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OCCURENCE OF THE PHALLOSTETHID FISH *PHENACOSTETHUS SMITHI* MYERS IN SOUTHERN JOHOR, PENINSULAR MALAYSIA, WITH SOME OBSERVATIONS ON ITS ANATOMY AND ECOLOGY

Angus D. Munro and **Elizabeth Y. M. Mok**

ABSTRACT. - The phallosthethid fish, *Phenacostethus smithi* Myers, 1928, previously reported only from eastern Thailand, has been found in some river systems of the Johor, southern Peninsular Malaysia. The external appearance of the thoracic area is described, based on scanning electron microscope observations. The structure of the skeletal system of the priapium is apparently identical to that of Thai populations of *P. smithi*, as is the external appearance of the mature male genital papilla. Pertinent aspects of the histology of the gonads and brain are described.These studies are consistent with the proposition that phallostethoids are atherinomorphs. A preliminary account is given of various aspects of the ecology of *P. smithi*.

INTRODUCTION

The phallostethids (superfamily Phallastethoidea) constitute an atherinomorph group which is both unique to southeast Asia and unique in its anatomical specialisations (in particular the gular copulatory organ, or priapium, of males). According to some workers (Roberts, 1971a; cf. Parenti, 1984), phallostethoids comprise two distinct families, the Phallostethidae (Regan) (*sensu stricto*) and the Neostethidae (Aurich), primarily on the basis of differences in priapial morphology and concommitant modifications of derivatives of the pelvic girdle and ribs in the male.

The present note reports an extension in the range of *Phenacostethus smithi* Myers, 1928, a phallostethid previously only recorded from freshwaters in southeastern Thailand (Myers, 1928; Roberts, 1971b). Also included are some general observations on its morphological peculiarities, together with some aspects of its general ecology in a river system in southern Peninsular Malaysia. These observations are a preliminary to more detailed future behavioural and anatomical studies on these fishes.

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MATERIALS AND METHODS

Source of fish. - Three sites in southwest Johor provided the majority of specimens. The first was from a river just downstream of the bridge for the road between Kulai and Kota Tinggi (just before the Kota Tinggi 24 km-milestone), sampled in February (by hand-netting) and March 1990 (by seine-netting). The second (March, June and September, 1990) and third (July and September, 1990) sites were at a nearby river, Sungei Sayong, respectively upstream and downstream of the bridge on route 91 (to Bandar Tenggara) about 450 m from its junction with the Kulai-Kota Tinggi road; in each case, sampling was done using hand-nets. A fourth site, from a river (possibly higher up the Sungei Sayong) on the left side of the Kulai-Kota Tinggi road near the Kota Tinggi 30 km-milestone, was sampled in September, 1990.

A solitary specimen, provisionally included in the present description, was caught in January 1990 by hand-netting during a routine (monthly) sampling of a stream (Sungei 'Kulai') at the Kulai 10 km-milestone on the Kulai-Kota Tinggi road.

Specimens were routinely fixed in Baker's formal calcium. However, some were instead fixed in glutaraldehyde-formaldehyde (Karnowsky, 1965) for electron microscopy.

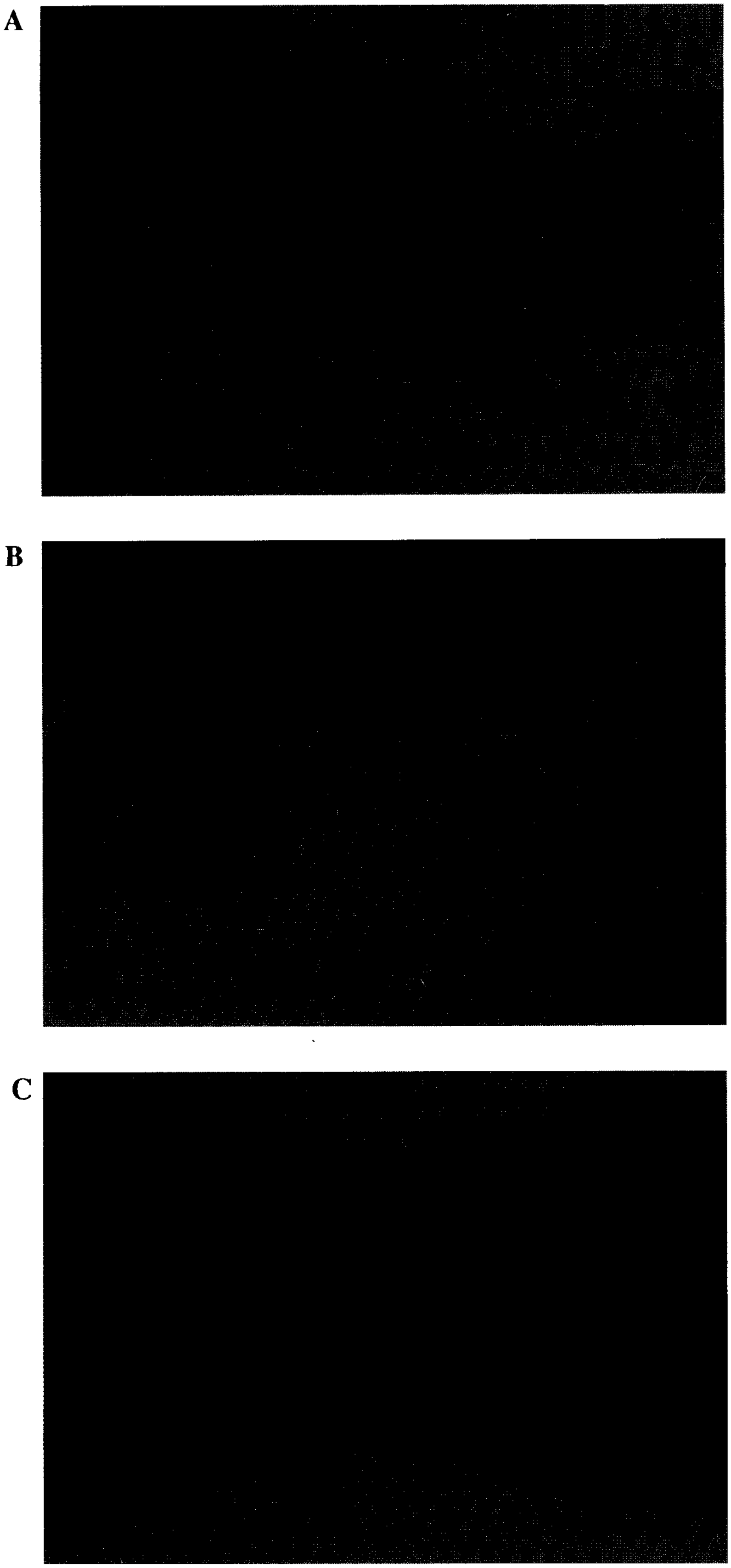
Alcian blue-alizarin red (AB-AR) preparations. - Specimens fixed in Baker's were processed according to the technique of Dingerkus and Uhler (1977).

Scanning electron microscopy (SEM). - After at least 14 days fixation at 4°C, specimens were transferred to 0.1 M sodium cacodylate (pH 7.2), followed by a slow progressive dilution to distilled water. Thereafter, they were slowly dehydrated through an alcohol series to absolute, then taken through several changes of liquid carbon dioxide before critical point-drying. The specimens were then coated with carbon followed by gold (ca. 35 nm thickness), and examined on a Jeol T220 scanning electron microscope.

Histology. - After fixation in Baker's formal-calcium for one to two days, specimens were transferred to 70% alcohol for storage. They were subsequently post-fixed (and decalcified) for seven days in Bouin's fixative, dehydrated and embedded for routine wax histology. Some specimens were double-embedded (Pantin, 1969) by passing through methyl benzoate-celloidin before embedding in wax. Serial sections (7 µm) were stained with Mayer's haemalum or Heidenhein's azan.

Pl. 1. **A, B.** Views of the aproctal (A) and proctal (B) sides of a mature male *P. smithi* stained with Alcian blue and Alizarin red. Nomenclature based on Bailey (1934) and Roberts (1971b). ai, anterior infrasulcar; ap, antepleural; ax, axial; bp, basipenial; c, cristate; cl, cleithrum; co, coracoid; ct, ctenactinium; d, dentary; p, penial; pa, papillary; pd, paradentary; pi, posterior infrasulcar; pp, prepapillary; pv, pulvinulus; r, priapial rib; sc, scapula; ta, toxactinium; u uncus. C Dorsal view of the anterior head of a male *P. smithi* stained with Alcian blue and Alizarin red. The mouth was held open by inserting a thin glass tube (g). Nomenclature based on Roberts (1971a). d, dentary; me, mesethmoid; m, maxilla; pm, premaxilla; pr, pararostral; ps, parasphenoid; pv, prevomer; r, rostral; sm, submaxillary.

Colour Plate 20



RESULTS AND DISCUSSION

Morphological observations

General. - A line of regularly-spaced, rostrocaudally-elongated melanophores extends a variable distance forwards from the middle of the caudal peduncle towards an area slightly posterodorsal of the pectoral fin. A second row of punctate melanophores runs on each side of the ventral surface, from immediately in front of the base of the anal fin back to the base of the caudal peduncle. Other melanophores are located in patches on top of the head in front of each eye; on the midline behind (above the posterior optic tectum and cerebellum of the brain); and at the base of the pectoral fin. These patches of melanophores were variable in the number of component cells, and in the degree of pigment dispersal. In life, the caudal peduncle has a faint yellow spot (as is also the case in the neostethids *Ceratostethus bicornis* and *Neostethus lankesteri*, unpublished data; cf. Roberts, 1971b). Thus the colour pattern is identical to that described and illustrated by Roberts (1971b) for *P. smithi*, with the exception that there was no obvious silver spot on the dorsal retina.

The gular region of the female. - The anal and, behind, oviducal openings lie on the ventral surface immediately behind the pectoral area. A pair of minute pelvic fins is present immediately behind the oviducal opening; typically, their tips are apposed on the midline (Fig. 1). Although histological studies indicate that the ureter must discharge through a small opening behind that of the oviduct (cf. Parenti, 1986), this was generally not visible in SEM specimens, possibly because it was obscured by the pelvic fins.

Structure of the priapial complex in the male. - The priapial complex is an assymmetrical keel-like bulge of the ventral throat area, which is supported by a complex skeleton, and which contains the terminal portions of the gut, ureter and sperm duct. Following other workers (e.g. Roberts, 1971a, b; Parenti, 1984), the anal opening (Fig. 2) is used as a marker to identify one side as the proctal side, in contradistinction to the opposite, aproctal side.

The appearance and disposition of the supportive skeletal elements in AB-AR preparations of the present material (Pl. 1A, B) is indistinguishable from published descriptions for *P. smithi* by Bailey (1936) and Roberts (1971b).

The anterior portion of the priapial complex is represented externally by the pulvinulus, an ovoid structure which is displaced towards the proctal side of the midline (Fig. 2). AB-AR and histological preparations indicate that it is supported by a ring of cartilage; and that its ventral surface comprises a connective tissue sheath which encloses a fluid-filled space. With SEM, the discoid ventrolateral surface typically has an elevated margin. The enclosed area is variable in appearance (possibly the result of fixation artefacts through collapse of the underlying fluid-filled space), being smooth in some specimens, whilst bearing a few to many irregularly-distributed longitudinal ridges in others (Fig. 2). There was a general tendency for the central portion to be elevated (possibly due to the connective tissue sheath collapsing to rest against the underlying articulation of the axial bone with the toxactinium).

A smooth, hook-shaped toxactinium emerges from the anterolateral face of the pulvinulus, recurving forwards towards the aproctal side underneath the lower jaw. The toxactinium is widened immediately distal to its point of emergence, with a distinct

Fig. 1. Ventral view of the thoracic region of a female *P. smithi*, to show the anal (A) and oviducal (O) openings, immediately in front of the pelvic fins (P); ff, midventral fin-fold. X100.

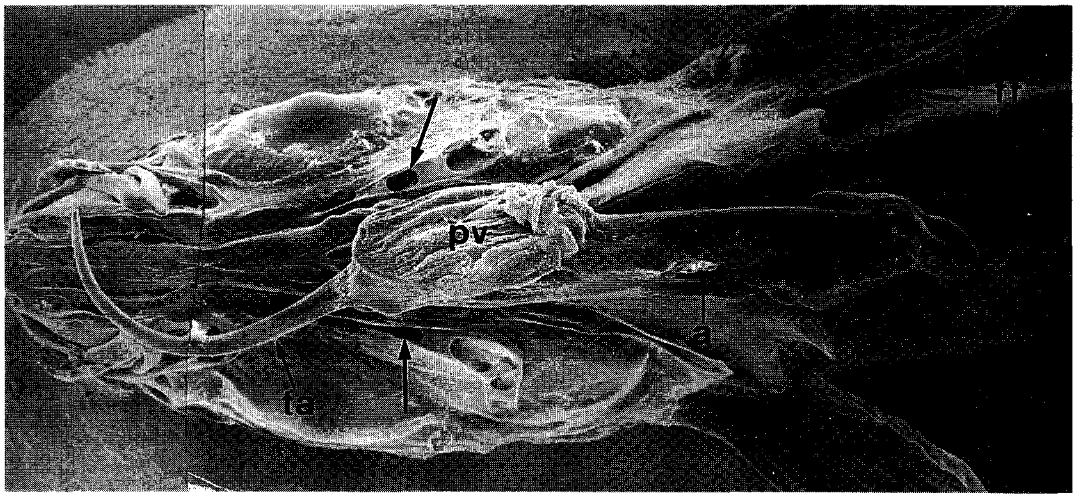


Fig. 2. Montage ventral view of the anterior end of a male *P. smithi*, to show the components of the priapial complex; note the assymmetry of the preopercular components of the lateral line system (arrows). a, anal opening; ct, ctenactinium; ff, midventral fin-fold; pa, genital papilla; pc, pectoral fin; pv, pulvinulus; ta, toxactinium. X35.

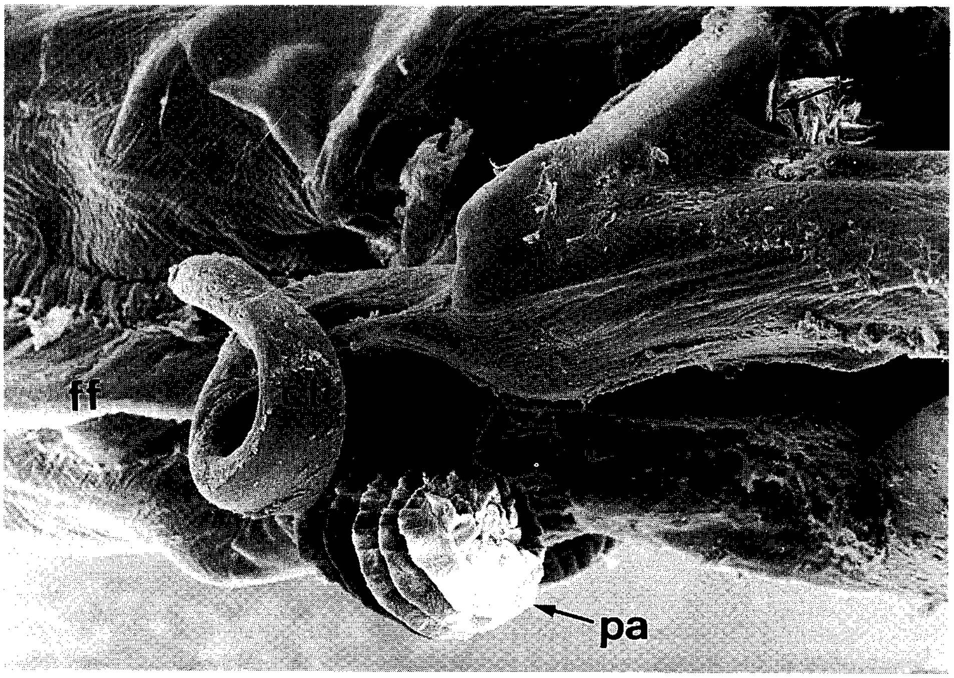


Fig. 3. Proctoventral view of the posterior priapium of a mature male to show the genital papilla (pa), with its well- developed ruffled terminal portion, and the ctenactinium (ct); a, anal opening; ff, ventral fin-fold. X100.

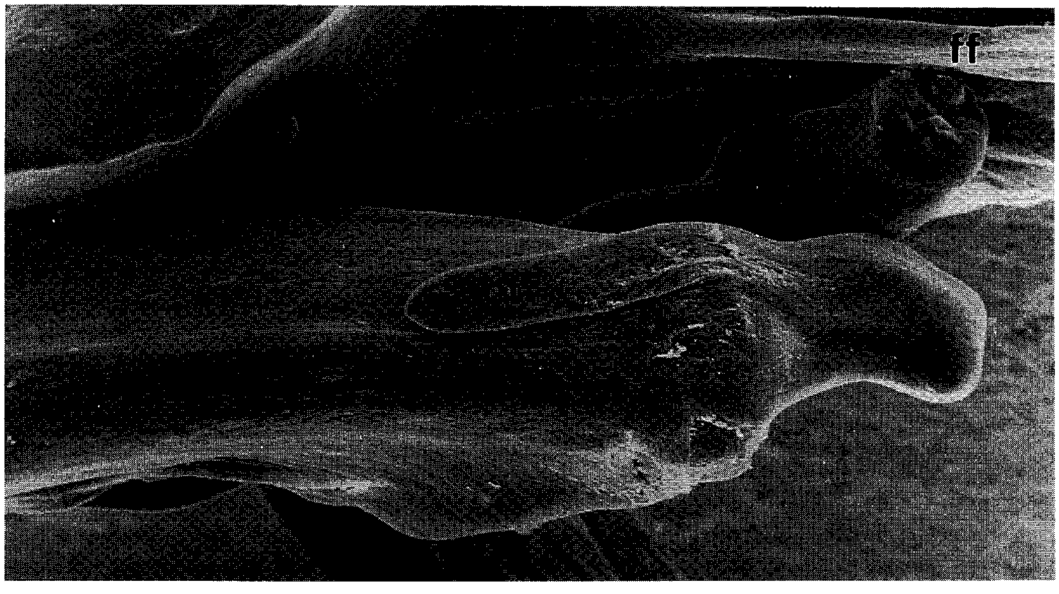


Fig. 4. View of the posterior priapial complex of the same specimen as in Fig. 2, but tilted towards the aproctal side to show the relationships of the genital papilla (pa) and the ctenactinium (ct); ff, midventral fin-fold; pg, pectoral girdle; x, unidentified extraneous artefact. X100.

protuberance on the lateral side (Fig. 2); there may also a fluting on its ventral side. The surface of this structure was smooth in apparently undamaged specimens, with no obvious structural specialisations.

A keel extends from the pulvinulus to the back of the priapial complex, at which level it becomes thinner ventrally. Again, the epithelium covering the general surface shows no glandular or other specialisations, including neuromasts or other such sensory structures. The dorsal portion of the posterior face gives rise to the genital papilla; whilst the ventral portion narrows before expanding caudally to form the base of the ctenactinium (Figs. 2-4).

Of the males examined with SEM, only one had a well-developed genital papilla (Fig. 3) comparable with that described by Roberts (1971b) for *P. smithi* from Thailand. In this specimen, the tip of the papilla extended over the aproctal face of the priapium. Its terminal portion was ringed by eight folds of tissue which lacked any obvious surface specialisations; the only unusual feature was that the distal four pleats showed intense charging-up under the electron beam (Fig. 3), suggesting problems with carbon- and/or gold-coating, but the underlying reason for this is not apparent. The opening of the sperm duct was located apically. Prior to critical point drying, the ctenactinium of this specimen projected rostroventrolaterally out from the aprocto-caudal face of the priapium, being only slightly curved; however, there was some subsequent distortion so that, with SEM, it was coiled.

Histological studies have established that males are fully mature (in the sense that the sperm ducts leading to the genital papilla are full of sperm) by the time that the toxactinium has completed development (at about 14 mm SL). Nevertheless all of other 'mature' males (on the basis of the development of the toxactinium and being 15 to 17 mm SL) examined with SEM lacked a well-developed genital papilla. Instead, the papillae of the other fish were shorter, pointing aprocto-caudally, with their tips generally close to the proctal face of the ventral fin fold. The tip, bearing the opening of the sperm duct, was surrounded by a few apical swellings in some (but not all) specimens (Fig. 4). The ctenactinium in these fish extended forwards, close to the aproctal side of the priapium (Figs. 4, 5). SEM studies indicate that it is variable in shape: whilst it is straight in some specimens (Figs. 4, 5), it may have one or more undulations in others (presumably the result of distortions resulting from tissue processing for SEM: see above).

From an examination of smaller males, the priapial complex initially appears as a triangular keel whose apex extends forwards into the gular region, with the (bilaterally-assymmetric) base between the level of the pectoral fins and the origin of the ventral fin-fold. There appears to be considerable variation in the sequence of subsequent metamorphic events. In some individuals (Fig. 6A), the lateral migration of the anal opening precedes the differentiation of the posterior priapial complex; whilst in others, the opposite is the case (Fig. 6B). The relative time of appearance of the pulvinulus appears to be similarly variable with respect to the differentiation of the posterior priapial complex (Figs. 6A, B). Available evidence suggests that the development of the basic genital papilla (i.e. without the rufous terminal segment) is completed prior to that of the ctenactinium. It would seem that, as the ctenactinium approaches its final adult length, a distinct narrowing of the sulcar region develops between its base and the main portion of the priapium (compare Figs. 4 and 5); thereafter, observations on other material by light microscopy indicate that

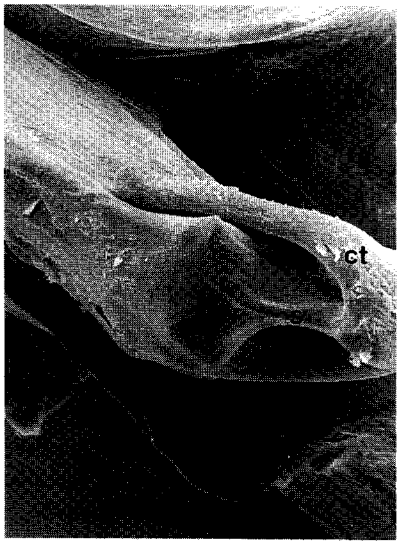


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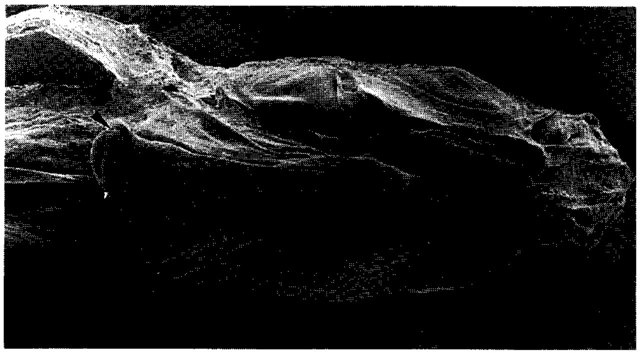
Fig. 5. Ventrolateral view of the posterior priapial complex of a male *P. smithi*, to show the thinning of the sulcar area (s) at the base of the ctenactinium (ct) characteristic of more mature specimens. X100.



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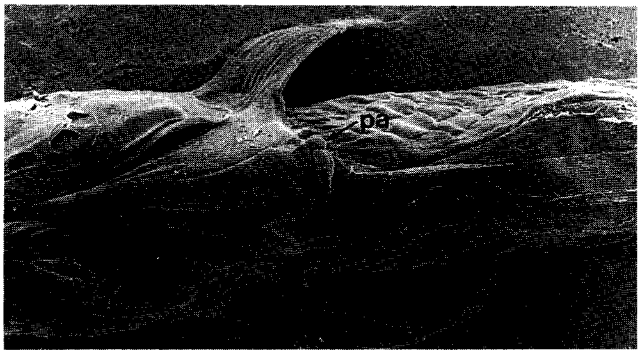


6B

Fig. 6. Ventral views of two immature male *P. smithi*. In A, the priapial complex has developed as a keel, with the anal opening (a) displaced towards the proctal side; an incipient pulvinulus (pv) is evident; whilst the posterior portion comrises a single fluted protrusion towards the aproctal side with the apical opening of the sperm duct (arrow). In B, on the other hand, the anal opening (a) has shown little lateral displacement and the pulvinulus is much less distinct; however, the posterior priapial complex has differentiated into a distinct genital papilla (pa) with, below, a developping ctenactinium (ct). Both X35.

the tip of the (still straight) ctenactinium subsequently swings outward, to point rostroaproctally.

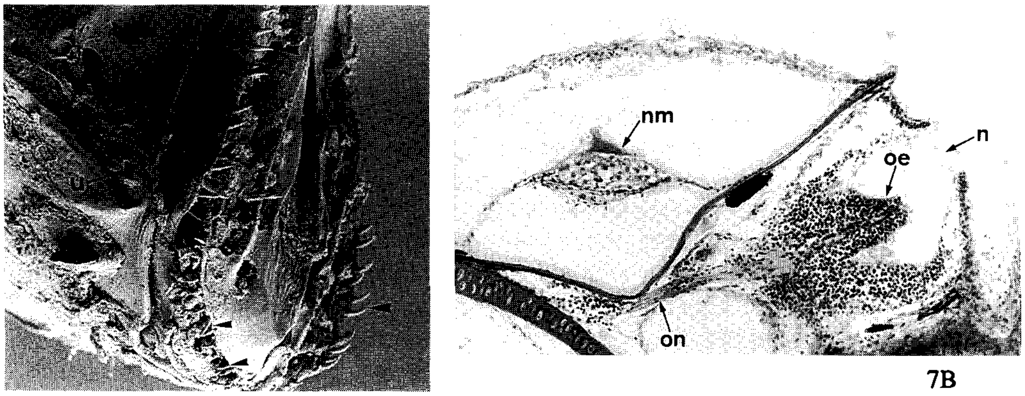
Despite its large size (17 mm), the single large specimen caught from Sungei 'Kulai' was clearly an immature male with an aberrant pattern of development of its secondary sexual characteristics. Thus, in contrast to immature males from other locations, the pulvinulus-toxactinium was fully differentiated (although the toxactinium was unossified and malformed), whereas more posterior elements of the priapium were not apparent, either externally or after staining with AB-AR. Since subsequent monthly samplings have failed to catch any more specimens of this fish at the same location, it could be either a stray (perhaps a result of accidental egg-transport by birds or other agents); or else a relict of a previously-established population (the stream in question has recently changed in character, previously being shaded by mature oil palms: P. N. K. Ng, pers. comm.). The abnormal development of its secondary sexual characters might suggest that the development of various elements of the priapial complex is under multiple control mechanisms (endocrine or otherwise); and that the normal expression of one or more of these controls was disrupted by the prevailing environment of Sungei 'Kulai'. This possibility would also provide a working hypothesis for the observed variability in the sequence of the development of the priapial complex observed for males from the other locations (see above).

Meristic counts. - These are broadly comparable with published data for *P. smithi* from Thailand, except for the lower pectoral fin-ray counts (Table 1). As in the latter species (Roberts, 1971), there is a small first dorsal fin, represented by a single spine; and the ventral fin-fold from the post-anal area back to the start of the anal fin is not covered with scales (e.g. Figs. 1-4).

Table 1. Meristic counts

	Kota Tinggi-24km (N = 4)	Sungei Sayong (N = 2)	Kulai-10km (N = 1)	Roberts (1971b) (N = 2)
Second dorsal	II 4	II 4	II 5	II $4^1/_2$
Anal	II 12	II 13-14	I 12	II $12^1/_2$-13
Pectoral	I 7	I 7	I 7	I 9
Caudal	7/7-5/12 (7/7-5/11)*	7/7-5/12	7/7-5/10	7 I 6-5 II 10 8 II 4-6 I 9
Abdominal vertebrae	14 (15)*	14	14	14
Caudal vertebrae	$19^1/_2$-$20^1/_2$	$20^1/_2$-$21^1/_2$	$20^1/_2$	$19^1/_2$-$20^1/_2$

* : In one specimen.



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7A

Fig. 7. A: Rostrolateral view of the head of a mature male *P. smithi*, to show the single nostril (n); the small teeth on the medial dentary (small arrow); and the large, protruding teeth on the lateral premaxillary (large arrow). X75. B: Parasagittal section through the naris and rostrolateral neuromast chamber. The naris comprises a single ridge of olfactory epithelium (oe), in contact with the exterior through a small pore (n); a small olfactory nerve (on) passes back from the olfactory organ. The rostrolateral neuromast chamber has a neuromast (nm) in its membranous floor, with its cupula protruding into the chamber above. Azan, X280.

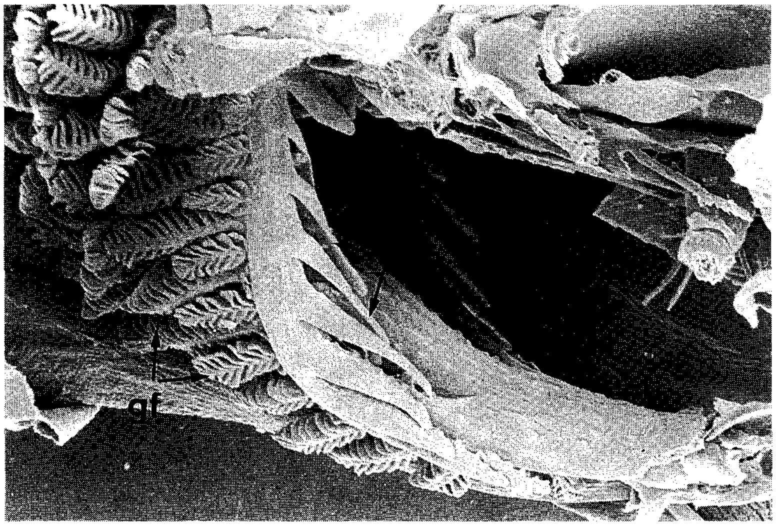

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Fig. 8. View of the exposed gill-arches of a mature female *P. smithi*, to show the gill filaments (gf) and gill-rakers (gr) of the first ceratobranchial: note the unusual intercalation of large and small gill-filaments (not apparent in all such preparations). X100.

The jaws and teeth. - Compared to the descriptions of Bailey (1936) and Roberts (1971), there are some differences in dentition. First, there are eight teeth on the rostromedial portion of each premaxillary (Pl. 1C), as reported for *P. smithi* by Roberts (1971b), but not Bailey (1936). Secondly, these teeth are similar in size to, rather than larger than (Roberts, 1971b), those on the rostromedial dentary in AB-AR preparations and with SEM (Fig. 7). Thus the largest teeth are another group of eight located on the caudo-lateral arm of the premaxillary (Pl. 1C); in scanning electron micrographs, these extend outside the lower jaw when the mouth is closed (Fig. 7).

As in other phallostethoids (Roberts, 1971a; Parenti, 1984), a paradentary lies lateral to each dentary (Pl. 1A). In the present material, however, the paradentaries are cartilaginous, as well as being untoothed (cf. *Phallostethus dunckeri* Parenti, 1984). During buccal opening, they support the ventrolateral portions of the aperture of the resultant buccal tube, as in neostethids (Roberts, 1971a).

The submaxillary of other phallostethoids (including *Phallostethus dunckeri*, Parenti, 1984) appears to be represented here by a cartilaginous (AR-negative) connection between the maxillary and the rostral neurocranium (Pl. 1C). Roberts (1971a) reported that *P. smithi* lacked submaxillaries on the basis of alizarin red studies; the present observations suggest that this may merely be because they (like the paradentaries) are not ossified.

The present observations confirmed those of Bailey (1936) that the gill-rakers were best developed on the first gill-arch (Fig. 8). They were elongate, with knobs on the dorsal face of the (longer) lowermost members. One difference between Bailey's and the present observations is the lower number of rakers on the first ceratobranchial in the present material (9 to 10 rather than 12).

The octavo-lateralis system. - There was no trunk lateral line, and surface neuromasts are virtually absent, on the basis of SEM and histological observations. The single exception was the presence of a few scattered neuromasts on the scale-less area lateral to the oviduct opening of one female prepared for SEM (Fig. 9), with a few other neuromasts on what appeared to be a specialised scale immediately behind in the same fish (Fig. 10). Those on the lateral, scale-less area are comparable in number and position with those described by TeWinkel (1934). These neuromasts were not recognised in the other suitable females examined; it is not clear whether this is due to differences in sexual maturity, the result of preparation damage (the scales are very dehiscent, as also noted by Roberts (1971b) and others), or other factors.

The cephalic portion of the lateral line was present, with components corresponding to the supra-, pre- and infra-orbital, together with preopercular-mandibular, systems of other teleosts. There was some evidence for an assymmetry of the preopercular series in male *P. smithi* (e.g. Fig. 2), but this requires further investigation.

Histological examination of the characteristic dome-shaped forehead of *P. smithi* confirmed TeWinkel's (1934) and Roberts, (1971b) observations that this encloses a fluid-filled space. A membranous partition along the midline divides the cavity bilaterally. Anteriorly, the floor of the cavities is thin, overlying another fluid-filled space (Fig. 7B); at more posterior levels, the membrane is apposed to the frontal bones of the skull (Figs. 11A, B).

Fig. 9. Ventral surface of a female *P. smithi*, to show the cluster of neuromasts (arrowed in inset) rostrolateral to the opening of the oviduct (O). X200 (inset X1000).

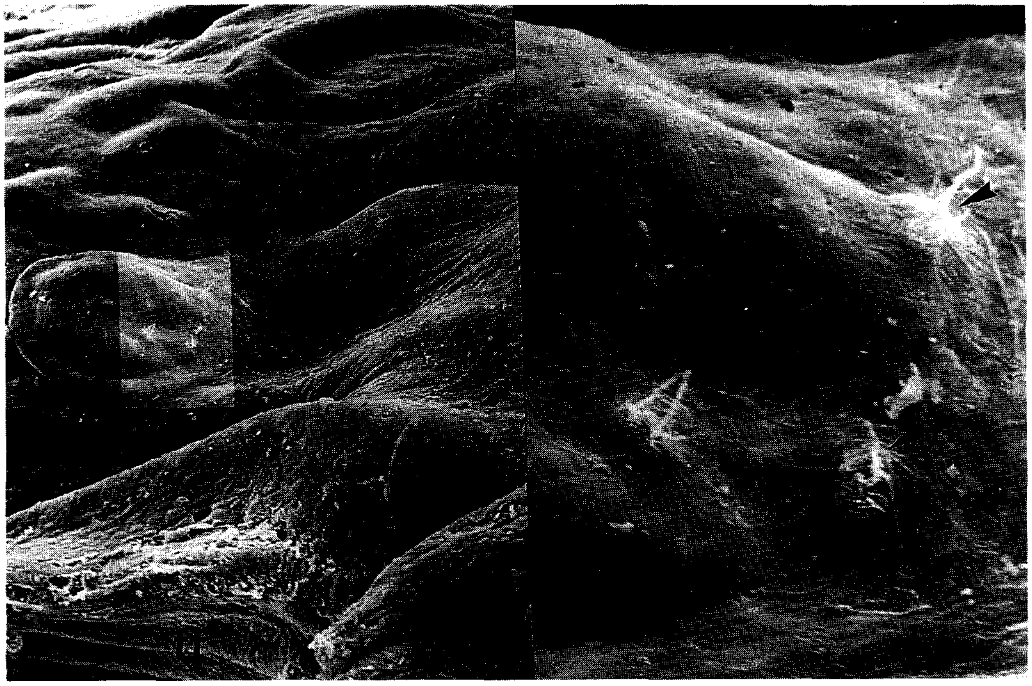

Fig. 10. Scale on the ventrolateral surface, just behind the pectoral girdle of the same female as in Fig. 9, showing a group of neuromasts (arrowed in inset); ff, ventral fin-fold; P, pelvic fins. X150 (inset X750).

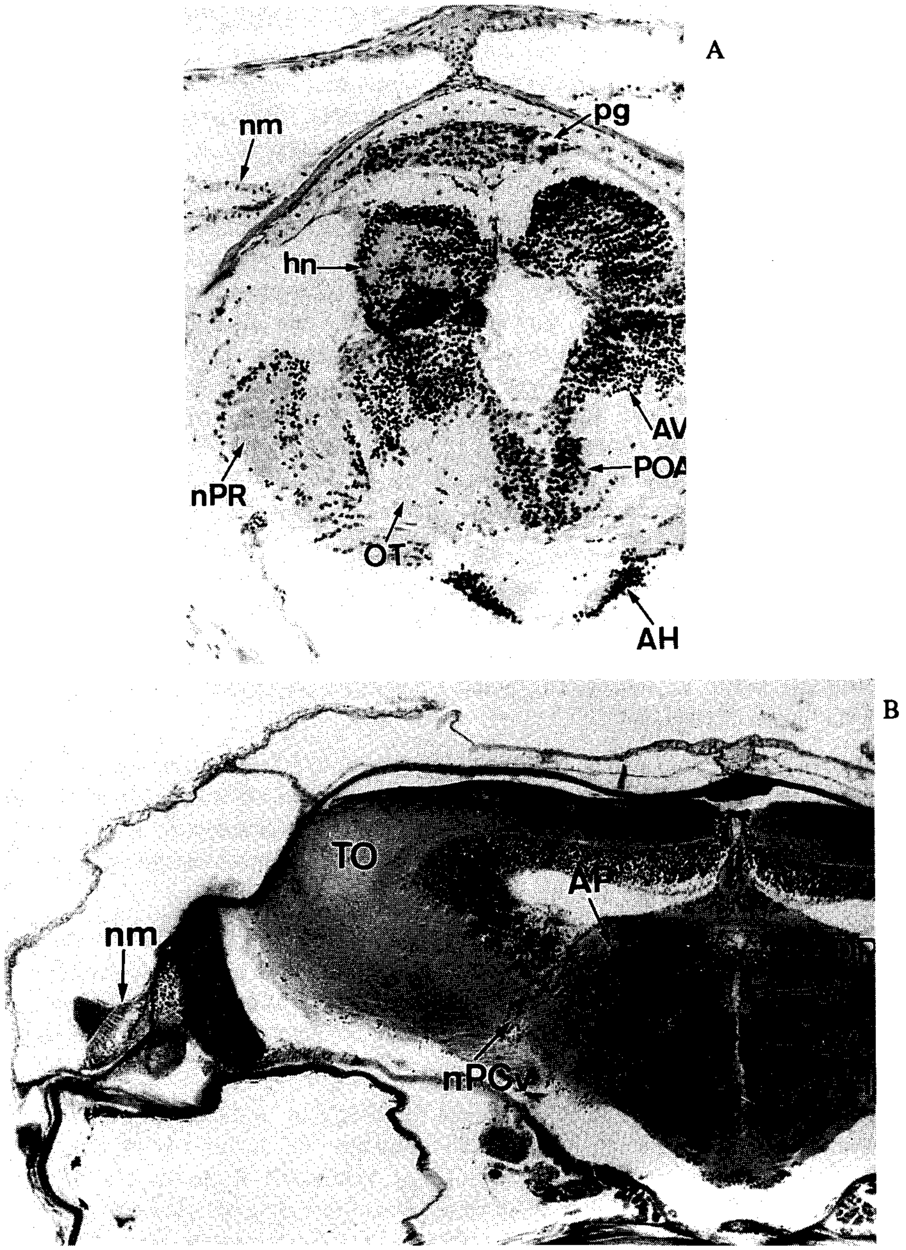

Fig. 11. A: Transverse section through the rostral diencephalon of a mature male *P. smithi*, to show the well-developed pineal gland (pg), habenular nuclei (hn) and rostral pretectal nucleus (nPR); also visible are the cells of a neuromast (nm) in the chamber above. AH, anterior hypothalamus; AV, anteroventral thalamus; OT, optic tract (indistinct); POA, preoptic area (caudal portions). Haemalum, X140. B: Transverse section through the optic tectum (TO) and caudal diencephalon of a mature male *P. smithi*; note the regional variations in both of the primary tectal layers. The caudoventral pretectal nucleus (nPCv) is a well-developed group of large cells. Note also the neuromast (nm) in the lateral chamber. AH, anterior hypothalamus; AP, *area pretectalis*; AV, antero-ventral thalamus; DP, dorso-posterior thalamus; VP, ventro-posterior thalamus. Azan, X140.

Each of these chambers is further subdivided by one complete partition into two subchambers. The first of these is a rostromedial one, extending from the level of the nostril back to the level of the anterior orbital area. One elongate neuromast is present in the posterior portion of this chamber; horizontal sections indicate that the cupula of this neuromast is oriented rostromedio-caudolaterally, at an angle of about 30° to the transverse axis.

The second chamber extends the length of the head, from the level of the nostril to the posterior margin of the optic tectum; this chamber moves medially to displace the rostromedial chamber above the eye, expanding laterad over the dorsolateral surface of the head behind the eye. Behind the middle of the eye, two short rostrocaudal partitions are present, creating rostrolateral and caudolateral diverticula. As a whole, this chamber contains six neuromasts: one anteriorly (Fig. 7B; perpendicular to the longitudinal axis of the fish, and in parallel with that in the rostromedial chamber); a series of three close to the midline from the level of the middle of the eye back to the rear margin of the optic tectum, with their cupulae at different orientations to the sagittal plane of the fish; and a pair of more lateral neuromasts, one behind the other (respectively in front of and within the caudolateral diverticulum - Fig. 11B).

No patent pores could be observed, either histologically or with the SEM, connecting either of the chambers to the exterior: the pores of the pre-orbital canal (Fig. 7A) are clearly not connected with this supra-cephalic system. There is no clear histological evidence that, in life, the neuromast cupulae are in direct physical contact with the overlying epithelial membrane.

General observations on the structure of the brain. - The olfactory bulbs are sessile, being relatively small (Fig. 12) with an olfactory tract running forward from the lateral surface of each towards the olfactory epithelium. The last, undetected by Roberts (1971b), comprises a single lamella (Fig. 7B) in the base of a pit communicating with the exterior by a single small naris (Figs. 7A, B).

The telencephalon is relatively small and poorly developed, particularly in the case of the area dorsalis: in the main, neurones are restricted to a thin periventricular band, with relatively few migrated neurones compared to other teleosts (Figs. 12, 13), although this is not as marked as in *C. bicornis* (unpublished data). By contrast, the anterior commissure is relatively large.

No attempt is made here to subdivide the preoptic area, at the transition between the telencephalon and the hypothalamus, into its component nuclei: this requires a more thorough analysis (Munro & Mok, in prep.). The main point of interest is that there is a pronounced sexual dimorphism in the development of this area, with an unusual hypertrophy of rostroventral portions in males (Fig. 13). This hypertrophy is, furthermore, assymmetrical: neurones of the ventral preoptic area extend ventrally down the optic chiasma on the right side of the brain irrespective of the fish's proctal handedness (Fig. 13B). The significance of this phenomenon is not clear: however, anterior and lateral portions of the preoptic area have been implicated in the control of reproductive behaviour in teleosts, as in other vertebrates (Munro & Pitcher, 1983; Koyama *et al.*, 1984; Satou *et al.*, 1984). Whilst sexual dimorphism in the development of this area has been documented for various mammals (e.g. Bleier *et al.*, 1982), the present report would appear to be the first for a fish; it would also appear to be the first of a clear bilateral assymmetry of this area in any vertebrate.

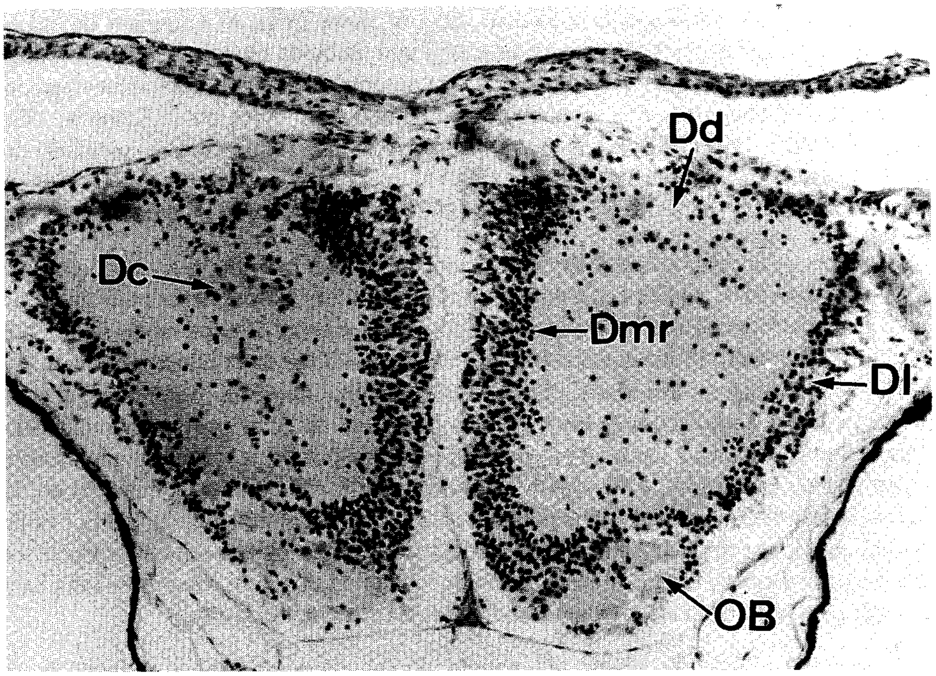

Fig. 12. Transverse section of the rostral telencephalon, to show the olfactory bulbs (OB); above are the central (Dc), dorsal (Dd), lateral (Dl) and rostromedial (Dmr) components of the *area dorsalis telencephali* (Munro & Dodd, 1983). Haemalum; X280.

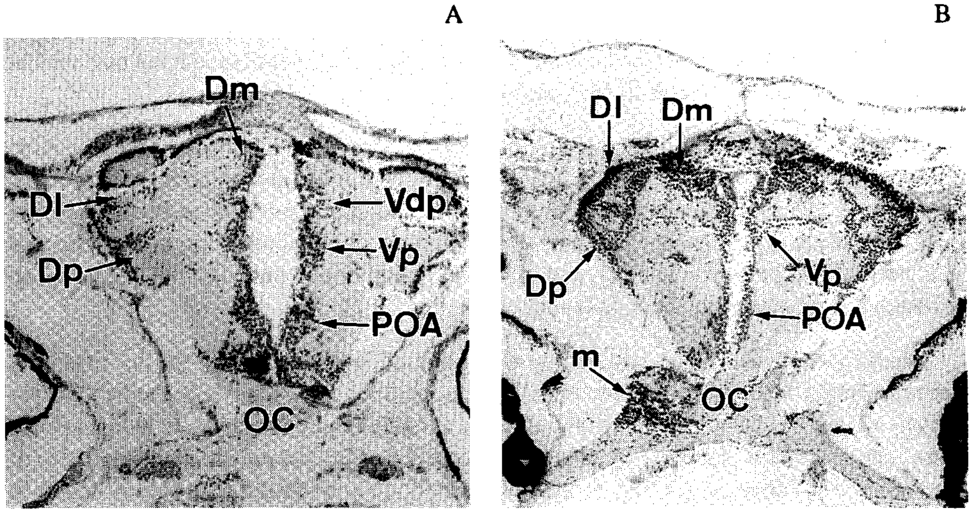

Fig.13. Transverse sections of the forebrain of (A) a mature female and (B) a mature male, at the level of the optic chiasma (OC). Note the presence of a distinct migrated mass (m) of neurones and neuropile from the preoptic area (POA) in the male. Above (Munro & Dodd, 1983) are the lateral (Dl), medial (Dm) and posterior (Dp) components of the *area dorsalis telencephali*; and the postero-dorsal (Vdp) and post-commissural (Vp) components of the *area ventralis telencephali*. Haemalum; X140.

The *nucleus glomerulosus* is a circular shell of nerve cells, of uncertain functional significance, which appears to originate as a migrated thalamic group of neurones (Munro & Dodd, 1983). On the basis of its characteristic appearance, it would seem to be restricted to acanthopterygians, including atherinomorphs (Schnitzlein, 1962; Ito & Kishada, 1975; Munro & Dodd, 1983). A typical (*sensu* Munro & Dodd, 1983) *nucleus glomerulosus* would appear to be present in *P. smithi* (Fig. 14; glomerular areas are visible in Azan preparations), belonging to Ito and Kishada's (1975) 'incompletely-laminated' type.

The optic tectum is well-developed; it is unusual in that both the periventricular grey (*stratum periventriculare*) and the more superficial, mainly fibrous layers are thicker at rostral and ventrolateral levels, becoming progressively thinner dorsomedially (Figs. 11B, 14). Given the pattern of retinotectal projections described for other teleosts (Vanegas & Ito, 1983), this suggests that visual inputs from the ventral quadrant of the retina (i.e. the dorsal visual field) are relatively the least important in terms of sensory processing. Such a conclusion is somewhat surprising, given the evidence that these fish are predominantly surface-feeders (see below). One possibility is that the pineal supplants the bilateral retinae as a (low-resolution 'shadow') dorsal photodetector of predators and, possibly, prey (Munro & Dodd, 1983; see below). An additional (or alternative) sensory input is from the supracephalic neuromast system (see below), the development of whose chambers may be at the expense of the dorsal optic tectum, in order to preserve streamlining.

As in other acanthopterygians, the magnocellular and parvocellular pretectal nuclei (*sensu* Braford & Northcutt, 1983: both are important targets for retinal projections) are conspicuous (Figs. 11A, B). This, together with the above observations on the optic tectum, again serve to emphasise the importance of vision. This contrasts with the congeneric *P. trewavasae*, where the minute lens must greatly limit visual acuity (Parenti, 1986).

The pineal is well-developed, as is the habenular nucleus, its principal efferent target (Fig. 11A). In contrast to *Ceratostethus bicornis* (unpublished data), the pineal end vesicle does not extend outside the skull in *P. smithi.*

All lobes of the cerebellum are well-developed, consistent with this being an active fish requiring high manoeuvrability. Considering the extreme sexual dimorphism in the structure of the secondary sexual characteristics (and the associated muscular systems involved), there would appear to be no evidence for a clear dimorphism in the structure and size of the potential sensory and motor systems in the medulla and spinal cord, although a more complete analysis is required.

Gonad structure. - The structure of the unpaired testis is similar to the restricted spermatogonial testis-type of Grier (1981). Thus, spermatogonia are restricted to the periphery of the ventro-posterior testis, with successive stages occuring in progressively more anterodorsal zones (Fig. 15). This type of arrangement is characteristic of atherinomorph fishes, in contrast to the unrestricted spermatogonial distribution found in other teleosts, where the different stages of spermatogenesis are scattered throughout the testis (Grier, 1981).

The ovary shows a continuous range in the distribution of oocyte diameters and stages of growth. This is consistent with females being small batch spawners, laying small numbers of eggs at frequent intervals (de Vlaming, 1983). Ovarian histology further indicates that, as in *Gullaphallus mirabilis* (fide Villadolid & Manacop, 1934) and

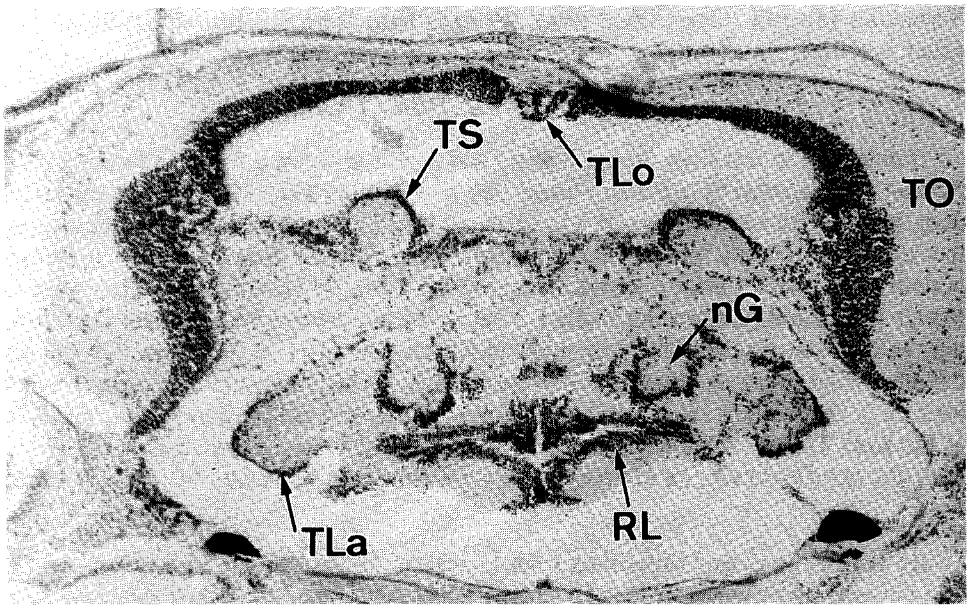

Fig. 14. Transverse section of the mesencephalon and caudo- ventral diencephalon of a mature male *P. smithi*. Note the presence of a typical *nucleus glomerulosus* (nG); and the regional variations in the thickness of the major layers of the optic tectum (TO). RL, lateral hypothalamic recess; TLa, hypothalamic torus lateralis; TLo, torus longitudinalis; TS, torus semicircularis of the midbrain. Haemalum, X140.

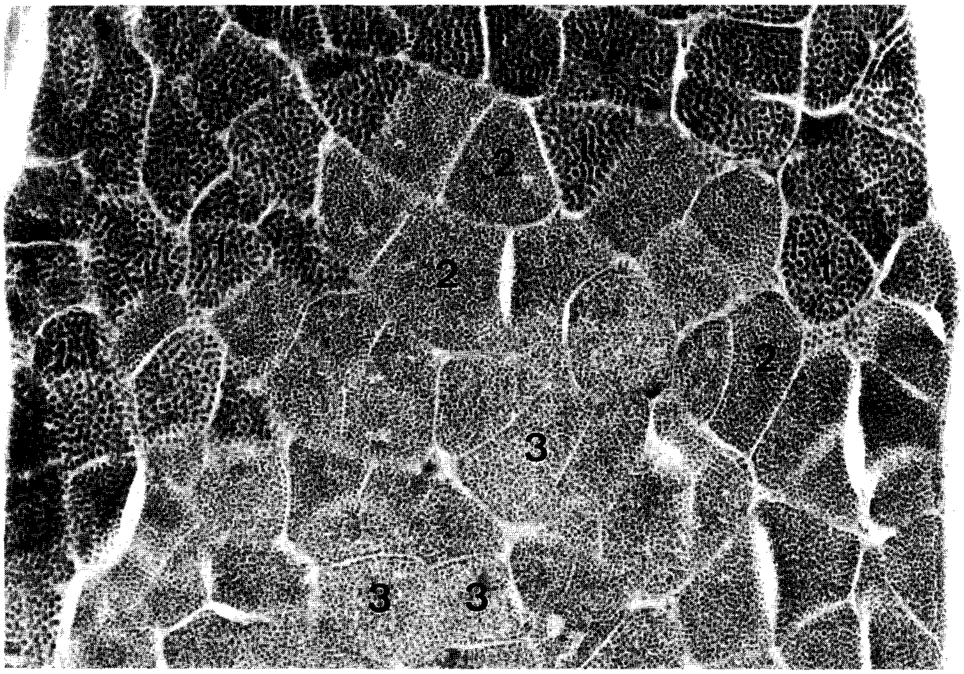

Fig. 15. Horizontal section of the testis of a mature male *P. smithi* (posterior to the right). Note the discrete spatial localisation of the cysts of secondary spermatogonia (1), primary (2) and secondary (3) spermatocytes. Azan, X280.

Neostethus bicornis (unpublished data), the eggs have large numbers of fibrils on the external surface.

ECOLOGICAL OBSERVATIONS

Habitat. - The fish from Sungei Sayong and nearby sites were found in turbid, slow-flowing rivers shaded by secondary forest. The river at Kota Tinggi 24 km was relatively deep, and the fish could be seen in a loose shoal; they were in the upper layers, but not at the surface, and mostly far from the bank. Comparing the catch data for the two sampling periods (by hand net from the bank, February 21, 1990; by seine net in deeper waters, March 29) suggests that 'mature' males (i.e. including those where the papilla is unruffled) may be found nearer the banks than other individuals. The two sites on Sungei Sayong, on the other hand, were shallower; the fish were caught by hand in backwater areas (behind logs, etc.), although the catch pattern again suggests that they occurred in groups. The fish at the Kota Tinggi 30 km-site were caught in grassy shallows at the edge of the river. Thus the species occupies a diversity of habitats, characterised mainly by sluggish water flow.

By contrast, the single specimen from Sungei 'Kulai' was caught in a stream which was typically clear, relatively fast-flowing with few shallows or other sheltered areas, and not shaded by trees.

Population structure. - The male:female sex ratio for 'mature' fish was 18:15 for the Kota Tinggi 24 km population, 47:20 for Sungei Sayong, and 9:8 for the Kota Tinggi 30 km sample. The ratio of left:right-sided males (i.e. the handedness of the aproctal side - Roberts, 1971b) was 8:10 for the Kota Tinggi 24 km population; 21:8 for the upstream location on Sungei Sayong; 2:2 for the downstream location; and 4:5 for Kota Tinggi 30 km. About the only difference between the upstream location and the other three sites is that the former is on a sharp left-hand river bend, whereas the others are relatively straight sections. It is at least tempting to speculate that, under such circumstances, left-sided males might be at a hydrodynamic advantage when maintaining station or swimming upstream.

Overall, the fish from Sungei Sayong were larger than those from the Kota Tinggi 24 km population (modal lengths 15 mm vs. 11 mm), although the maximum sizes - 17 mm for both sexes - were the same. In all populations, sexual 'maturity' was attained at 14 mm for both males (on the basis of having completed development of the toxactinium; but see above) and females (based on the presence of a few large eggs in the ovary). There was little evidence for any sexual dimorphism in adult size, in contrast to the (larger) neostethids from Singapore mangroves (*Ceratostethus bicornis* and *Neostethus lankesteri*), where the males are larger (unpublished data).

It would seem strange that only a small proportion of males were fully mature, in the sense of having not only active spermatogenesis (with spermiation) but also a rufous terminal portion to the papilla (< 10 to 33% of all 'mature' males, depending on sample). Their relative scarcity may reflect habitat shifts with the attainment of full maturity; or increased mortality consequent upon maturity; or it may reflect that different individuals may use different reproductive strategies to achieve mating (differences in gonopodial morphology at the inter-specific level have been associated with the practice of different reproductive strategies in poeciliids: Constantz, 1989).

The absence of distinct size-classes (albeit over a limited sampling period) might suggest year-round breeding, to be expected in a fish of such a small adult size (Munro, 1990). Likewise, there is evidence for year-round spawning in a Philippine freshwater neostethid, although there was a suggestion of seasonal fluctuations in the proportion of mature females (Villadolid & Manacop, 1934: however their data apparently do not take into account the impact of any inter-month variations in the male:female sex ratio in calculating the proportion of fish with mature ovaries).

Diet. - An analysis of the gut contents of seven fish from Sungei Sayong and 14 fish from Kota Tinggi 24 km indicated that the food items taken at both locations consisted predominantly of the larvae and, especially imagines of allochthonous insects (various dipterans, homopterans and thrips); such items were found in the guts of all individuals examined. Another component at both locations was mites (three of the fish from Kota Tinggi 24 km; one of the fish from Sungei Sayong). In addition, the sample from Kota Tinggi 24 km also fed on cladocerans (six fish) and rotifers (five fish). Similarly, Villadolid and Manacop (1934) reported that the freshwater Philippine neostethid *Gulaphallus mirabilis* fed largely on animal matter, particularly as adults: the main items were insect larvae and imagines, with crustaceans, plant matter and other items as minor components.

GENERAL CONCLUSIONS

The present specimens appear to be identical with Thai specimens of *P. smithi* on the basis of the apparent identity of the arrangement of the priapial supportive skeleton and the external appearance of the genital papilla in some of the mature males. This suggests that they are the same species. Meristic data (Table 1) are, for the most part, consistent with this.

The structure of the testis, and the presence of distinct magnocellular pretectal nuclei and a *nucleus glomerulosus*, are consistent with phallostethoids being atherinomorphs.

P. smithi would appear to be an opportunistic carnivore, with a well-developed visual system. As in other surface-feeders (Bleckmann *et al.*, 1989), the cephalic system of neuromasts appears to be specialised for the detection of surface waves; however, as in other aspects of its biology, the unusual structural organisation of this neuromast system appears to provide yet another example of the range of unique characteristics shown by phallostethoids in general.

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