventral sucker, containing 20–30 fusiform

to guttiform pockets arranged in a crescent and containing strongly refractive material. Numerous small gland-cells associated with gonotyl and pockets. Protruded gonotyl with openings of pockets basally and with small sclerotised knobs

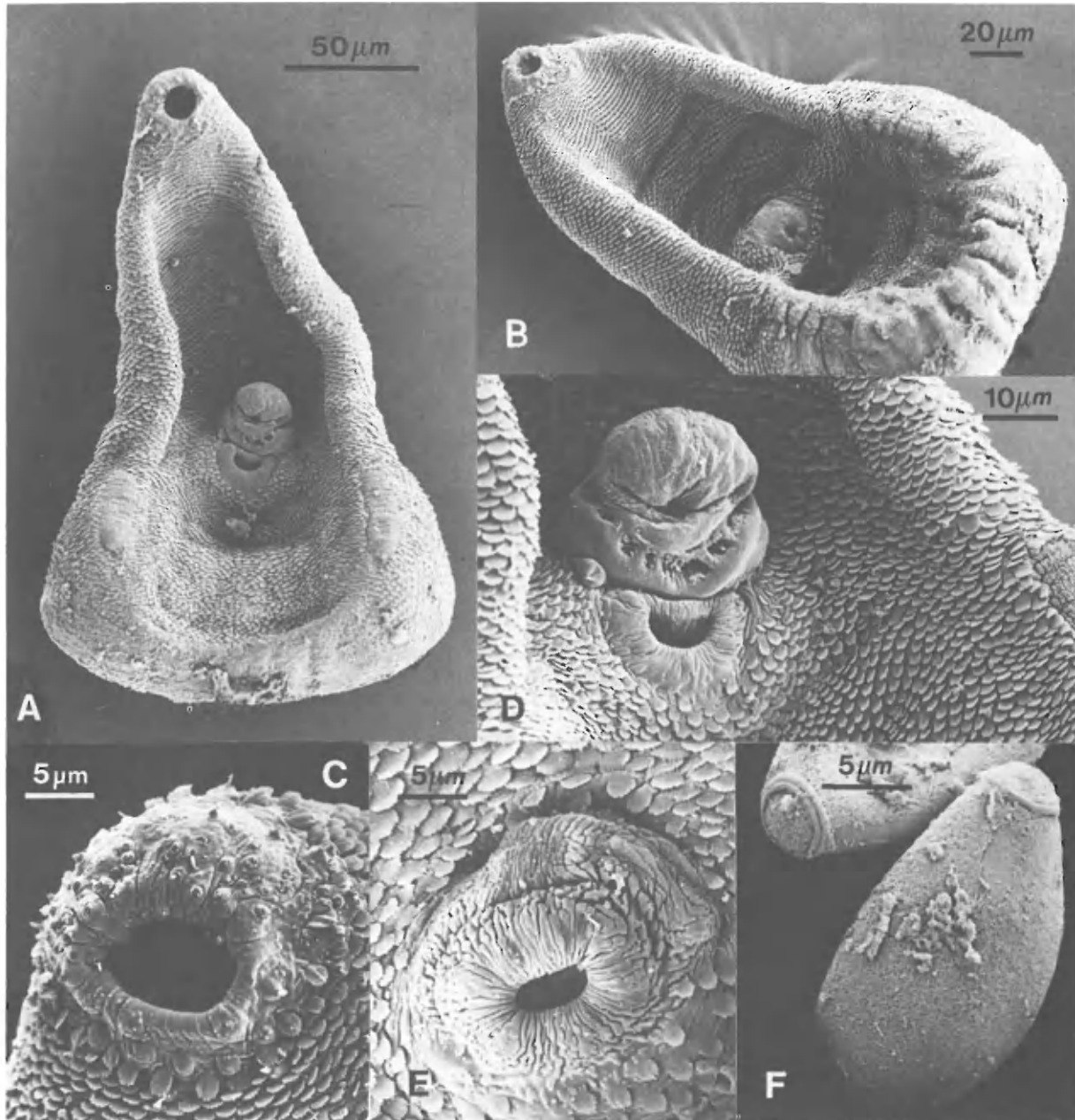


Fig. 3. *Pygidiopsis ardeae* n. sp. SEM micrographs. A. Ventral view of specimen with protruded gonotyl. B. Ventro-lateral view of specimen with gonotyl in genital sac. C. Anterior, ventral view, showing circumoral spines, smaller body spines and supposed sensory structures. D. Gonotyl, ventral sucker and part of ventro-lateral region devoid of spines. E. Ventral sucker and opening of genital sac. F. Eggs removed from uterus.

Eggs operculate, most often collapsed, nearly colourless when newly formed but golden yellow in more distal portion of uterus. Surface with fine irregular pattern. Opercular line between 2 annular thickenings. Mature eggs contain miracidium.

Discussion

Pygidiopsis ardeae n. sp. is similar in general morphology to the type-species, *P. genata* Looss, 1907 (Table I), as described by Looss (1907). Eight species of *Pygidiopsis* have been described (Yamaguti, 1971; Wang, 1982).

No *Pygidiopsis* species has been described from northern Europe. Carrère (1938) recorded *Pygidiopsis* sp. in Ardeiformes from southern France, but as the species was neither described nor figured its identity is unknown. The specimens examined from piscivorous birds in Great Britain were in too poor a condition to be compared with the present specimens. Two species, *P. genata* Looss, 1907 and *P. piclaumoreli* Dollfus & Capron, 1958, have been described from the Mediterranean area and Senegal, respectively. The remaining species are from the Philippines, North and South America and Asia. *P. ardeae* differs from *P. piclaumoreli* by being smaller and in the position of the vitelline follicles, these being lateral to the testes in the former and pre-testicular in the latter. *P. ardeae* differs from *P. plana* in its smaller size (Table I) and its absence of a wide, sac-like pre-pharynx. *P. ardeae* differs from *P. summa* Onji & Nishio, 1916, which has been recorded in Asia only, by its smaller size and by the different shape of the retracted gonotyl. *P. summa* has two separate groups of "strongly refractive rod-like bodies" on the gonotyl (Yamaguti, 1939). According to Looss (1907) *P. genata* has the ventral sucker smaller than the oral sucker, whereas in Witenberg's description of this species the ventral sucker is larger than the oral sucker. The specimens of *P. genata* examined in the present study also had the ventral sucker slightly larger than the oral sucker. However, *P. genata*, *P. summa* and *P. ardeae* are clearly separated by the morphology of the metacercariae and their choice of the first intermediate host. The cercaria of *P. genata* develops in *Melania tubercu-*

lata (Cerithiacea, Melaniidae) (Boulos *et al.*, 1981), the cercaria of *P. summa* occurs in *Tympanotonus microptera* (Cerithiacea, Potamididae) (Chai *et al.*, 1986), whereas that of *P. ardeae* is found in *Hydrobia* spp. (Rissoacea, Hydrobiidae) (Kjøie, 1990). Neither *M. tuberculata* nor *T. microptera* live in northern Europe. The encysted metacercariae of *P. genata* and *P. summa* are spherical (unpublished illustrations by Dr Boulos, pers. comm.; Chai *et al.*, 1986), whereas those of *P. ardeae* alone are elongate (Kjøie, 1990). Estuarine fishes are intermediate hosts for all three species. The cercaria of *P. genata* is pleurolophocercous and thus differs from that of *P. ardeae* (see Kjøie, 1990). The life-cycle of *P. genata* has been worked out experimentally (Boulos *et al.*, 1981; Dr Boulos, pers. comm.). The cercaria of *P. summa* (see Yamaguti, 1975) has no similarity with that of *P. ardeae*. Christensen & Roth (1949) found specimens of *Pygidiopsis* (identified as *P. genata*) in dogs experimentally fed on plaice *Pleuronectes platessa* and flounders *Platichthys flesus* from Danish inlets. Since in the present study the metacercariae of *P. ardeae* were experimentally (and naturally – see Kjøie, 1990) obtained in estuarine fishes, including flatfishes, sticklebacks and gobies, it seems very likely that Christensen & Roth's specimens from the dogs belong to this species.

The single row of circumoral spines was overlooked in the original description of *P. genata* by Looss (1907), but mentioned by Witenberg (1929) in his redescription of the species. Examination of *P. genata* (present study) revealed that the oral spination was identical with that of *P. ardeae*, i.e., a single row with 16 circumoral spines and a posterior dorsal row with 4 slightly smaller spines. Ostrowski de Nuñez (1974) also described a similar oral spination of *P. pindoramensis* Travassos, 1929 from Argentina. The circumoral spines of *Pygidiopsis* are often recorded as only occurring in live specimens (Chai *et al.*, 1986). As the circumoral spines are attached to the oral sucker they are most often withdrawn into the sucker on fixation. After death the circumoral spines are lost before the remaining body spines. The circumoral spines were easily recognisable in specimens live-fixed, cleared in lactophenol and

Table 1. Measurements (in μm) of *P. genata*, *P. piclaumorelli*, *P. plano* and *P. sumina*.

Name	<i>P. ardrea</i>		<i>P. genata</i>		<i>P. piclaumorelli</i>		<i>P. plano</i>		<i>P. sumina</i>	
	No. of specimens	I (holotype) R (paratypes)	?	Egged specimens	1	1	1	1	?	?
Condition	Unflat, whole-mounts				4	7	7	1		
Length	360	358 (275-467)	310-500		908	785	670		550-910	
Width	220	212 (160-280)	210-220		263	263	320		250-400	
Forebody as % of body-length	50.0	50.9 (45.0-57.1)	[54.3]		[54, 60]		[48, 11]		[53, 3]	
Oral sucker	45 \times 40	41 (36-48) \times 38 (30-44)	40 diam.		55 diam.	53 diam.	30 \times 40		30-54 diam.	
Pharynx	34 \times 20	35 (30-42) \times 18(14-20)	33 \times 24		50 \times 38	33 \times 19	30 \times 27		30-40 \times 27-33	
Ventral sucker	38 \times 45	39 (30-48) \times 45 (36-50)	37-39 diam.		69 diam.	77 \times 63	36 \times 45		48-60 diam.	
Sucker-width ratio	1:1.13	1:1.18 (1:1.05-1.31)	1:0.93-0.98		1:1.25	1:1.19	1:1.13		-	
Testis, right	30 \times 65	33 (24-46) \times 60 (44-80)	[30 \times 65]		87 diam.	98-102 \times 76	96 \times 54		42-75 \times 75-120	
Testis, left	30 \times 55	33 (26-45) \times 60 (45-74)					50 \times 45			
Ovary	54 \times 46	38 (24-52) \times 44 (30-65)	[28] diam.			[37, 39] diam.	72 \times 63		45-83 diam.	
Eggs	18 (17-19) \times 9 (8-10)	19.3 (17-22) \times 9.4 (7-11)	21 \times 11		26-27.7 \times 14-17.5		20 \times 12		21-23 \times 11-14	
Host	<i>Ardea cinerea</i>		<i>Pelecanus onocrotalus</i>		max. 32 \times 16.6					
Type-locality	Zealand, Denmark		Cairo, Egypt		<i>Sterna bergii</i>		<i>Butorides viviscens</i>		<i>Milvius nigricans lineatus</i>	
Source of data	Present study		Looss (1907)		Rubisque, Senegal		Woods Hole, USA		Japan	
					Dollfus & Capron (1958)		Linton (1928)		Yamaguti (1939)	

* Measurements of three eggs from each specimen.

† Measurements in brackets taken from published illustrations.

mounted in glycerine jelly, and they also appeared on SEM fixed material.

Examination of *P. genata* (present study) revealed that they have two ventro-lateral unspined fields on the hindbody, underlying glandular cells, and an anteriorly directed fold (fornix-like fold of Pearson, 1973) at the junction between the prepharynx and pharynx, features which were also found in *P. ardeae*. The prepharynx and pharynx of *P. genata* and *P. ardeae* are thus similar to those of some species of the heterophyid genus *Galactosomum* (see Pearson, 1973). During the act of ingestion the pharynx moves forward within the prepharynx and may half enter the lumen of the oral sucker: it then returns to the resting position. In the process the pharynx engulfs material from the lumen of the oral sucker (Pearson, 1973).

The observation by Chai *et al.* (1986) that the gonotyl of *P. summa* is provided with spines needs confirmation. Yamaguti (1939) described strongly refractive rod-like bodies in the genital plug of *P. summa* and Ciurea (1933) described the gonotyl of *P. genata* as "une ventouse génitale dégradée, tapissée par une cuticule qui forme des plis ayant l'apparence des petits bâtonnets, disposés d'une manière radiaire". Similar bodies in the gonotyl of *P. ardeae* represent cavities with a glandular material which may be expelled when the gonotyl is protruded.

Several heterophyid trematodes and among these at least two *Pygidiopsis* species, *P. genata* and *P. summa*, are reported from humans, where they cause intestinal heterophyiasis (Boulos *et al.*, 1981; Seo *et al.*, 1981). It is not known whether or not *P. ardeae* may infect humans, but the occurrence of metacercariae of *P. ardeae* in plaice and flounders, and its successful development in dogs, makes it a possible human pathogen if viable metacercariae are ingested.

Acknowledgements

The author wishes to thank Dr Leila M. Boulos, Alexandria, Egypt, Dr D.I. Gibson, British Museum (Natural History), Prof. A.G. Chabaud, Museum National d'Histoire Naturelle, Paris,

and Dr J.R. Lichtenfels, U.S. Department of Agriculture, Beltsville, for the loan of specimens of *Pygidiopsis genata*, *Pygidiopsis* sp., *P. piclaumoreli* and *P. plana*, respectively. Dr D.I. Gibson, BM(NH), kindly read and suggested improvements to the draft manuscript.

References

- Boulos, L.M., Abdou, L.A. & Girgis, R.S. (1981) Histopathological and histochemical studies on experimentally infected hamsters with *Pygidiopsis genata*. *Journal of the Egyptian Society of Parasitology*, **11**, 67–76.
- Carrère, M.P. (1938) Recherches expérimentales et épidémiologiques sur les trématodes de quelques poissons marins. *Soixante et Onzième Congrès des Sociétés Savantes*, pp. 293–295.
- Chai, J.-Y., Seo, B.-S., Lee, S.-H. & Hong, S.-T. (1986) Growth and development of *Pygidiopsis summa* in rats and mice with a supplementary note on its morphological characters. *The Korean Journal of Parasitology*, **24**, 55–62.
- Christensen, N.O. & Roth, H. (1949) Investigations on internal parasites of dogs. *Kongelige Veterinær- og Landbohøjskoles Aarskrift*, 1949, 1–73.
- Ciurea, I. (1933) Les vers parasites de l'homme, des mammifères et des oiseaux provenant des poissons du Danube et de la Mer Noire. *Archives Roumaines de Pathologie Expérimentale et de Microbiologie*, **6**, 5–134.
- Dollfus, R.P. & Capron, A. (1958) Un *Pygidiopsis* (Trematoda Heterophyidae) de *Sterna* de la côte du Sénégal. *Bulletin de l'Institut Française d'Afrique Noire*, **20**, Série A. 2, 306–310.
- Kjøie, M. (1987) Scanning electron microscopy of rediae, cercariae, metacercariae and adults of *Mesorchis denticulatus* (Rudolphi, 1802) (Trematoda, Echinostomatidae). *Parasitology Research*, **73**, 50–56.
- Kjøie, M. (1990) The life cycle of *Pygidiopsis ardeae* Kjøie, 1990 (Digenea, Heterophyidae). *Journal of Parasitology* (In press).
- Linton, E. (1928) Notes on trematode parasites in birds. *Proceedings of the United States National Museum*, **73**, 1–36.
- Looss, A. (1907) Notizen zur Helminthologie Ägyptens. V11. Über einige neue Trematoden der ägyptischen Fauna. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene*. I. Originale. **43**, 478–490.
- Ostrowski de Nuñez, M. (1974) Estudio sobre estados larvales de trematodes digeneos de peces Cyprinodontiformes. *Physis, Buenos Aires*. Sección B, **33**(86), 45–61.
- Pearson, J.C. (1973) A revision of the subfamily Haplorchinae Looss, 1899 (Trematoda: Heterophyidae) II. Genus *Galactosomum*. *Philosophical Transactions of the Royal Society of London*, **266**, 341–447.
- Seo, B.-S., Hong, S.-T. & Chai, J.-Y. (1981) Studies on intestinal trematodes in Korea. III. Natural human infections of *Pygidiopsis summa* and *Heterophyes heterophyes nocens*. *The Seoul Journal of Medicine*, **22**, 228–235.
- Wang, Pu-qin (1982) [Notes on some digenetic trematodes of

- birds in Fujian Province.] *Wuyi Science Journal*, **2**, 75-90 (In Chinese with English summary).
- Witenberg, G. (1929) Studies on the trematode family Heterophyidae. *Annals of Tropical Medicine and Parasitology*, **23**, 131-239 .
- Yamaguti, S. (1939) Studies on the helminth fauna of Japan. Pt. 25. Trematodes of birds, IV. *Japanese Journal of Zoology*, **8**, 129-210.
- Yamaguti, S. (1971) *Synopsis of digenetic trematodes of vertebrates*. Tokyo: Keigaku Publishing Company, Vol 1, 10 pp.
- Yamaguti, S. (1975) *A synoptical review of life histories digenetic trematodes of vertebrates*. Tokyo: Keigaku Publishing Company, 590 pp.

THE UNIVERSITY OF CHICAGO
LIBRARY
540 EAST 57TH STREET
CHICAGO, ILL. 60637
TEL: 773-936-3000

B-1323