

DETECTION OF A PREVIOUSLY UNRECOGNIZED MORMYRID FISH (*MORMYRUS SUBUNDULATUS*) BY ELECTRIC DISCHARGE CHARACTERS

by

John D. CRAWFORD (1) and Carl D. HOPKINS (2)

ABSTRACT. - This paper characterizes the electric discharges of the recently described mormyrid *Mormyrus subundulatus* Roberts, and is presented as a companion to the species description (Roberts, 1989). *M. subundulatus* was shown to differ significantly from its sympatric congener, *M. rume*, in several features of the electric discharge. The use of electric characters in systematic studies of the Mormyridae is discussed. Ecological data are also presented on these two *Mormyrus* forms, for localities in the Bandama River Basin, Côte d'Ivoire.

RÉSUMÉ. - Cet article concerne les décharges électriques de *Mormyrus subundulatus* Roberts récemment décrit, et complète la description de l'espèce (Roberts, 1989). *M. subundulatus* diffère de son congénère sympatrique, *M. rume*, par plusieurs particularités de la décharge électrique. L'utilisation des caractères électriques dans les recherches systématiques effectuées sur les mormyres est discutée. Des données écologiques sur ces deux *Mormyrus*, relatives à des localités dans le bassin de la rivière Bandama, en Côte d'Ivoire, sont aussi présentées.

Key-Words: Mormyridae, *Mormyrus subundulatus*, Ivory Coast, Electric discharge.

Mormyrus subundulatus Roberts is a newly described species from West Africa (Roberts, 1989). We encountered this form while making a survey of the mormyrid fishes of the Bandama River Basin, Cote d'Ivoire, in 1985 and have analyzed its electric discharge (EOD) in the context of systematic taxonomy. This paper is presented as a companion to the species description of Roberts (1989), including electric and ecological data on the specimens discussed by Roberts.

Mormyrids produce weak electric discharges which function in spatial orientation and in communication (Lissmann and Machin, 1958; Hopkins, 1986). Since mormyrid EODs (electric organ discharges) are generally species-specific (Hopkins, 1981; Hopkins, 1986) we have made a quantitative comparison of the EODs of *M. subundulatus* and those of its only known sympatric congener, *M. rume*. This analysis of electric characters has been used together with more conventional characters to recognize and describe *M. subundulatus*. To our knowledge, this is the first time that electric characters have been used in the description of a new species of electric fish.

(1) Reprint requests: Parmly Hearing Institute, Loyola University, 6525 N. Sheridan Rd., Chicago, IL, 60626 USA.

(2) Neurobiology and Behavior, Cornell University, Ithaca, NY, 14853 USA.

MATERIALS AND METHODS

Collecting sites

The Bandama River flows south-east through Côte d'Ivoire to the Atlantic Ocean. The Nzi River is a major tributary of the Bandama, flowing south to join the Bandama a few km north of Tiassale. Our collecting expeditions were made from Station Scientifique de Lamto, near Ayeremou, Côte d'Ivoire.

Mormyrus were collected in the Bandama River within a few km of Lamto. Collections were also made in small streams flowing into the Bandama and Nzi rivers. We travelled north to Tamoudi and east from Tamoudi to Dimbokro, sampling streams intersecting the roadway. This work was conducted during November, 1985, following the September-October rainy season.

Collecting methods

In daylight, mormyrids were found by placing an electrode probe in the water and listening for electric discharges. Battery powered amplifiers, with audio monitors, were used to amplify electric discharges picked up by Ag/AgCl electrodes. An oscilloscope (Tektronix 323) was used to observe EOD waveforms.

In rocky habitats (in the Bandama near Lamto), rotenone was used to disperse and immobilize fish. Live specimens were placed into fresh aerated water, taken at the site before adding rotenone. Several of our larger specimens were collected with gill nets, but died before EODs could be recorded. At sites where mormyrids were found amongst vegetation along the banks of streams, fish were localized by EOD detection, a circular net (diam. = 0.7 m) was placed 0.5 m downstream and the fish was flushed into the net.

Ecological measurements

Dissolved oxygen concentration and pH were measured using a Hatch test kit. Electrical conductivity was measured with a Cole-Parmer 1481-50 conductivity meter calibrated against Cole-Parmer 12880 $\mu\text{S}/\text{cm}$ standard. Notes on water depth, stream width, flow rate and substrate composition were taken at each site.

EOD recording

Fish were transported to the laboratory at Lamto and maintained in stream or river water until their EODs were recorded (within 24 h). Each fish was placed in a plexiglas holding tank (34.0 x 9.0 x 17.5 cm high, inside) containing 4 liters of water from the collection site. Water temperature was 25°C (25.23°C \pm 1.2 SD). Conductivity ranged from 75-130 $\mu\text{S}/\text{cm}$ and the pH from 6.8-7.5. The tank was equipped with an Ag/AgCl pick-up electrode at each end and discharges were examined while the fish faced the positive electrode. Observations of many species have confirmed that EOD waveforms, examined in this manner, were the same as those recorded from undisturbed fish in their natural habitat (with an electrode probe).

A sequence of 10 EODs was recorded from each specimen. EOD's were recorded on magnetic tape (Scotch 209) with a NAGRA IV SJ at 37 cm/s. The pass-band of this recording system was 50 Hz to 25 KHz. For every specimen, the EOD was examined on the oscilloscope both directly from the pick-up electrodes and from the output of the tape recorder. Recorded signals were accurate representations of the EODs viewed directly on the oscilloscope. The power spectra confirmed that the power in the recorded EOD's fell-off well before the high-frequency corner of the recording system. Thus, there was no indication that the waveforms were distorted by the band limits of the recording system.

Tape recorded EODs were digitized, at 200 KHz, with a Tektronix 5223 digitizing Oscilloscope, stored to disk on a PDP 11/34 computer and plotted with a HP 7220T plotter. We did not attempt to measure absolute or relative EOD ampli-

tudes. EOD's were scaled to the same peak-to-peak amplitude for analysis and for plotting.

RESULTS

Ecology

Both *Mormyrus rume* and *M. subundulatus* were collected in the Bandama River, within 2 Km of Lamto. Animals were most often localized in shallow (1-2 m deep) waters amidst rocks. These sites were in the main channel of the river (approximately 0.5 Km wide) and in smaller side branches (approximately 0.25 Km wide or less). *Mormyrus* spp. were detected in water as deep as 6 m. The flow in these areas was swift, without turbulence, and the water was black but not turbid. Surface temperatures were measured at 29.0°C (temperature profiles were not made). Conductivity was 93.5 $\mu\text{S}/\text{cm}$, dissolved O_2 was 3.0 ppm and pH was 6.8.

Mormyrus rume were also found in small streams flowing east south-east into the Nzi River. *M. subundulatus* were not found in these streams. Numerous collections were made in streams intersecting the route north from Lamto to Toumodi and east of Toumodi toward the Nzi River. These streams ranged from 1 to 7 m in width and most were swift with some turbulence, had rock bottoms and dense vegetation along their banks (near the road cuts). Depths ranged from 0.2 to 3.0 m. The water was turbid, with several of the streams east of Toumodi carrying red sediment as did the Nzi River. Water temperature ranged from 25-26°C., conductivity ranged from 66 to 129 $\mu\text{S}/\text{cm}$, pH from 6.8 to 7.1 and dissolved O_2 was measured, for one site, at 7 ppm (25°C).

Mormyrus rume were found spaced out along streams at approximately 4 m intervals and were generally solitary. They were most commonly located amongst vegetation along the stream margin but occasionally amidst rocks on the bottom or under within submerged dead trees. Although fast running streams were the most common habitat, *M. rume* were found in one turbid red stream, with no observable flow, near the Nzi River (8.6 Km east of Toumodi).

Figure 1 includes the sizes of the fish we collected, along with their discharges. All of the specimens were small compared with the largest *Mormyrus* specimens reported in the literature and gonadal examination showed all of our specimens to be immature.

Electric characters

The electric organ discharges of *Mormyrus rume* and *M. subundulatus* are illustrated in Figure 1 and their power spectra in Figure 2. Within an individual, the EOD was extremely stereotyped as has been observed for many species of mormyrids (see Hopkins, 1981; Hopkins, 1983a). For each specimen recorded, we have presented a single EOD (Fig. 1). In both species the first phase of the biphasic EOD (P1) was positive and the second phase (P2) was negative. Intraspecific variability has been illustrated by plotting all the EODs superimposed at the bottom of each column in Figure 1. Although there was some intraspecific variability, this was small compared to differences between species.

One striking difference in the two waveform types was in the relative amplitudes of the phases (Fig. 1). In *M. rume* the amplitude of P1 was relatively greater than that of *M. subundulatus*. This difference is reflected in the ratio of P1 to P2. The average ratio of P1 to P2 for *M. rume* was 0.445 (range: 0.398 - 0.494) and for *M. subundulatus* 0.144 (range: 0.113 - 0.203). These data were normalized by taking the natural log of each ratio and then compared with a Student's t-test (Ryan *et al.*, 1976). This difference between EOD types is highly significant ($P < 0.0001$, $t = 13.518$, $DF = 12$).

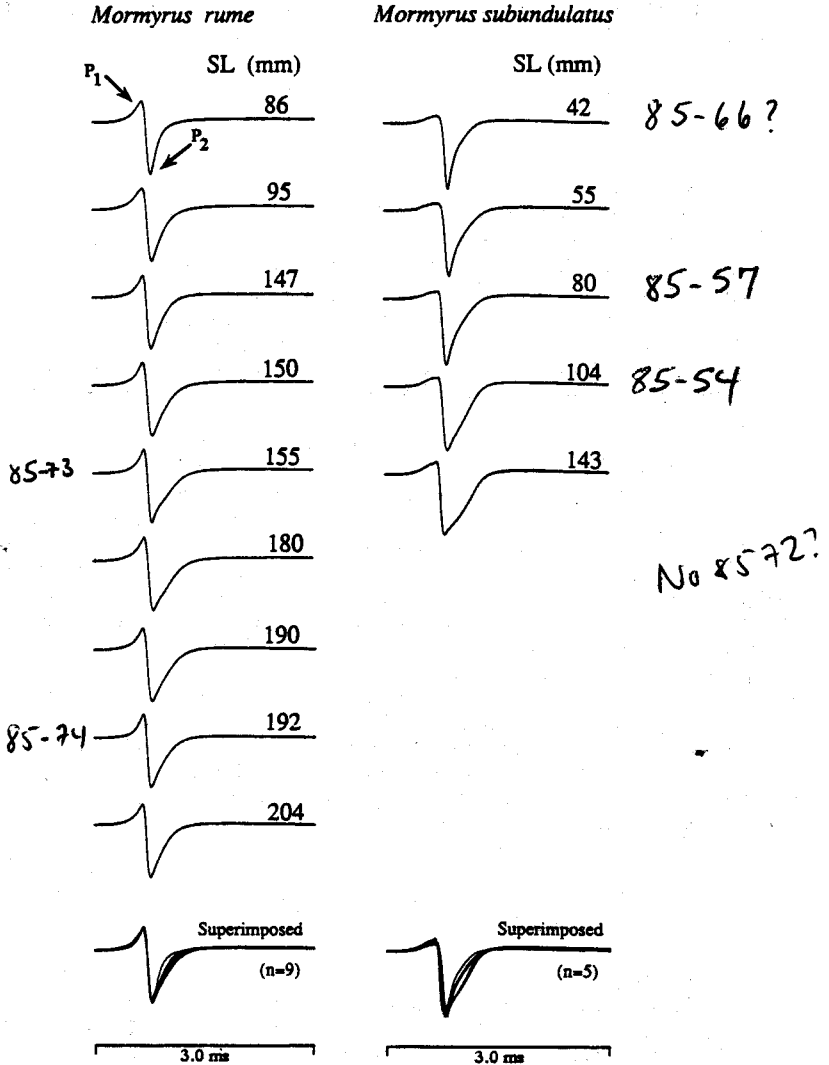


Fig. 1: Electric Organ Discharges (EODs) of *Mormyrus rume* (left) and *Mormyrus subundulatus* (right). The standard length (SL), of the specimen from which the EOD was recorded, is given to the right of each EOD in mm. At the first EOD (top left) the two phases, P1 and P2, are indicated. At the bottom of each column, all the EODs are replotted, superimposed, to illustrate the range of variability in each sample. These EODs illustrate the non-reproductive state for these two species but do not show sex differences, though sex differences are likely to exist in reproductive adults.

A second difference between the two EOD types was in the initial positive slope of Phase 1 (Fig. 1, P1). This initial rise rate was a factor of 2.2 faster in *M. rume* than in *M. subundulatus*. Slope was measured as the ratio of the amplitude of P1 to the interval between the first departure of 2.0% from base line and the

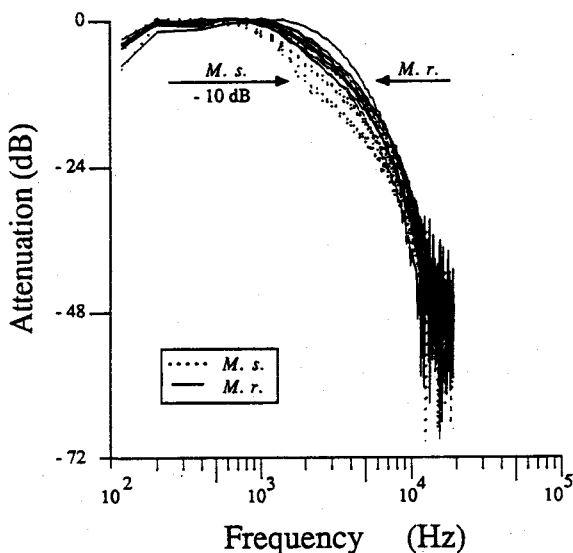


Fig. 2. Power Spectra for EODs illustrated in Figure 1. Each EOD was subjected to a Fast Fourier Transform (FFT), its power spectrum was calculated and plotted. Power spectra for *Mormyrus subundulatus* EODs are dotted ($n=5$ curves) and for *Mormyrus rume* the spectra are solid ($n=9$ curves). The horizontal axis is logarithmic in Hz and the vertical axis is decibels of attenuation (0 dB = maximum power).

time at which P1 reached its maximum amplitude. EODs were scaled to the same peak to peak amplitude, rise was measured in units of percent of peak-to-peak amplitude, and slope as rise per ms. For *M. rume* the average slope was 81.2 ± 1.13 (SD)%/ms and for *M. subundulatus* the slope was 36.82 ± 1.15 %/ms. The difference was highly significant ($P < 0.0001$, $t = 8.759$, $DF = 12$, analysis of natural log transformed slopes).

EOD duration was about 1.0 ms in both species; duration was measured from the point the trace first rose to 2.0% above base line to the point where it returned to within 2.0% of base line (at the terminal part of phase 2). For *M. rume* duration was 0.966 ± 0.109 ms and for *M. subundulatus* duration was 0.961 ± 0.093 ms. In this parameter, differences between species were not significant.

Figure 2 shows the power spectra calculated from Fast Fourier Transforms (FFT's) of the EODs. In both species the distribution of power was very broad with a peak at about 1 KHz. The *M. rume* spectra showed slightly more power in the higher frequencies than did the *M. subundulatus* spectra. This difference was quantified by measuring the frequencies (on the high frequency side of each spectrum peak) at which the power had dropped by 10 dB. On average, this parameter was 1.1 KHz lower for *M. subundulatus*, reflecting the readily observed differences between the two species in the 1 to 10 KHz range. The difference between the two species in this parameter was significant ($P < 0.006$, $t = 3.329$, $DF = 12$).

DISCUSSION

While observing living *Mormyrus* specimens in the field, it became clear to us (Roberts, Crawford and Hopkins) that there were two forms. They differed in their body coloration and morphology, there was ecological separation between types and the EOD waveforms were different. Our subsequent quantification of these observations confirmed that the electric organ discharges of these two sympatric *Mormyrus* forms were significantly different and that these differences agreed with other morphometric and meristic characters useful in species diagnosis (Roberts, 1989).

Our findings support the hypothesis that characteristics of the mormyrid electric discharge are valuable in the systematic taxonomy of this group, as proposed earlier by Hopkins (Hopkins, 1980; 1983b; 1986). We conclude that, when possible, future species descriptions, and keys to these fishes, should include characteristics of the discharge. These conclusions are further supported by the observations that 1) species specificity in the EOD appears to be the rule (Bennett, 1971; Moller *et al.*, 1979; Hopkins, 1981) and 2) the EOD plays a role in species isolation for at least one species (Hopkins and Bass, 1981).

The *Mormyrus* forms discussed here (and by Roberts, 1989) are doubtless also distinguished by anatomical features of their electric organs. Studies of the mechanisms by which mormyrids produce electric discharges have revealed morphological diversity in electric organs comparable to the diversity seen in the waveforms they generate (Bass, 1986). Electric organ morphology and physiology have been examined in *Mormyrus* (Szabo, 1961; Bennett and Grundfest, 1961; Bennett, 1971). From the EOD waveforms shown by Bennett (1971) it appears that his "*M. rume*" were actually *M. subundulatus* as described here. Physiology showed that the large negative-going phase of the EOD arose from the anterior face of the electrocyte and that the smaller positive-going phase arose from the stalk. Since stalk morphology is a close correlate of species differences in EOD waveforms (Bass, 1986), we expect a greater physiological contribution of the stalk to the EOD of *M. rume* and that stalk morphology may also distinguish these two *Mormyrus* species.

EOD in species diagnosis

In our experience, there has been excellent agreement between electric data and traditional diagnostic characters, both for described species and in the case of identifying *M. subundulatus*. However, it is clear that additional work will be required to rigorously evaluate the utility of electric characters in systematics, since important questions regarding heritability, environmental influences during ontogeny, and geographic variability have not yet been addressed.

There are several practical issues which should be considered in the description of mormyrid electric discharges. First, discharge waveforms are relatively simple and are changed by even the smallest phase-shifts introduced by recording apparatus. This is not such a serious problem in comparisons between forms, recorded identically as in this study, but can be a source of confusion when comparing data presented by different authors using different methods. The best methods involve low impedance electrodes (e.g. carbon rods) and digital devices that can sample at 0.5 to 1.0 MHz (see Kramer and Weymann, 1987 for further discussion).

A second important variable is water conductivity (Harder *et al.*, 1964; Bell *et al.*, 1976; Bratton and Kramer, 1988). Good biological descriptions of EOD waveforms should be done with conductivity within the natural range for the species, and preferably in the water in which the fish was collected. Since waveform depends upon conductivity, EOD data without conductivity data are not very useful.

A third consideration is that of intraspecific variability. Hormonally-controlled sex-differences in the EODs of adult mormyrids are well known (Lucker and Kramer, 1981; Hopkins, 1981; Westby and Kirschbaum, 1982; Bass and Hopkins 1983; 1985; Bratton and Kramer, 1988). Females and juveniles usually have similar EODs while breeding males (or individuals treated with androgens) often produce longer duration EODs or EOD's with a different waveshape. A complete description of a species' electric characteristics should include data on juveniles and on breeding adults of both sexes, though this will often be difficult to obtain (larval mormyrids also have a distinct discharge, produced with a special larval electric organ, Kirschbaum, 1975; Westby and Kirschbaum, 1977; 1978). As all of the specimens used in the present study were immature, it remains to be seen how the EODs of mature individuals may differ from the EODs presented here.

Conclusions

The Mormyridae is a fascinating group, exhibiting impressive morphological, ecological and behavioral diversity. At present, there are over 200 described species in this group (Taverne, 1972) but there will likely be more described as investigators look more closely at the group and explore different regions of Africa. The EOD has proven to be a distinguishing character for *Mormyrus subundulatus*, drawing our attention to a suite of characters which distinguish it from *M. rume*. We suspect that the EOD can provide valuable information for future systematic studies of the Mormyridae.

Acknowledgements: We thank Dr. Roger Vuattoux and the staff of the Station Biologique de Lamto for their help and hospitality. We are grateful to B. Baldwin, J. Gittleman and T. Natoli for assistance in preparing for the field work and to A. R. McCune, K. Adler, G. Harned and two referees for criticizing the manuscript. This research was supported by NIMH grant MH37972 to C. Hopkins and NIMH grant S5T3215793 to J. Crawford.

REFERENCES

- BASS A.H., 1986. - Species differences in electric organs of mormyrids: substrates for species-typical electric organ discharge waveforms. *J. Comp. Neurol.*, 244: 313-380.
- BASS A.H. & C.D. HOPKINS, 1983. - Hormonal control of sexual differentiation: changes in electric organ discharge waveform. *Science*, 220: 971-974.
- BASS A.H. & C.D. HOPKINS, 1985. - Hormonal control of sex differences in the electric organ discharge (EOD) of mormyrid fishes. *J. Comp. Physiol.*, 156:587-604.
- BELL C.C., BRADBURY J. & C.J. RUSSELL, 1976. - The electric organ of a mormyrid as a current and voltage source. *J. Comp. Physiol.*, 110: 65-88.
- BENNETT M.V.L. & H. GRUNDFEST, 1961. - Studies on morphology and electrophysiology of electric organs. III. Electrophysiology of electric organs in mormyrids. In: Bioelectrogenesis (C. Chagas & A.P. Carvalho, Eds.). pp. 113-135. Elsevier, Amsterdam.
- BENNETT M.V.L., 1971. - Electric organs. In: Fish Physiology, (W.S. Hoar & D.J. Randall, eds.), 5: 347-491.
- BRATTON B.O. & B. KRAMER, 1988. - Environmental influence upon the dimorphic discharge of a pulse-type electric fish, *Pollimyrus isidori* and *Petrocephalus bovei* (Mormyridae, Teleostei) and their dependence on water conductivity. *Exp. Biol.*, 47: 227-238.
- HARDER W., SCHIEF & H. UHLEMANN, 1964. Zur funktion des elektrischen organs von *Gnathonemus petersii* (Gthr. 1862). *Z. Vergl. Physiol.*, 48: 302-331.
- HOPKINS C.D., 1980. Evolution of electric communication channels of mormyrids. *Behav. Ecol. Sociobiol.*, 7: 1-13.
- HOPKINS C.D., 1981. On the diversity of electric signals in a community of mormyrid electric fish in West Africa. *Amer. Zool.*, 21: 211-222.
- HOPKINS C.D., 1983a. - Neuroethology of species recognition in electroreception. In: Advances in Vertebrate Neuroethology (J.P. Ewert, R.R. Capranica & D.I. Ingle, eds.). NATO ASI series A, 56: 871-881. Plenum Publ., New York.

- HOPKINS C.D., 1983b. - Functions and mechanisms in electroreception, *In: Fish Neurobiology*. Vol. 1. (R.G. Northcutt & R.E. Davis, eds.). p. 215-259. Univ. Michigan Press, Ann Arbor, Michigan.
- HOPKINS C.D., 1986. - Behavior of mormyridae. *In: Electroreception* (T.H. Bullock & W. Heiligenberg, eds.). pp. 527-576. J. Wiley and Sons, Inc., New York, New York.
- HOPKINS C.D. & A.H. BASS, 1981. - Temporal coding of species recognition signals in an electric fish. *Science*, 212: 85-87.
- KIRSCHBAUM F., 1975. - Development of the electric discharge in mormyrid and gymnotid fish (*Marcusenius* sp and *Eigenmannia virescens*). *Experientia*, 31: 1290-1292.
- KRAMER B. & D. WEYMANN, 1987. - A microprocessor system for the digital synthesis of pulsed and continuous discharges of electric fish (or animal vocalizations). *Behav. Brain Res.*, 23: 167-174.
- LISSMAN H.W. & K.E. MACHIN, 1958. - The mechanisms of object location in *Gymnarchus niloticus* and similar fish. *J. Exp. Biol.* 35: 451-486.
- LUCKER H. & B. KRAMER, 1981. - Development of a sex difference in the preferred latency response in the weakly electric fish, *Pollimyrus isidori* (Cuvier et Valenciennes) (Mormyridae, Teleostei). *Behav. Ecol. Sociobiol.*, 9: 103-109.
- MOLLER P., SERRIER J., BELBENOIT P. & S. PUSH, 1979. - Notes on ethology and ecology of the Swashi River mormyrids (Lake Kainji, Nigeria). *Behav. Ecol. Sociobiol.*, 4: 357-368.
- ROBERTS T.R., 1989. *Mormyrus subundulatus*, a new species of mormyrid fish with a tubular snout from West Africa. *Cybium*, 13(1): 51-54.
- RYAN T.A., JOINER B.L. & B.F. RYAN, 1976. Minitab student handbook. PWS Publishers, Boston Massachusetts.
- SZABO T., 1961. - Les organes électriques des mormyrides. *In: Bioelectrogenesis* (C. Chagas & A.P. Carvalho, eds.). pp. 20-24. Elsevier, Amsterdam.
- TAVERNE L., 1972. - Ostéologie des genres *Mormyrus* Linné, *Mormyrops* Müller, *Hyperopisus* Gill, *Isichthys* Gill, *Myromyrus* Boulenger, *Stomatorhinus* Boulenger, et *Gymnarchus* Cuvier. Considérations générales sur la systématique des poissons de l'ordre des Mormyriiformes. *Annls Mus. r. Afr. cent.*, 200: 1-194.
- WESTBY G.W.M. & F. KIRSCHBAUM, 1977. - Emergence and development of the electric organ discharge in the mormyrid fish, *Pollimyrus isidori*. *J. Comp. Physiol.*, 122: 251-271.
- WESTBY G.W.M. & F. KIRSCHBAUM, 1978. - Emergence and development of the electric organ discharge in the mormyrid fish, *Pollimyrus isidori*. II. Replacement of the larval by the adult discharge. *J. Comp. Physiol.*, 127: 45-59.
- WESTBY G.W.M. & F. KIRSCHBAUM, 1982. - Sex differences in the waveform of the pulse-type electric fish, *Pollimyrus isidori* (Mormyridae). *J. Comp. Physiol.*, 145: 399-403.

Reçu le 25.02.1989.

Accepté pour publication le 13.09.1989.