

## Guanine-Type Retinal Tapetum of Three Species of Mormyrid Fishes

Hiroaki Somiya

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**Abstract** The eye of mormyrid fishes (*Marcusenius* and *Gnathonemus*) contains a retinal tapetum composed of guanine crystals. In *Marcusenius*, the quantity of guanine is about  $2 \text{ mg cm}^{-2}$  of the retinal surface area. The retina is duplex, and the cones and rods are grouped in bundles. Each bundle is surrounded by pigment epithelial cell processes which contain numerous guanine reflectors. Two kinds of reflector are present: brick-shaped and rodlet. Mormyrids may use their high sensitivity for nocturnal activities. The retinal features of mormyrid fishes were compared with those of other fish species belonging to the Notopteroidei such as the Hiodontidae, Notopteridae and Gymnarchidae, and related to the chemical nature of notopterid and gymnarchid tapetum.

Mormyrids are primitive osteoglossomorph fishes which have a unique electrosensory system for social communication and spatial orientation (Moller, 1976; 1980; Moller et al., 1982). Recent research on the visual behaviour of these fishes showed that they seem to be functionally "blind" under bright daylight (Defazio, 1979; Teyssedre and Moller, 1982). McEwan (1938) examined the retina of *Petrocephalus* and *Gnathonemus* histologically and found that the fishes have unusual retinal features for dim light vision, that is, a grouped receptor retina and a prominent retinal tapetum. She suggested that the reflecting tapetal materials may be guanine (McEwan, 1938).

Reflection by the tapetum depends upon its chemical nature and its structural organization. In fish, at least seven chemicals are found: guanine, uric acid, pteridine, lipid, astaxanthin and two kinds of melanoid substance (Nicol, 1981). In earlier literature, the tapetal material of walleye (*Stizostedion vitreum*) was believed to contain guanine. However, it has been shown that the walleye tapetum does not contain guanine but 7, 8-dihydroxanthopterin, a reduced pteridine (Zyznar and Ali, 1975). Furthermore, it has been found that the hiodontids (*Hiodon*), a family related to mormyrid fish, have the uric acid type retinal tapetum, although it was formally believed to contain guanine (Zyznar et al., 1978).

In this paper, I examined the tapetal reflector materials of three species of mormyrid fishes spectrophotometrically and paper-chromatographically, presenting definite evidence that they are mainly guanine. A brief description of the struc-

tural organization of the tapetal materials is also included. The retinal features of the mormyrid fishes are compared with those of other fish species belonging to Notopteroidei and related to the chemical nature of the tapetum.

### Materials and methods

One specimen of each of *Marcusenius isidori* (100 mm total length), *M. longianalis* (97 mm total length) and *Gnathonemus petersii* (102 mm total length) was obtained from a commercial supplier. The fishes were killed by decapitation. The *Marcusenius* species were used for spectrophotometric analysis of the tapetal materials, while *Gnathonemus* was used for paper-chromatographic analysis and electron microscopic examination of the tapetum.

**Spectrophotometry:** Extraction of the reflecting substances from the tapetum was carried out as follows. The cornea, lens and most of the vitreous body were removed from the eyeball. The reflector material remaining in the pigment epithelium was sucked directly into a hypodermic syringe. This material was then dissolved in 0.1 N NaOH. This solution was centrifuged and the supernatant was retained for analysis. To identify the reflecting substance, the ultraviolet (UV) absorption spectrum of the tapetal extract in 0.1 N HCl was measured in a Hitachi recording spectrophotometer and compared with those obtained of guanine and uric acid. Guanine content in the tapetum was estimated by the differential extinction technique (Bendich, 1957).

**Paper-chromatography:** The tapetal extract (in 0.1 N NaOH) of *Gnathonemus petersii* was run vertically on filter paper (Toyo Roshi No. 2) at room temperature. Guanine (in 0.1 N NaOH) and uric acid (in 0.1 N NaOH) were used as standards along with the tapetal sample. The solvent system consisted of a 4:1:1 v/v/v mixture of n-Butanol, acetic acid and water. The chromatogram was examined with light from a short wavelength UV lamp.

**Ultrastructure:** The retina was pre-fixed in 2.5% glutaraldehyde and post-fixed in 2% osmium tetroxide solution. After dehydration the retina was embedded in Quetol 812. Thin sections were made with a Soval Porter-Blum MT-I ultramicrotome and double stained with Uranyl acetate and lead nitrate solutions. The sections were examined with a JEM-100C EM.

## Results

### Biochemical identification of tapetal material.

**Spectrophotometry:** The UV spectra of the tapetal extracts from *Marcusenius longianalis* and *M. isidori* were measured and compared with those of guanine and uric acid in 0.1 N HCl. Figure 1 shows that the spectrum of the tapetal extracts of *Marcusenius* is the same as that of guanine. Using the differential extinction technique, the guanine content in the tapetum was calculated (Table 1). The tapeta of *M. longianalis* and *M. isidori* contained 0.37 and 0.34 mg of guanine, respectively. This corresponds to a guanine distribution of approximately  $2 \text{ mg cm}^{-2}$  of the retinal surface area ( $4\pi r^2/2$ ,  $r$ =radius of the eyeball).

**Paper-chromatography:** The tapetal extracts from *Gnathonemus petersii* moved at the same rate on paper as the guanine standards in this solvent system. The  $R_f$  values for the tapetal extract, guanine and uric acid were 0.57, 0.57 and 0.34, respectively.

### Eye shine and structure of the retinal tapetum.

Both *Gnathonemus* and *Marcusenius* display strong eye shine when photographed by photoflash light (Fig. 2). The histological structure of the retina of *Gnathonemus* is shown in Fig. 3. The main features of the retina are as follows. 1) Pigment epithelial cells are thick and their processes are well developed. 2) Photoreceptors are grouped in bundles. 3) The outer nuclear layer (rod

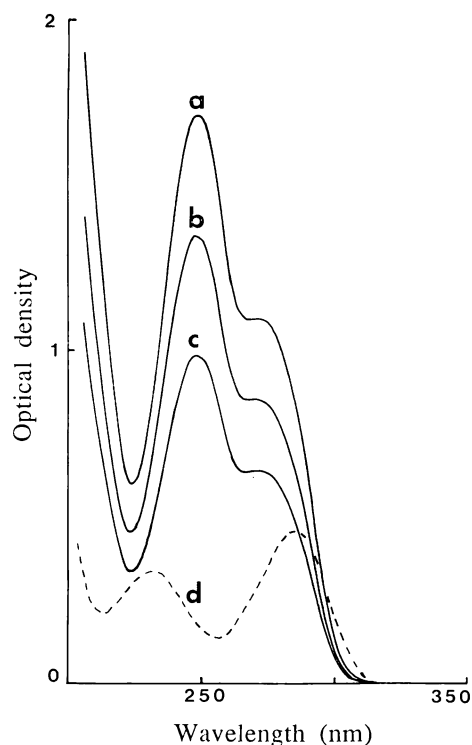


Fig. 1. Ultraviolet absorption spectra of the tapetal extracts of *Marcusenius longianalis* (a) and *M. isidori* (b), authentic guanine (c) and uric acid (d) in 0.1 N HCl.

nucleus) is rather thick. 4) The horizontal cell system is poorly developed.

The tapetum lies in the pigment epithelial cells which contain numerous guanine reflectors and few melanosomes (Fig. 4). An unstained section of a part of the cytoplasm of a pigment epithelial cell shows the numerous guanine crystals (Fig. 5). In a stained section, they easily disappear leaving clear spaces (crystal sacs). Two kinds of guanine reflectors are observed, brick-shaped (crystallites,  $0.4 \times 0.5 \mu\text{m}$  in size) and rodlets ( $0.2 \times 2 \mu\text{m}$  in size) (Fig. 6). The brick-shaped reflectors are distributed throughout the entire

Table 1. Amounts of guanine in tapetum.

| Species<br>(Total length,<br>mm)        | Eye<br>diameter<br>(mm) | Guanine contents |                                      |
|---|-------------------------|------------------|--------------------------------------|
|   |                         | mg/eye           | mg/retinal<br>area ( $\text{mm}^2$ ) |
| <i>Marcusenius<br/>longianalis</i> (97) | 3.2                     | 0.37             | 0.023                                |
| <i>M. isidori</i> (100)                 | 3.5                     | 0.34             | 0.018                                |

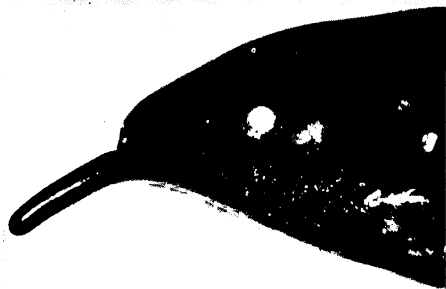


Fig. 2. *Gnathonemus petersii* (102 mm total length) showing strong eye shine when photographed by photoflash light.



Fig. 3. Histological structure of the retina of *Gnathonemus petersii* stained with toluidin blue. 1, pigment epithelium; 2, photoreceptor layer; 3; outer nuclear layer. Arrow indicates horizontal cell layer. Bar = 50  $\mu$ m.

cell, while the rodlet reflectors are arranged in an orderly manner on the periphery of the vitread half of the cell.

Numerous mitochondria ( $0.5 \times 2 \mu$ m in size) are observed in the basal part of the pigment epithelial cells (Fig. 4). The melanin granules are elongated ellipsoids, circular in section and about  $0.4 \times 1.4 \mu$ m in size (Fig. 6). The retina is duplex; the cones and rods are grouped in bundles and each bundle is surrounded by the pigment epithelial cell processes which contain numerous guanine reflectors (Fig. 7).

## Discussion

The reflecting material in the retinal tapetum of the mormyrids is mainly guanine in crystalline form. The quantity of guanine measured ( $2 \text{ mg cm}^{-2}$ ) is similar to that found in the tapeta of chlorophthalmid deep-sea fish and elopid fish (Somiya, 1980; Ito and Nicol, 1981), but is 4 times greater than that in the retinal tapetum of the anchovy (Zyznar and Nicol, 1973).

It is generally known that the tapetum is placed just behind the photoreceptor layer so that the light which is not absorbed by the photosensitive pigments in its first passage is reflected back through them and, thus, has a second chance of being absorbed (Denton, 1970). Usually in the retinal-type tapetum, the pigment epithelial cells contain both tapetal materials and a mass of melanosomes. In such fish eyes, melanin act as an occlusive pigment of the tapetal reflector in the light-adapted state.

In the case of the mormyrid eyes, only a few melanosomes were observed, so that they may not function as occlusive pigments. Teyssedre and Moller (1982) reported that the optomotor response ceased to occur under bright light (540 lx). Thus, the mormyrid fishes may have eyes which are adapted only for nocturnal or dim light vision. In a field study of mormyrid behaviour, Moller et al. (1979) found that they become active after sunset. Possibly mormyrids use their sensitive eyes at night.

Ultrastructurally, the tapetal organization of the reflecting materials of the mormyrids resemble that of the hiodontids in the following respects. 1) Well-developed pigment epithelial cells with processes extend nearly to the outer nuclear membrane. 2) Two kinds of tapetal reflectors are present, brick-shaped and rodlet (Best and Nicol, 1979; Braekevelt, 1982). However, there is a definite difference between the mormyrid and hiodontid tapetum in that hiodontids use uric acid (Zyznar et al., 1978) but that the mormyrids examined use guanine as the tapetal material.

According to Greenwood (1971), the Notopteroidei are composed of four living families: the Hiodontidae, Notopteridae, Mormyridae and Gymnarchidae. All these families have the grouped receptor retina together with the retinal tapetum as examined so far (McEwan, 1938; Engström, 1963; Ali and Anctil, 1976; Wanger and Ali, 1978).

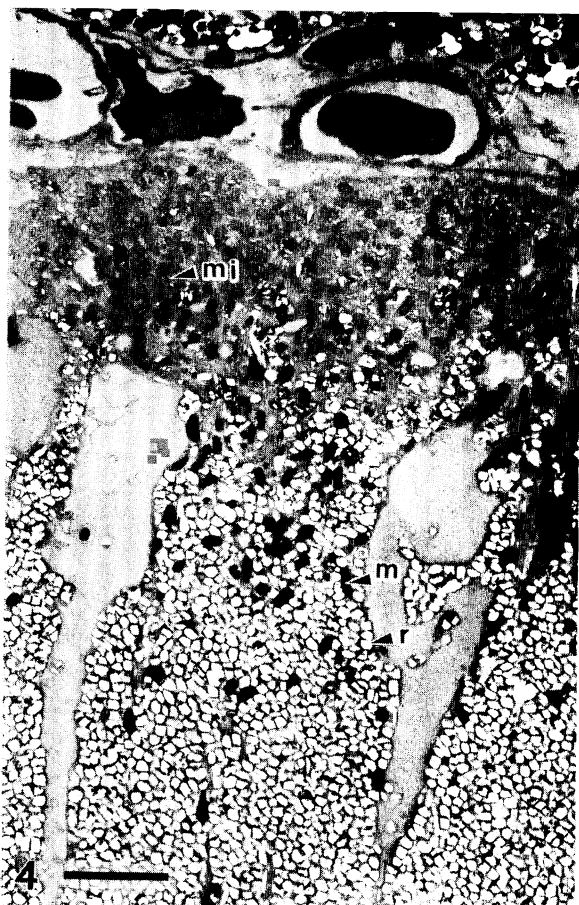


Fig. 4. Pigment epithelial cells of *Gnathonemus petersii* containing numerous guanine reflectors (r) and few melanosomes (m). Numerous mitochondria (mi) are shown at the basal (sclerad) part of the cell. Bar = 5  $\mu$ m.



Fig. 5. An unstained section showing part of the cytoplasm of a pigment epithelial cell from *Gnathonemus petersii* with numerous guanine crystals. Bar = 1  $\mu$ m.

However, there is no literature showing the chemical nature of the notopterid and gymnarchid tapetum. To discuss the possible chemical types of the notopterid and gymnarchid tapetum, the retinal features of some fish species belonging to these groups are summarized from the earlier reports (McEwan, 1938; Engström, 1963; Ali and Anctil, 1976; Wagner and Ali, 1978; Zyznar et al. 1978) and listed in Table 2.

Table 2 shows that only the hiodontid has the following retinal features: a poorly-developed outer nuclear layer (ONL) and a well-developed horizontal cell layer (HCL). The opposite histological features of a well-developed ONL and a poorly-developed HCL are observed in the eyes of the remaining families. The retinal characters listed

in Table 2 thus divide the Notopteroidei into two family groups: one including Hiodontidae, the other Mormyridae, Notopteridae and Gymnarchidae. This agrees with the view that the Notopteroidei comprise two superfamilies, the Hiodontoidea (Hiodontidae and fossil Lycoperidae) and the Notopteroidea (Notopteridae, Mormyridae and Gymnarchidae) (Greenwood, 1973). Greenwood presented this view mainly on the basis of the anatomy of the inner ear.

The histological similarity of these two retinal elements in the eyes of the latter three groups in Table 2 is striking enough to suggest that notopterid and gymnarchid fishes may have the guanine type tapetum. It also indicates that the retinal characters such as the chemical nature of tapetal

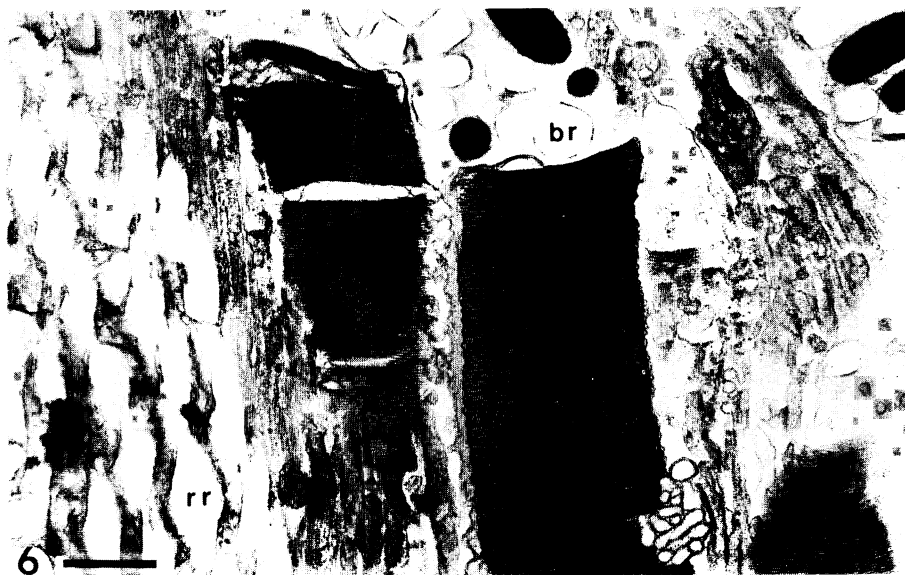


Fig. 6. Two kinds of guanine reflectors, brick-shaped (br) and rodlet (rr), in the pigment epithelial cell of *Gnathonemus petersii*. Bar=1  $\mu$ m.

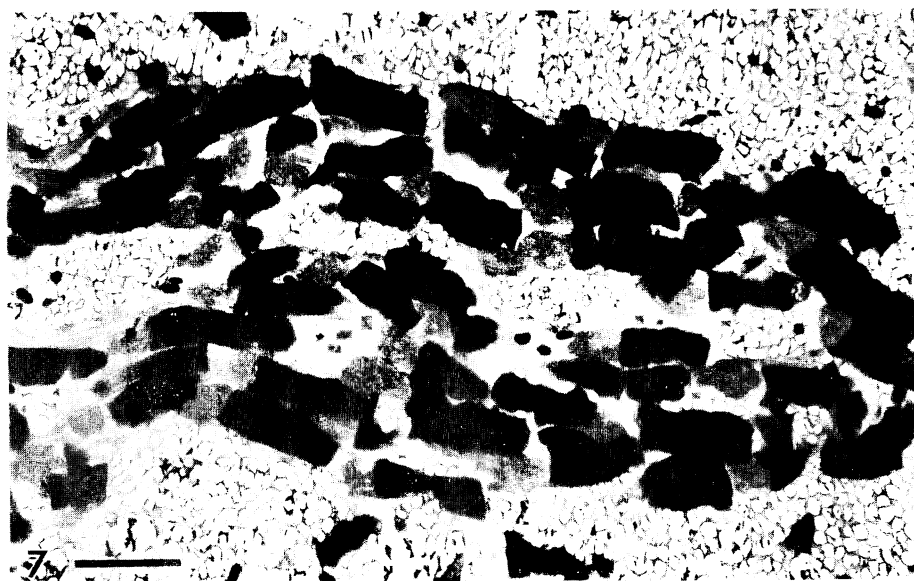


Fig. 7. Slightly oblique tangential section of the photoreceptor layer of *Gnathonemus petersii*. Photoreceptors are grouped in bundles, and each bundle is surrounded by numerous guanine reflectors. Bar=5  $\mu$ m.

materials, grouped receptors, ONL, HCL etc. of this fish group may have some taxonomic value for analyzing interrelationships. Conversely, if notopterids and/or gymnarchids have the uric

acid type tapetum in their retinae, it will throw a new light on the current concepts of the phylogenetic relationships of the Notopteroidei (Lauder and Liem, 1983). These points will be the sub-

Table 2. Retinal features of Notopteroidei. G, grouped receptor retina; +, poorly-developed; ##, well-developed; a, Ali and Ancil (1976); b, Wagner and Ali (1978); c, Zyznar et al. (1978); d, present study; e, Engström (1963); f, McEwan (1938); ?, no chemical evidence.

|                    | Receptor arrangement | Outer nuclear layer | Horizontal cell layer | Chemical type of tapetum | References |
|--------------------|----------------------|---------------------|-----------------------|--------------------------|------------|
| Hiodontidae        |                      |                     |                       |                          |            |
| <i>Hiodon</i>      | G                    | +                   | ##                    | uric acid                | a, b, c    |
| Notopteridae       |                      |                     |                       |                          |            |
| <i>Notopterus</i>  | G                    | ##                  | +                     | ?                        | a          |
| <i>Xenomystus</i>  | G                    | ##                  | +                     | ?                        | a          |
| Mormyridae         |                      |                     |                       |                          |            |
| <i>Marcusenius</i> | G                    | ##                  | +                     | guanine                  | d, e       |
| <i>Gnathonemus</i> | G                    | ##                  | +                     | guanine                  | d, f       |
| Gymnarchidae       |                      |                     |                       |                          |            |
| <i>Gymnarchus</i>  | G                    | ##                  | +                     | ?                        | e          |

ject of future research.

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(Department of Physiology, School of Veterinary Medicine, Azabu University, Sagami-hara 229, Japan)

#### モルミルス科魚類 3 種のグアニン型タペータム

宗宮弘明

モルミルス科魚類 (Mormyridae) の 3 種 (*Marcusenius*

*isidori*, *M. longianalis* と *Gnathonemus petersii*) はその眼に網膜タペータム (retinal tapetum) を持つことが明らかとなった。網膜タペータムを構成する反射物質の主成分はグアニンであり、その量は *Marcusenius* 属で、網膜 1 cm<sup>2</sup> あたり約 2 mg であった。グアニンは色素上皮細胞の中に小反射板として存在する。反射板はその結晶形状からブロック状と針状の 2 種に分けられた。以上のことから、Mormyridae は薄明環境において、その微量な光を有効に利用できることが推定された。また、これらの網膜の特徴を既往の文献に従って他のナギナタナマズ亜目 (Notopteroidei) のものと比較した。その結果、同亜目は次の 2 群に大別された。第 1 群は Hiodontidae からなり、第 2 群は Mormyridae, Notopterygidae, Gymnarchidae からなるグループであった。第 1 群に属する *Hiodon* (Hiodontidae) は、タペータムの主成分が尿酸 (uric acid) で、今のところ尿酸型のタペータムを持つ唯一の硬骨魚類である。第 2 群に属する魚類は相互によく似た網膜形質を持つ。このことから、Notopterygidae と Gymnarchidae は、その眼に尿酸型ではなく、Mormyridae と同様のグアニン型タペータムを持つ可能性が示唆される。

(229 相模原市淵野辺 1-17-71 麻布大学獣医学部獣医学科)