

A Histologic Survey of Diencephalic Circumventricular Organs in Teleosts with Special Reference to Osteoglossomorphs

Kazuhiko Tsuneki

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Abstract Diencephalic circumventricular organs of various teleosts were studied histologically. Special attention was paid to osteoglossomorphs. The neurohypophysis of osteoglossomorphs (*Arapaima*, *Notopterus*, *Xenomystus*, and *Gymnarchus*) is well differentiated into the median eminence and the neural lobe. The pituitary organization of these species is an intermediate between that of holosteans and of more advanced teleosts. The saccus vasculosus is absent in *Pantodon* and *Gymnarchus*, but it is well developed in *Notopterus* and *Xenomystus*. The light microscopically discernible pineal is absent in *Gymnarchus*: this may be the only species that lacks the pineal among teleosts. The paraphysis is found in various species including most osteoglossomorphs and some perciforms. In advanced teleosts such as gobiids and tetraodontids, the saccus dorsalis and velum transversum are absent, but the diencephalic choroid plexus is well developed instead. Some evolutionary trends are apparent in the occurrence and organization of these circumventricular organs among teleosts.

Specialized structures appearing on the ventricular surface of the brain are together called circumventricular organs (Leonhardt, 1980). Although a few circumventricular organs occur outside the diencephalon, most organs exist in the diencephalon, either in the hypothalamus or epithalamus. The median eminence, neural lobe (pars nervosa), saccus vasculosus, and pineal may be well-known examples.

In a previous paper (Tsuneki, 1986), I surveyed the occurrence of about seventeen circumventricular organs in entire vertebrate classes and discussed the phylogeny of these organs. In that paper, I studied various groups of vertebrates, but my main attention was paid to chondrichthyans, non-teleostean osteichthyans, and amphibians. Among teleosts, only eight species were included: *Osteoglossum bicirrhosum* (Osteoglossidae), *Gnathonemus petersi* (Mormyridae), *Anguilla japonica* (Anguillidae), *Sardinella zunasi* (Clupeidae), *Hypomesus nipponensis* (Osmeridae), *Silurus asotus* (Siluridae), *Poecilia reticulata* (Poeciliidae), and *Odontobutis obscura* (Gobiidae). The number of species might be too small to make generalizations about teleosts. Even in this restricted array of species, however, some evolutionary trends on the occurrence and organization of circumventricular organs in teleosts were apparent (Tsuneki, 1986). (1) Species belonging to the Osteoglossomorpha (*Osteoglossum* and

Gnathonemus) possess a distinct median eminence that is clearly distinguishable from the neural lobe. (2) In osteoglossomorphs, the saccus vasculosus is either absent (*Osteoglossum*) or is very small (*Gnathonemus*). (3) The paraphysis occurs in species belonging to relatively primitive groups (*Osteoglossum*, *Gnathonemus*, and *Anguilla*). (4) The saccus dorsalis and velum transversum are developed in species belonging to relatively primitive groups, but these structures are absent in species belonging to relatively advanced groups (*Silurus*, *Poecilia*, and *Odontobutis*). In the latter groups, the diencephalic choroid plexus is developed instead.

The present study is an attempt to verify these evolutionary trends of circumventricular organs in teleosts by studying a larger number of species belonging to various phylogenetic groups. Special attention is placed on osteoglossomorphs, because this group is situated in a phylogenetically important place at the base of teleost phylogeny (Nelson, 1968; Greenwood, 1973) but its circumventricular organs have been scarcely described in the literature.

Materials and methods

The following osteoglossomorphs were obtained from commercial sources. Total length of fish studied is indicated in parentheses. Osteoglossiformes: pirarucu, *Arapaima gigas* (40 cm)

(Osteoglossidae), clown knifefish, *Notopterus chitala* (10 and 11 cm) (Notopteridae), African knifefish, *Xenomystus nigri* (13 and 16 cm) (Notopteridae), and freshwater butterfly fish, *Pantodon buchholzi* (6 and 7 cm) (Pantodontidae); Mormyriiformes: aba aba, *Gymnarchus niloticus* (18, 22, 24, 31, and 54 cm) (Gymnarchidae).

All non-osteoglossomorph teleosts were captured from their natural habitats in Shimane or Tottori Prefecture in Japan. Total length of fish studied is indicated in parentheses. In the following species, the hypothalamus was studied: Cypriniformes: *Pungtungia herzi* (7 and 8 cm) (Cyprinidae), *Zacco temmincki* (9 and 10 cm) (Cyprinidae) and *Cobitis biwae* (8, 9, and 10 cm) (Cobitidae); Perciformes: *Sillago japonica* (20 cm) (Sillaginidae), *Leiognathus nuchalis* (10 and 11 cm) (Leiognathidae), *Pagrus major* (8 and 9 cm) (Sparidae), *Girella punctata* (7 and 8 cm) (Girellidae), *Pseudolabrus japonicus* (14 cm) (Labridae), *Parapercis* (= *Neopercis*) *sexfasciata* (17 cm) (Mugiloididae), *Pictiblennius* (= *Blennius*) *yatabei* (8 cm) (Blenniidae), *Dictyosoma burgeri* (21 and 28 cm) (Stichaeidae), *Repomucenus* (= *Callionymus*) *richardsoni* (12 cm) (Callionymidae), *Acanthogobius flavimanus* (14 cm) (Gobiidae), and *Leucopsarion petersi* (4.6 and 4.8 cm) (Gobiidae); Scorpaeniformes: *Sebastes inermis* (10 cm) (Scorpaenidae) and *Hypodytes rubripinnis* (7 cm) (Congiopodidae), Pleuronectiformes: *Paralichthys olivaceus* (29 cm) (Paralichthyidae), Tetraodontiformes: *Thamnaconus* (= *Navodon*) *modestus* (25 cm) (Monacanthidae) and *Takifugu* (= *Fugu*) *niphobles* (7 and 8 cm) (Tetraodontidae). In the following species, the epithalamus was also included in the study; *Pungtungia*, *Zacco*, *Cobitis*, *Leiognathus*, *Pagrus*, *Girella*, *Pseudolabrus*, *Parapercis*, *Repomucenus*, *Acanthogobius*, *Leucopsarion*, and *Takifugu*. The epithalamus of the other non-osteoglossomorphs was partly damaged and thus was not examined. For convenience, here I use only generic names for the species I studied, as if they represented species.

All fishes were sacrificed without any experimental manipulation. The brain with or without surrounding tissues was fixed in Bouin's solution and embedded in paraffin. In osteoglossomorphs except for *Arapaima*, serial sagittal and serial transverse sections (7 μ m thickness) of the brains were prepared. In *Arapaima* and all non-osteoglossomorphs, serial sagittal sections (7 μ m

thickness) of the brains were made. The sections were stained with Masson-Goldner's method with pre-staining with paraldehyde fuchsin (AF).

Results and discussion

The neurohypophysis (median eminence and neural lobe). In the organization of the neurohypophysis, three types could be distinguished among the species studied. The caudal part of the neurohypophysis is consistently stained with AF.

Type I. The caudal part of the neurohypophysis penetrates into the pars intermedia, while the rostral part of the neurohypophysis does not penetrate into the pars distalis but is simply apposed to the pars distalis. The ventral region of the rostral neurohypophysis is not stained with AF and its ventral surface is abundantly supplied with blood vessels. From topological relations to the adenohypophysis and AF stainability, the caudal neurohypophysis may correspond to the neural lobe while the rostral neurohypophysis may correspond to the median eminence. Type I is represented by *Arapaima* (Figs. 1, 2), *Notopterus* (Figs. 3-5), and *Xenomystus*. The neurohypophysis of *Gymnarchus* may also belong to this type, although the rostral neurohypophysis slightly penetrates into the pars distalis (Fig. 6). In *Gymnarchus*, the ventral surface of the hypothalamic floor situated rostrally to the pituitary gland is also supplied with abundant blood vessels. This region may represent the rostral extension of the median eminence and may correspond to the peculiar median eminence described in some silurids by Sathyanesan (1972). Among the species examined in the previous paper, *Osteoglossum* and *Gnathonemus* belong to type I (Tsuneki, 1986). This type is never represented by non-osteoglossomorphs.

Type II. The entire neurohypophysis penetrates into the adenohypophysis, but the rostral neurohypophysis is scarcely or only weakly stained with AF. The neurohypophyses of *Pantodon* and most non-osteoglossomorphs studied belong to type II (Figs. 7, 8).

Type III. The entire neurohypophysis penetrates into the adenohypophysis and is densely stained with AF except for the ventral thin layer of the rostral neurohypophysis that contacts the pars distalis. This type is represented only by *Repomucenus* (Fig. 9), *Thamnaconus*, and *Takifugu*.

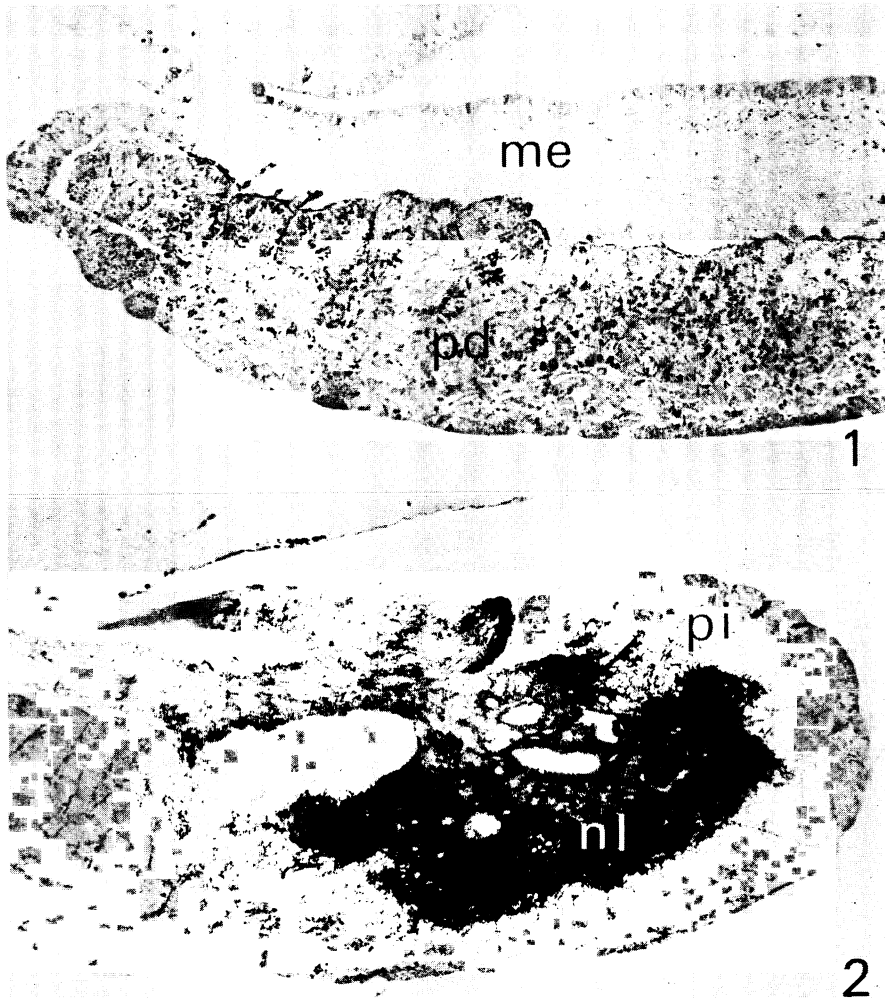


Fig. 1. A sagittal section of the rostral part of the pituitary of *Arapaima gigas*. me, median eminence; pd, pars distalis. $\times 85$.

Fig. 2. A sagittal section of the caudal part of the pituitary of *Arapaima gigas*. Note numerous fuchsinophilic beaded fibers in the pars intermedia (pi). nl, neural lobe. $\times 85$.

Detailed accounts of the pituitary organization of tetraodontids are found in Chiba (1984).

Type I is represented exclusively by osteoglossomorphs, one of the most primitive groups of teleosts, while type III is represented by highly advanced teleosts. Therefore, type I may represent the primitive condition and type III may represent the advanced condition of the teleost neurohypophysis (Fig. 10). Type I is actually an intermediate condition between holosteans and non-osteoglossomorph teleosts. If in *Lepisosteus* the connective tissue between the median eminence and the pars distalis diminishes in amount and

the highly vascularized ventral surface of the median eminence slightly undulates and contacts the pars distalis, then type I in osteoglossomorphs is easily induced. In any event, trend (1) (see Introduction) appears to be verified: the neurohypophysis of most osteoglossomorphs is clearly divided into the median eminence and the neural lobe, but the neurohypophysis of non-osteoglossomorphs cannot be clearly separable into the median eminence and the neural lobe.

In some non-osteoglossomorphs (*Sillago*, *Pagrus*, *Girella*, *Sebastes*, and *Paralichthys*), the ventral thin layer of the caudal neurohypophysis

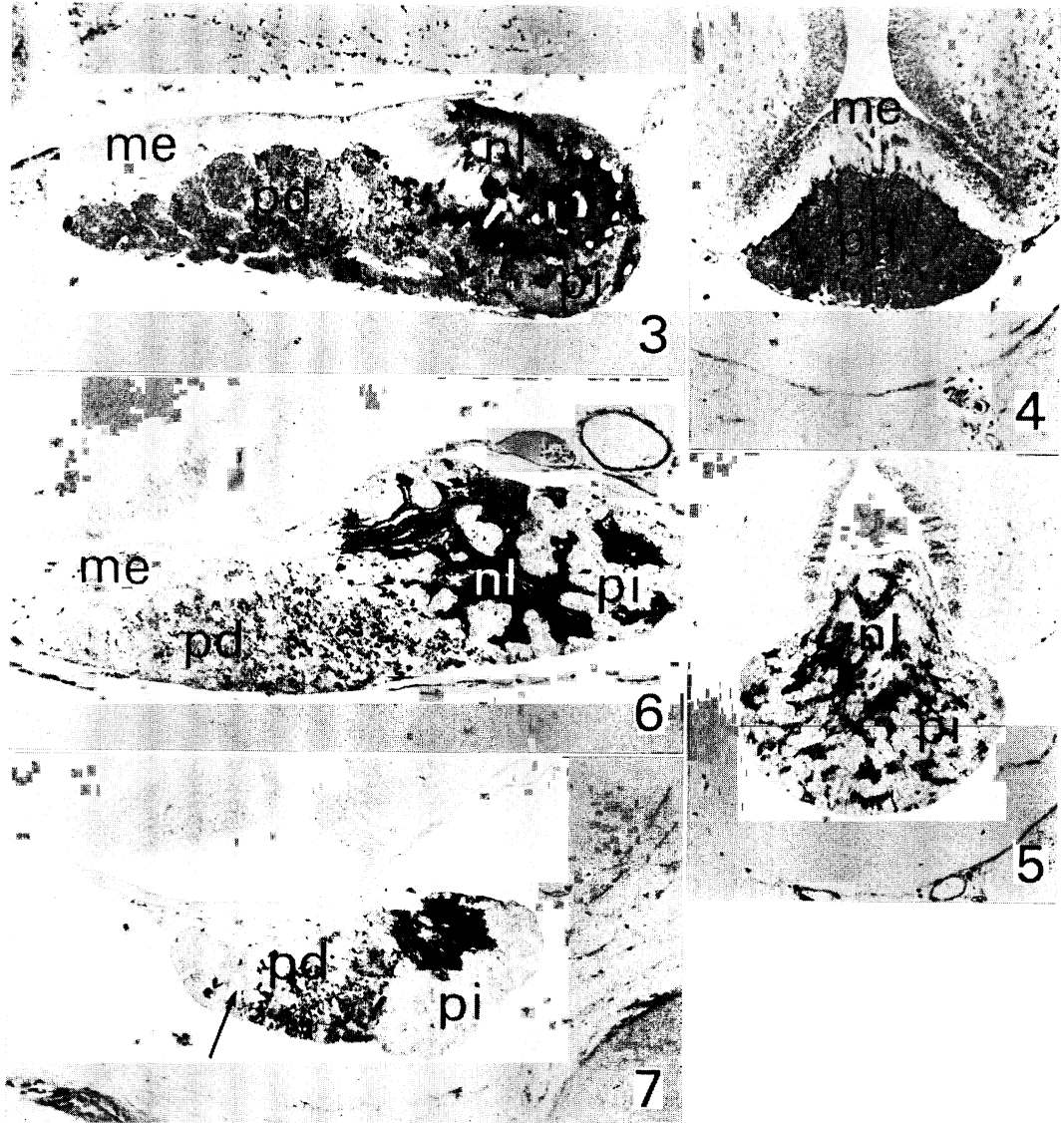


Fig. 3. A sagittal section of the pituitary of *Notopterus chitala*. me, median eminence; nl, neural lobe; pd, pars distalis; pi, pars intermedia. $\times 95$.

Fig. 4. A transverse section of the rostral part of the pituitary of *Notopterus chitala*. me, median eminence; pd, pars distalis. $\times 95$.

Fig. 5. A transverse section of the caudal part of the pituitary of *Notopterus chitala*. nl, neural lobe; pi, pars intermedia. $\times 95$.

Fig. 6. A sagittal section of the pituitary of *Gymnarchus niloticus*. me, median eminence; nl, neural lobe; pd, pars distalis; pi, pars intermedia. $\times 70$.

Fig. 7. A sagittal section of the pituitary of *Pantodon buchholzi*. The rostral neurohypophysis partly penetrates (arrow) into the pars distalis (pd). pi, pars intermedia. $\times 100$.

that contacts the pars intermedia is not stained with AF and is rather chromophobic (Fig. 11). In these species, the pars intermedia does not appear to be invaded by AF-positive nerve fibers.

It may be interesting to study the immunohistochemical distribution of biologically active substances in this thin layer of the caudal neurohypophysis in these species.

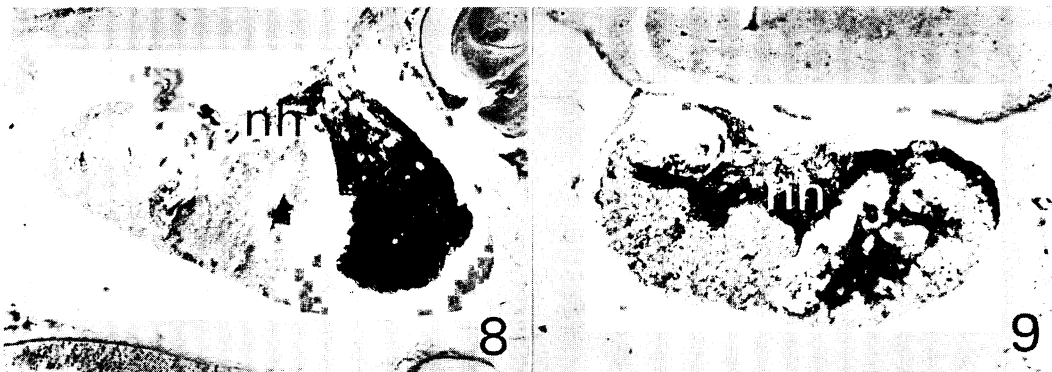


Fig. 8. A sagittal section of the pituitary of *Girella punctata*. The neurohypophysis (nh) consists of the poorly fuchsinophilic rostral part and the intensely fuchsinophilic caudal part. $\times 100$.

Fig. 9. A sagittal section of the pituitary of *Reponucenus richardsoni*. The neurohypophysis (nh) is densely fuchsinophilic as a whole. $\times 100$.

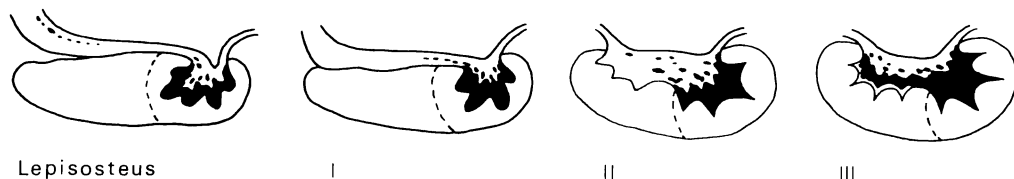


Fig. 10. Types of the neurohypophysis. *Lepisosteus* type was figured according to the author's own preparation. The solid area represents fuchsinophilic regions in the neurohypophysis. A dotted line marks the boundary between pars distalis and pars intermedia. The types on the left may represent primitive conditions and the types on the right may represent advanced conditions.

The pituitary organizations of many Japanese teleosts were diagrammatically illustrated by Kawamoto (1967). This is a good reference source concerning the pituitary organization of non-osteoglossomorph teleosts.

The saccus vasculosus. Among the examined osteoglossomorphs, *Pantodon* and *Gymnarchus* do not possess the saccus vasculosus. In *Arapaima*, there is a small ependymal tube in the posteroventral corner of the third ventricle. Although the tube was partly damaged in my preparation, the ependymal cells of the intact region of the tube are not provided with conspicuous protrusions ("crowns" or "coronets"). In all probability, the saccus vasculosus is absent in *Arapaima*. On the contrary, *Notopterus* and *Xenomystus* possess a large saccus vasculosus (Fig. 12). It seems peculiar that such a great diversity in the development of the saccus vasculosus is encountered in one order (Osteoglossiformes). There are many freshwater teleosts that do not possess the saccus vasculosus or possess only a small saccus vasculosus, while many seawater teleosts possess a

distinct saccus vasculosus (Dammerman, 1910; Dorn, 1955; Tsuneki, 1986; see also the following paragraph). All extant osteoglossomorphs are freshwater species. Therefore, the presence of the large saccus vasculosus in the Notopteridae is somewhat puzzling. The possibility of the rather recent seawater origin of this family could not be excluded. In *Osteoglossum*, the saccus vasculosus is absent and in *Gnathonemus* the saccus vasculosus is a small tube (Tsuneki, 1986). Trend (2) (see Introduction; absence or reduction of the saccus vasculosus in osteoglossomorphs) may be verified in the Osteoglossidae, Pantodontidae, and Gymnarchidae, but does not hold true in the Notopteridae.

Among the studied Cypriniformes, the saccus vasculosus is either small (*Pungtungia* and *Zacco*) or scarcely discernible (*Cobitis*). All other teleosts investigated in this paper are marine or brackish species and their saccus vasculosus is moderately or very well developed. The list provided by Dorn (1955) is a good source concerning the presence or absence of the saccus

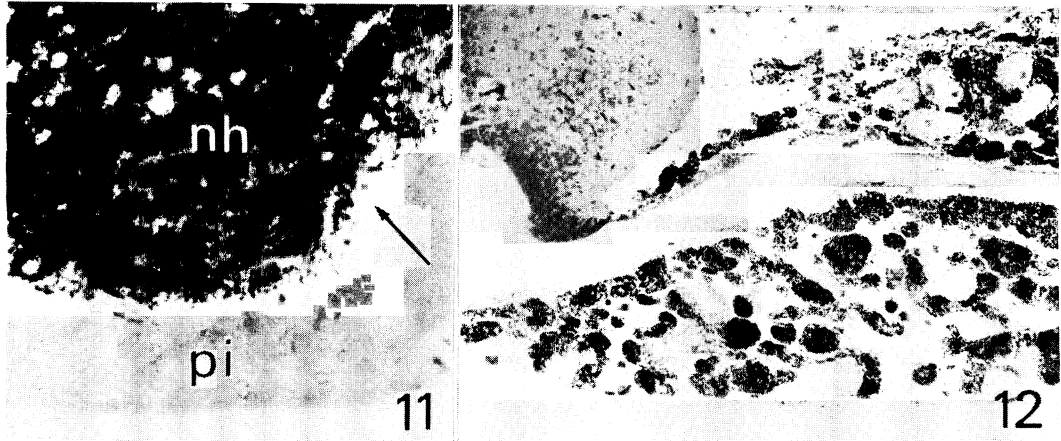


Fig. 11. The boundary between the neurohypophysis (nh) and the pars intermedia (pi) of *Girella punctata*. Note the AF-negative region (arrow) in the neurohypophysis. $\times 405$.

Fig. 12. A saggital section of the saccus vasculosus of *Notopterus chitala*. $\times 95$.

vasculosus in many non-osteoglossomorph teleosts.

It is possible that crown cells could be revealed by an electron microscopic study even in species in which the saccus vasculosus cannot be revealed by a light microscopic study. Even if this should happen, the above argument should be still valid because here we discuss primarily organs but not cells.

The pineal. The pineal is found in all species studied with the exception of *Gymnarchus*. The pineal of *Gnathonemus*, which belongs to the same order as *Gymnarchus* (Mormyriiformes), is tiny and consists of two parts which are not connected with each other at least as adults (Tsuneki, 1986). The pineal organization of *Gnathonemus* is summarized as follows. The proximal part (pineal stalk) is represented by a tiny dorsal evagination in front of the subcommissural organ. The distal part is a tiny end-vesicle that is partly surrounded by paraphyseal saccules and is located far rostrally. In *Gymnarchus*, there is no dorsal evagination in front of the subcommissural organ, except for the highly folded saccus dorsalis (Figs. 18, 19), and thus the proximal part of the pineal (pineal stalk) does not occur. In *Gymnarchus*, paraphyseal saccules are found far rostrally as in *Gnathonemus*, but the pineal end-vesicle as clearly identified as such cannot be recognized among paraphyseal saccules (Fig. 15). Advanced techniques to identify the pineal (electron microscopy, enzyme cytochemistry, opsin-immunocytochemistry etc.) were not carried out. All what I can say

is therefore that in *Gymnarchus* there is no light-microscopically discernible pineal. This is in high contrast to all other teleost species so far reported in the literature, including blind cave fish and deep-sea fish (Holmgren, 1959; Omura, 1975; McNulty, 1976). The pineal of all these teleosts can be identified easily with routine light microscopic histology. The purported absence of the pineal in the cyprinid teleost, *Labeo umbratus*, (Steyn and Webb, 1960) is somewhat doubtful and needs confirmation. The parapineal described by them may actually represent the pineal.

The brain organization of mormyrid teleosts is peculiar, especially in the large everted cerebellum (Nieuwenhuys, 1982). In *Gnathonemus*, the mesencephalon, diencephalon, and cerebral hemisphere are covered with the everted cerebellum. Therefore, the pineal development might be suppressed by the cerebellum and a tiny end-vesicle might extend far rostrally beyond the cerebellum in searching the sunlight, so to speak. However, this explanation is not applicable to *Gymnarchus*, because the cerebellum of this species does not cover the diencephalon and cerebral hemisphere although the cerebellum itself is large and partly everted.

The identification of the parapineal requires serial transverse sections of the brain and such sections were made only in four species belonging to the Osteoglossomorpha (*Pantodon*, *Notopterus*, *Xenomystus*, and *Gymnarchus*). However, the