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Electric organ discharges of South African *Marcusenius* species (Teleostei: Mormyridae) and their effectiveness as indicators of local species diversity

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Abstract

Recent morphological and genetic studies have revealed two new species of snoutfish in South Africa, *Marcusenius caudisquamatus* and *M. krameri*, which had been confused with *M. pongolensis*, the South African bulldog fish. All known mormyriform fish are nocturnal and emit electric organ discharges (EODs) for communication that are characteristic for their species. This paper examines whether or not the EODs of these three closely-related South African species can be differentiated from each other. An EOD pulse of a bulldog fish consists of a head-positive phase P, followed by a head-negative phase N of short duration. We measured and compared six variables of the EOD pulse waveform for South African samples for the three species from different locations using MANOVA, ANOVA and Discriminant Analysis, with *M. devosi* from Kenya as an outgroup. The EOD waveforms, normalized to the same P-phase amplitude, varied significantly from each other in four variables, most strongly in the amplitude of the N phase and the duration of the P phase. In two species, *M. devosi* and *M. krameri*, there was no evidence of difference between sexes, in contrast to *M. pongolensis* and *M. caudisquamatus* whose male pulses were of longer duration. *M. devosi* and *M. krameri* were statistically significantly independent of each other and of any other group studied. By contrast, the *M. pongolensis* specimens from different locations showed a high degree of variability amongst each other, including significant separation, and overlap with *M. caudisquamatus*.

Key words: communication, geographic differentiation, interspecific, intraspecific, polymorphism, sex difference

Introduction

Snoutfish (Mormyridae) are members of the largest fish family that is endemic to African freshwater bodies (Lowe-McConnell 1987: 28, 32, 34). All species that have been studied emit electric organ discharges (EODs) for intra- and interspecific communication, as well as electro-location, and are more active at night. EOD pulses are usually short (often less than 1 ms) compared to the [inter-discharge] intervals separating the pulses. EOD pulse rates vary greatly depending on the time of day, behavioural state, and the level of sensory arousal. The waveform of EOD pulses is highly stable for an individual and varies so little among individuals of the same species that it is considered characteristic for a species, and has been used as a taxonomic character. In some species male pulses tend to be of longer duration than female pulses (for reviews see Moller 1995; Bullock *et al*. 2005; Kramer 2009a-c; Wiltschko & Kramer 2009; Peters 2009).

In certain mormyrid genera, such as *Hippopotamyrus*, *Mormyrops* and *Pollimyrus*, closely-related species—as far as they have been studied—are more easily recognized by the marked and characteristic differences in their EOD pulse waveforms than by their morphology. In other mormyrid genera, such as *Petrocephalus*, the EOD waveforms of all species which have been studied are basically similar, and rather cryptic, species-characteristic differences are often revealed only by quantitative comparisons at high resolution (for example, Kramer *et al*. 2012; Lavoué *et al*. 2010). Yet another case is a single East-African species, *Mormyrus tenuirostris*, which differs

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radically in waveform type from all other known *Mormyrus* species throughout Africa, all of which display basically similar waveforms (Kramer 2013).

The present study is of three species of South African *Marcusenius*, commonly known as bulldog fish. *Marcusenius* is one of the mormyrid genera in which the waveforms of individual EOD pulses have been found to be basically similar among southern African and even West and East African species. Nevertheless, differences have been observed in most cases when studied with sufficient scrutiny. Mormyrids are extremely sensitive to the fine details of their EOD waveforms on a microsecond (µs) scale (Graff & Kramer 1992, Paintner & Kramer 2003). Electric signaling plays an important role in aggression, courtship and pair formation in *M. pongolensis* (Werneyer & Kramer 2002, 2005), in addition to acoustic signaling (Lamml & Kramer 2007). Southern African *Marcusenius* species and their electric signaling were also the subject of studies on prey-predator relationships (Hanika & Kramer 1999, 2000), intra- and intersexual selection (Hanika & Kramer 2005, 2008; Machnik & Kramer 2008a, 2008b, 2011; Machnik *et al*. 2010) and ontogenetic EOD waveform development (Werneyer & Kramer 2006).

Crass (1960) synonymized the South African bulldog fish, *Marcusenius pongolensis* (Fowler, 1934), with *Marcusenius macrolepidotus* Peters, 1852, the type region of which is the Lower Zambezi (Mozambique). Kramer *et al*. (2007) reinstated *M. pongolensis* but also recorded a high level of variability in morphology, molecular genetics, and EOD waveform among samples of bulldog fish from different South African localities, but were unable to establish a clear geographical and taxonomical pattern. A recent study by Maake *et al*. (2014) demonstrated the presence of three *Marcusenius* species in South Africa: the widespread *M. pongolensis,* as well as the south-eastern *M. caudisquamatus* and the northern *M. krameri*. The present study investigates the question of differentiation in EOD waveform among South African *Marcusenius* species since Maake *et al*. (2014) did not include EOD analysis in their study.

Material and methods

The taxonomy used follows Maake *et al*. (2014). We studied 136 fish for their electric organ discharges (EODs) from the following rivers and systems (Fig. 1): the Mokolo River, which forms part of the Limpopo System near the northern border of South Africa with Botswana, is home to *M. krameri;* the Incomati System (including the Sabie System), the Pongola (or Phongolo) System, and the Kosi River System are inhabited by *M. pongolensis;* and the Mhlatuze River in KwaMaZulu-Natal is the southern distribution limit for mormyrids in Africa, with *M. caudisquamatus* as the only snoutfish species present. Specimens of these species were lodged with the South African Institute for Aquatic Biodiversity (SAIAB), Grahamstown, South Africa and the Ichthyology Section of the Zoologische Staatssammlung München (ZSM), Abteilung Vertebrata, Sektion Ichthyologie, Munich, Germany. SL is the standard length of the fish.

***Marcusenius caudisquamatus* Maake, Gon and Swartz, 2014.** Holotype, SAIAB 88684, SL 16.6 cm, male, Mhlatuze River, under the bridge on road R102, 28°50'44.2" S, 31°51'59.6" E, South Africa, 8 November 2009, coll. P. Maake, B. Kramer and B. Mackenzie.

SAIAB 191225(6), that is, six specimens with field nos. PM09A238–PM09A243, SL range 8.8–17.5 cm, four females, two males, same data as for holotype; SAIAB 79149(4), field nos. B02D, B03D, B05D, B07D, SL range 6.6–12.9 cm, four females or juveniles; and ZSM 35090(3), field nos. B01D, B04D, B06D, SL range 8.1–10.8 cm, two females, one male, the latter two collection lots from KwaMaZulu stream close to where it flows into Goedertrouw Dam, part of the Mhlatuze System in KwaZulu-Natal, 28°25'30" S, 31°01'30" E, 12 Aug. 1999, coll. J. Engelbrecht and B. Kramer, locality 7 on Fig. 1B.

***Marcusenius devosi* Kramer, Skelton, van der Bank and Wink, 2007.** Holotype, SAIAB 79138, field no. Ta13na, SL 10.3 cm, female, Tana River, Tana Primate Research Centre, 1°52'38.1" S, 40°08'22.5" E, south of village Wenje, east of road B8, 48 m above sea level, Kenya, 3 September 2001, coll. L. De Vos and B. Kramer.

SAIAB 79139(14), ZSM 35091(3), ZSM 35092(1), ZSM 35093(4), ZSM 35094(7), in addition to holotype out of these: specimen field nos. Ta10na–Ta12na, Ta14na, Ta16na, Ta18na, Ta20na, Ta21na–Ta25na, Ta27na–Ta34na, Ta47na, SL range 8.2–12 cm, 17 females, five males, 3–6 September 2001, Kenya, coll. L. De Vos and B. Kramer. Locality 1 on map of Fig. 1.

***Marcusenius krameri* Maake, Gon and Swartz 2014.** Holotype, SAIAB 188295, SL 11.6 cm, male, Mogalakwena River (also known as the Nyl River), Modimolle town, Pretoria Street, above a drift, 24°42'04.2" S, 28°24'40.8" E, Limpopo River System, South Africa, 14 Oct 2010, coll. P. Maake and O. Gon, not studied.

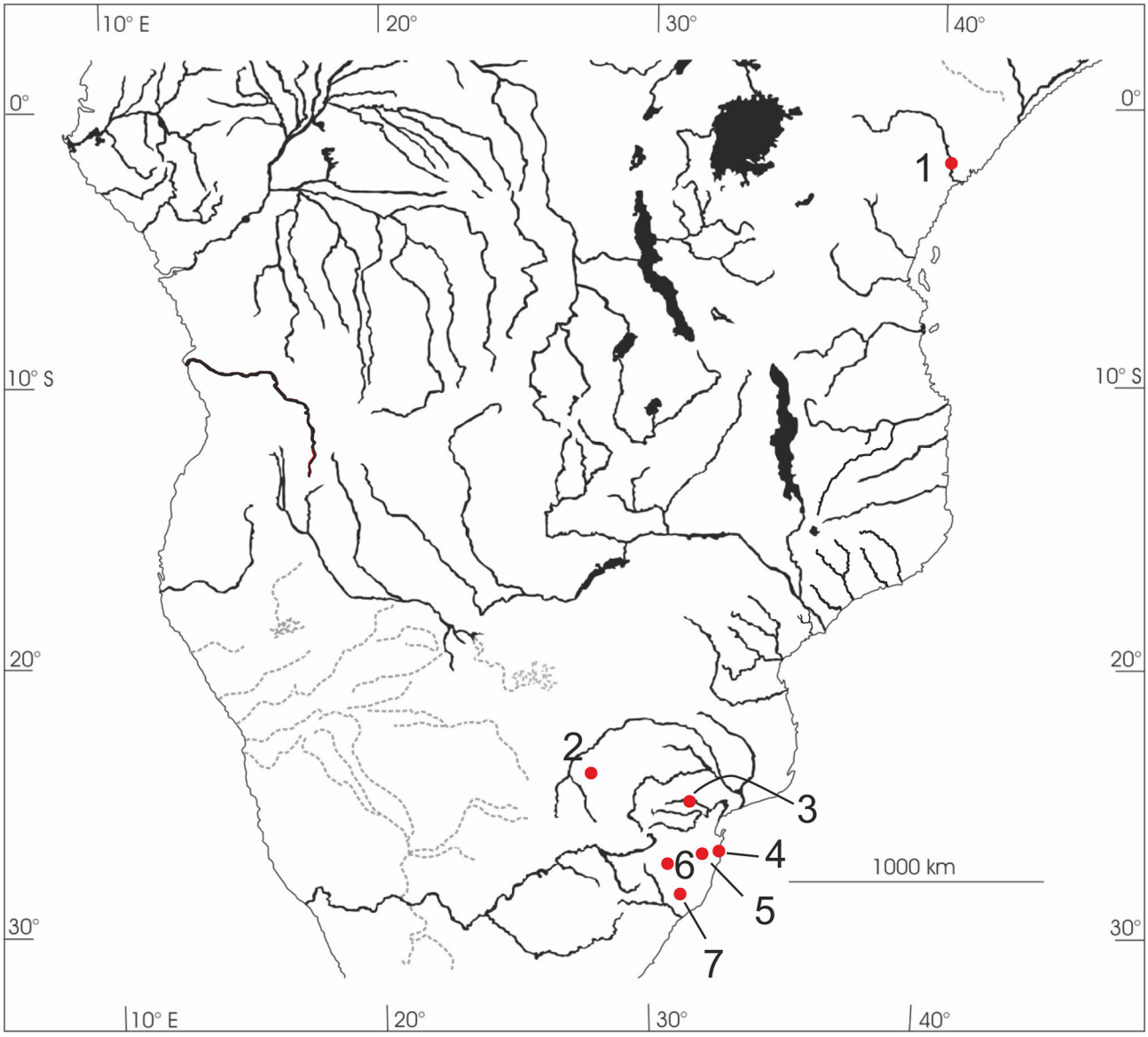

FIGURE 1. A, partial geography of southern Africa showing localities where fish were sampled. B, partial geography of South Africa. Locality 1, Tana River, Kenya, *Marcusenius devosi*. Locality 2, Mokolo River, *M. krameri*. Locality 3, Sabie River, *M. pongolensis*. Locality 4, Kosi Bay area, Kosi River system, *M. pongolensis*. Locality 5, Pongola River, *M. pongolensis*. Locality 6, Type locality for *M. pongolensis*. Locality 7, Mhlatuze River, *M. caudisquamatus*.

SAIAB 88845(15), field nos. Mogol01–Mogol04, Mogol15, Mogol16, Mogol25, Mogol26, Mogol29, Mogol32–Mogol34, Mogol37, Mogol38, Mogol57), SL range 6.8–9.7 cm, two males, 13 females or juveniles; ZSM 39535(7), specimen field nos. Mogol18, Mogol19, Mogol22–Mogol24, Mogol27, Mogol36, SL range 7.1–9.2 cm, four females, three males, both collection lots from Mokolo (also Mogol) River, Limpopo System, at road bridge, N of Hermanusdorings, 24°6'49.65" S, 27°48'9.11" E, elevation 932 m, 20 Oct 2008, South Africa, coll. A. Hoffman and B. Kramer. Locality 2 on map of Fig. 1.

***Marcusenius pongolensis* (Fowler, 1934), Pongola River.** SAIAB 79148(5), field nos Pon01, Pon02, Pon04, Pon05, Pon10, SL range 11.6–17.7 cm, two females, three males; ZSM 35087(5), field nos. Pon06–Pon09, Pon11, SL range 11.5–16.1 cm, three females, two males, both collection lots from Pongola River, bridge at road connecting Ndumo with Kosi Bay, 27°01'15" S, 32°18' E, 14 Aug. 1999, South Africa, coll. J. Engelbrecht and B. Kramer, locality 5 on map of Fig. 1. Locality 6 = type locality for *M. pongolensis* (Fowler, 1934).

Witrivier (Pongola System). SAIAB 88846 (2), field nos Wit01, female, SL 9.9 cm, Wit02, male, SL 18 cm; ZSM 39536, field no. Wit03, female, SL 8.2 cm, Witrivier (Pongola System), above Pongola Poort Dam, N of

Paulpietersburg / E of Piet Retief, within Mkhunyane Nature Reserve, 27°16'30.13" S, 31°05'22.88" E, altitude 747 m, 17 Oct. 2008, South Africa, coll. J. Engelbrecht, A. Linstroem and B. Kramer.

Kosi River system. SAIAB 88615(11), field nos. PM09A01, PM09A03–PM09A07, 25 Oct. 2009, and field nos. PM09A85–PM09A89, 28 Oct. 2009, SL range 6.2–17.8 cm, seven females, four males, Siyadla River, 27°04'53.0" S, 32°47'11.0 " E, elevation 29 m, South Africa, coll. P. Maake, E. Swartz, B. Kramer; SAIAB 88633(11), field nos. PM09A61–PM09A71, SL range 9.9–14.8 cm, at least five females and three males, Mahlambane River (Swamanzani System), 26°59'36.3" S, 32°44'34.3" E, Manguzi village, 27 Oct. 2009, South Africa, coll. P. Maake, E. Swartz, B. Kramer; SAIAB 88637(2), field nos. PM09A90, female 10.9 cm, and PM09A91, male 16.2 cm, Nkanini River at Kosi Bay Village, 26°56'57.9" S, 32°46'39.1" E, 28 Oct. 2009, South Africa, coll. P. Maake, E. Swartz, B. Kramer, all of the above near no. 4 on map of Fig. 1.

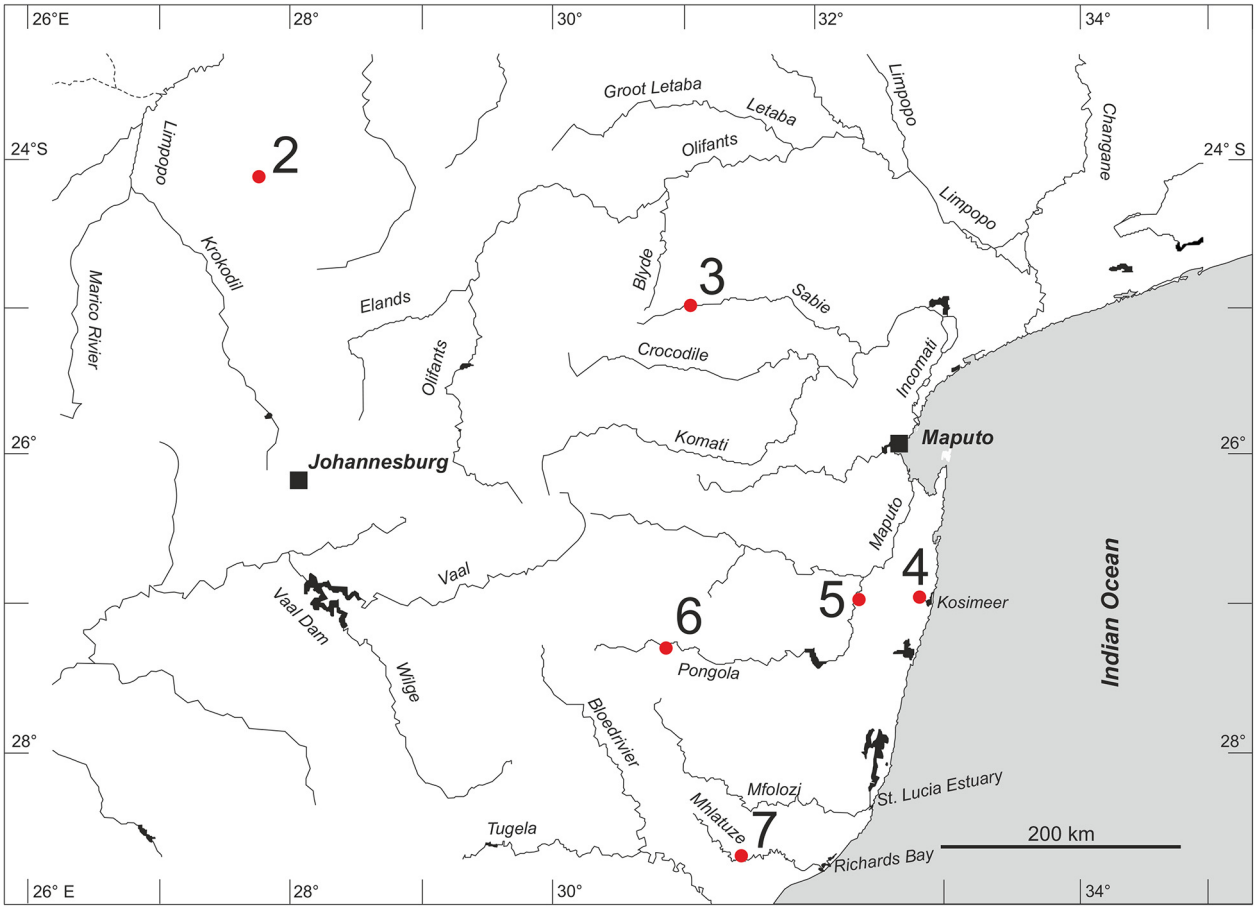

FIGURE 1B.

Sabie River System. SAIAB 54445, field no. 1Sabi, SL 8.6 cm, female, Sand River (tributary of the Sabie) at Londolozi, site 14, 24°47'31" S, 31°31'32" E; SAIAB 54446 (11), field nos. 2Sabi–12Sabi, SL range 5–16.8 cm, at least three females, four males, Sabie River above Hazy View, 25°02'S, 31°00' E; SAIAB 54447 (6), field nos. 13Sabi–18Sabi, SL range 7.4–15 cm, five females, one male, Sabie River above Skukuza just below the weir, site 8, 24°58'35" S, 31°35'05" E; SAIAB 54448 (12), field nos. 19Sabi–30Sabi, SL range 4.8–8.5 cm, three males, nine females or juveniles, Sabie River immediately above Hazy View (municipal picnic site), site 5, 25°01'48" S, 31°01'21" E, SAIAB 54445–54448, all of the above South Africa and coll. by P. Skelton and B. Kramer, 23–25 Sept. 1993; SAIAB 79144, field no. Sa01bi, SL 8.2 cm, female, from Sabie River, Sekurekwane, 24°59' S, 31°11' E, South Africa, coll. F.H. van der Bank and B. Kramer, 27 March 1996; SAIAB 79145, field no. Sa04bi, SL 14.4 cm, male, from the anastomosing reaches of the Sabie/Sand confluence at 24°57'22.7" S, 31°42'38.7" E, South Africa, coll. F.H. van der Bank and B. Kramer, 28 March 1996.

Thirteen specimens, field nos. Sa05bi, Sa09bi–Sa18bi, Sa25bi, Sa31bi, SL range 10.8–17.3 cm, ten females, three males, Sabie River, bridge near Lower Sabie tourist camp, 25°07' S, 31°55' E, South Africa, coll. F.H. van der

Bank and B. Kramer, 29–30 March 1996, EOD recorded in the field and fish then imported live to a European laboratory for behavioural studies; all of the above near locality 3 on Fig. 1. These fish were used in additional behavioural studies (see Introduction) and were then given either to National Zoological Garden, Pretoria, to 'electric fish' colleagues in Germany for their own studies, or public zoos in Germany (in this order of priority).

Electric Organ Discharges. This section is slightly modified from Kramer *et al*. (2016) for the present study. Electric organ discharges (EODs) were recorded in the field immediately after capture, in a 37-l plastic aquarium filled with river water where the fish was collected. Conductivity changes in the water possibly affecting EOD were, therefore, excluded.

Temperature (±0.1 °C) and water conductivity (±1 μS cm^{-1}) were constantly monitored using an electronic apparatus (LF92 by Wissenschaftlich-Technische Werkstätten, WTW, Germany). Fish were placed between a pair of carbon rod electrodes that were connected to a differential amplifier with variable gain (up to ×10; 0.2 Hz ... 100 kHz; filter slopes, -3 dB per octave; electronics workshop, Biology Department, University of Regensburg). Amplifier output was recorded with a digital storage oscilloscope (up to at least 10 MHz conversion rate, amplitude resolution 8 bit, 512 points per trace in the field, replaced by a 100 MHz/9 bit/10 000 points per sweep oscilloscope from 2003 on), and data were transferred onto a computer. Usually eight traces per fish were recorded. Field equipment was battery-operated.

Custom-designed computer programs were used to analyse the EODs (using a software package for signal analysis, Famos v3.1 to v7.0 by imc company, Berlin). When appropriate, EOD duration was corrected to 25 °C using a Q_{10} value of 1.5 (Kramer & Westby 1985) before data analysis.

Definition of EOD waveform variables (Fig. 2): Pamp, peak amplitude of positive P phase (i.e. from baseline to peak, which was set = 1 by definition); Namp, peak amplitude of negative N phase of EOD re: Pamp = 1; Pdur, Ndur, durations of P phase and N phase; PNsep, separation (or interval) between the peaks of the P and N phases; Parea, Narea, areas under the P and N phases. Durations in microseconds; amplitudes in relative Volts (re: P-phase amplitude = 1). Area measures, dimension (V × microseconds). Because of the asymptotic start and termination of an EOD, Pdur started at +5% of Pamp, and Ndur ended at -5% of Pamp. This threshold criterion was also used for Parea and Narea estimations.

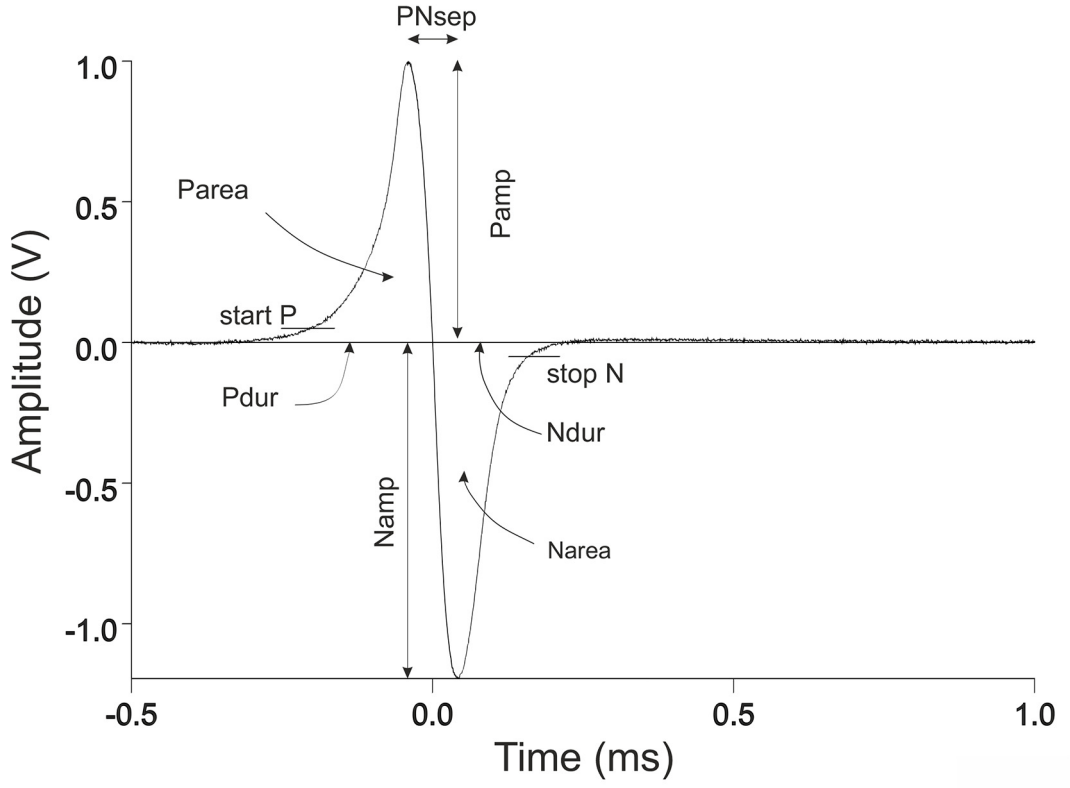

FIGURE 2. Electric organ discharge of a *Marcusenius krameri* with the positive peak amplitude normalized to 1 V, as an example for showing the characters analysed and their definitions. EOD shown was field-recorded from male specimen Mogol27 (ZSM 39535(7) from Mokolo River).

As detailed in Kramer *et al*. (2016), subsequent to EOD recording, fish were killed by an overdose of the anaesthetic 2-phenoxy-ethanol, their standard length (SL) determined using Vernier callipers, and fixed in 10% formalin for morphological studies. Fish were sexed by using the kink criterion of the anal fin base (a kink is absent in females and juvenile males, as confirmed histologically for certain bulldog fish, as described in Kramer (1997) and Kramer *et al*. (2007)).

Statistics. We tested our hypotheses that there were no differences between the species using Multivariate Analyses of Variance (MANOVA) and subsequent Analyses of Variance (ANOVA). Post-hoc tests followed the Games/Howell procedure. We also used Discriminant Analysis (with stepwise variable selection) as an extension of MANOVA. All *p* values are two-tailed. The software used was StatView v 5 and JMP v 11 (SAS Institute, USA).

Results

EOD recordings from specimens captured at different South African localities (Fig. 1) were compared within and among species to identify sex differences and species differences. Representative EOD examples are shown for bulldog fish from all locations, for a female and a male each (Fig. 3). We used a sample of *M. devosi* Kramer *et al*., 2007 from the distant Kenyan Tana River as the outgroup for statistical EOD waveform comparisons.

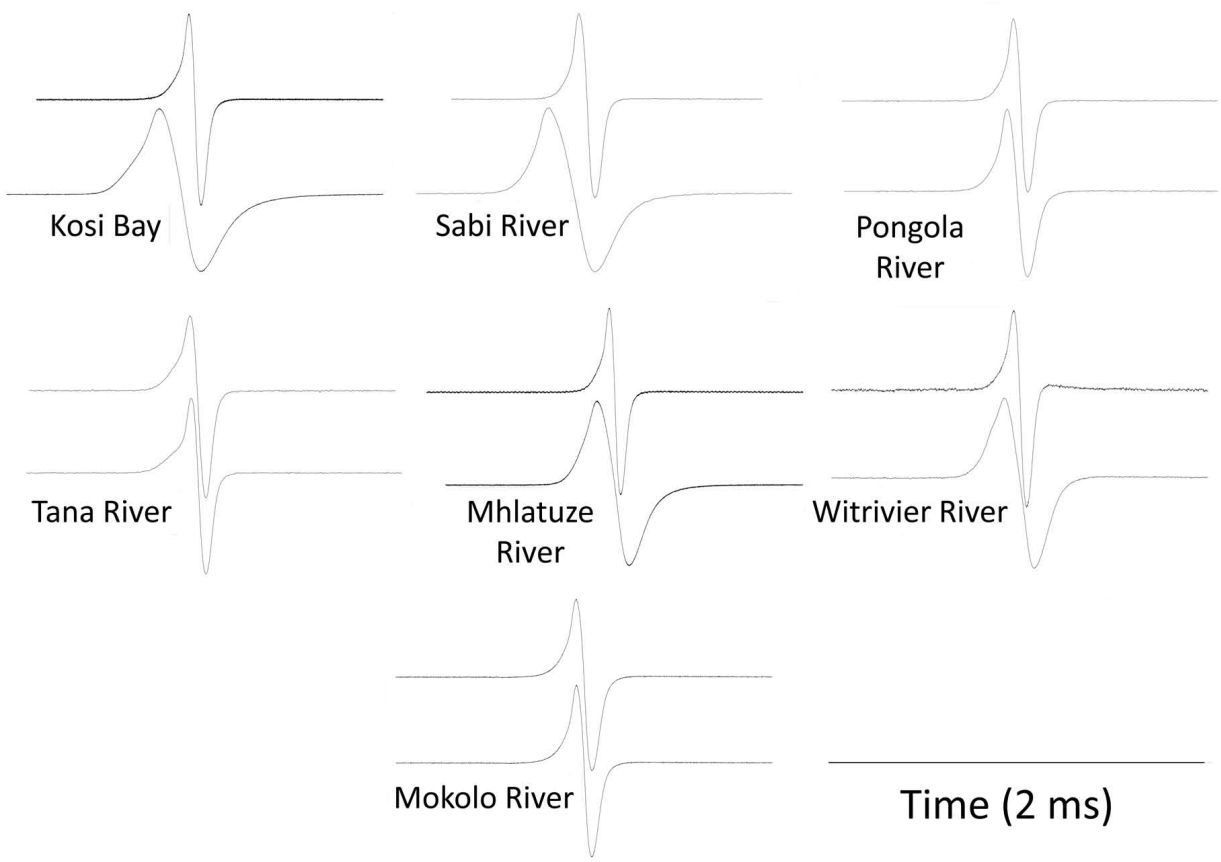

FIGURE 3. Electric organ discharges (EOD) of a male bulldog (below) and a female specimen (above) in each panel. Abscissa, time bar is 2 ms for all panels, ordinate, amplitude (V). EODs are normalised to the same positive peak amplitude from baseline = 1 V. Kosi Bay, *Marcusenius pongolensis* from the Kosi System, specimens PM09A90 (female, SL 10.9 cm) and PM09A91 (male, SL 16.2 cm), SAIAB 88637(2). Sabie River, *M. pongolensis*, specimens 8Sabi (male, SL 13.4 cm) and 9Sabi (female, SL 12.3 cm), both SAIAB 54446(11). Pongola River, *M. pongolensis*, specimens Pon02 (male, SL 18 cm), SAIAB 79148(5), and Pon09 (female, SL 15.5 cm), ZSM 35087(5). Witrivier River, *M. pongolensis,* specimen Wit 02, male, SL 18 cm, and Wit 01, female, SL 9.9 cm, both SAIAB 88846(2). Tana River, *M. devosi*, specimens SAIAB 79139(14), Ta32na, male, SL 10.1 cm, and ZSM 35092, Ta11na, female, SL 11.2 cm. Mhlatuze River, *M. caudisquamatus*, specimen PM09A242, male, SL 17.5 cm, and PM09A238, female, SL 10.2 cm, both SAIAB 191225(6). Mokolo River, *M. krameri*, specimen Mogol27, ZSM 39535(7), male, SL 9 cm, and Mogol29, SAIAB 88888(15), female, SL 9.7 cm.

TABLE 1. EOD waveform characters in females and juveniles combined (FF, females) compared to males MM, males) of seven *Marcusenius* samples from different South African and one Kenyan location.

Origin of samples	Namp (V)	Pdur (µs)	Ndur (µs)	PNsep (µs)	Parea	Narea(V×µs)	SL (cm)
krameri (*N*=22)							
FF Mean/Median* (17)	-1.292	197.65	149.49	75.86	79.11	92.87	8.2*
SE/SIQ*	0.021	3.66	3.94	1.78	1.61	2.17	0.65*
ANOVA *P*	>0.2	>0.5	>0.9	>0.9	>0.4	>0.3	6.8–9.7[1]
MM Mean/Median* (5)	-1.238	193.46	149.1	76.22	76.94	88.68	9*
SE/SIQ*	0.018	3.52	3.24	2.03	1.51	2.04	0.4*
							8.1–9.2[1]
pongolensis (Sabie) (*N*=45)							
FF Mean/Median*(35)	-1.186	179.451	140.032	78.571	74.354	84.189	8.8*
SE/SIQ*	0.013	2.961	2.763	1.613	1.074	1.326	0.25*
ANOVA *P*	0.0004	0.0076	0.0010	0.0023	0.0020	0.0035	4.9–15[1]
MM Mean/Median* (10)	-1.072	216.73	217.66	114.85	98.54	107.45	12.5*
SE/SIQ*	0.032	23.303	41.279	20.79	13.647	13.67	1.735*
							7.8–17.3[1]
pongolensis T (Pongola) (*N*=10)							
FF Mean/Median* (5)	-1.078	191.32	159.6	88.92	80.16	86.16	15*
SE/SIQ*	0.02	8.61	14.975	8.469	4.355	5.803	0.85*
ANOVA *P*	>0.5	>0.2	0.5	>0.5	>0.3	>0.5	8.2–15.5[1]
MM Mean/Median* (5)	-1.06	204.42	172.16	95.08	85.48	90.28	16.4*
SE/SIQ*	0.023	3.939	9.224	6.385	2.91	3.526	1.85*
							12–18[1]
pongolensis W (Witrivier)							
FF Mean/Median* (2)	-1.364	167.7	115.7	67.15	68.75	82.55	8.2,9.9
MM Mean/Median* (1)	-1.135	283.9	281.8	159.4	139	158.2	18
pongolensis (Kosi Bay) (*N*=21)							
FF Mean/Median* (13)	-1.181	182.746	151.154	77.231	76.669	86.308	11.23*
SE/SIQ*	0.025	3.427	8.16	2.189	1.658	1.68	1.5*
ANOVA *P*	0.0111	0.0135	0.0166	0.0068	0.0149	0.0128	8.2–12[1]
MM Mean/Median* (8)	-1.056	239	242.35	117.99	107.125	116.513	14.8*
SE/SIQ*	0.041	26.16	42.904	17.022	14.481	13.97	1.25*
							11.1–17.8[1]
caudisquamatus (*N*=13)							
FF Mean/Median*(10)	-1.153	174.32	145.49	76.45	74.49	82.94	9.2*
SE/SIQ*	0.015	4.074	2.96	2.754	2.251	1.365	2.4*
ANOVA *P*	-	-	-	-	-	-	6.6–13.5[1]
MM Mean/Median* (3)	-1.103	206.4	206.5	106.13	95.233	102.567	10.8*
SE/SIQ*	0.093	37.46	70.82	33.224	23.296	23.94	3.28*
							8.8–17.5[1]
devosi (*N*=22)							
FF Mean/Median*(17)	-1.392	239.365	142.959	75.794	90.824	93.012	11.1*
SE/SIQ*	0.03	6.353	5.133	2.144	2.62	3.034	0.585*
ANOVA *P*	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	8.2–12[1]
MM Mean/Median* (5)	-1.385	231.88	140.9	75.68	88.98	93.04	10.7*
SE/SIQ*	0.014	6.541	4.363	2.591	2.562	3.896	0.3135*
							10.4–11.5[1]

Abbreviations of EOD waveform characters, Material and methods. SE, standard error; * Median and SIQ (semi-interquartile range) for SL (standard length) only. SL also given as size range[1].

Comparisons within samples. In *M. devosi* none of the six EOD characters studied (Namp, Pdur, Ndur, PNsep, Parea, and Narea; Table 1) differed significantly between females ($N = 17$) and males ($N = 5$), as shown by ANOVA. A similar result was obtained for *M. krameri* from the Limpopo System (17 females, 5 males), indicating an absence of EOD sex difference in this species.

We studied four geographical samples of *M. pongolensis*, as re-described by Maake *et al*. (2014). The sample from the Sabie River (35 females, 10 males) matched the one from the Kosi System (13 females, 8 males) in showing statistically significant sex differences in each one of the six EOD characters studied (ANOVA, $p < 0.02$). For example, Namp was lower, and Ndur as well as Pdur greater in males than females. The *M. pongolensis* sample from the Pongola River (including the type locality) comprised only five males and five females. The results of the Pongola River sample were similar to the two preceding samples, as the mean Namp was smaller; all other mean values were greater in males than females. However, the differences between the sexes were not statistically significant in the Pongola sample. The smallest *M. pongolensis* sample (two females, one male) was from the Witrivier River, a tributary to the Pongola River. EOD characters differed between the two sexes in the same way as in the other *M. pongolensis* samples, but statistical difference testing was not possible. Combining the two Pongola System samples, so that 7 females were tested against 6 males, yielded a mean 218 µs (male) vs 187 µs (female) for Pdur ($p < 0.048$).

EOD mean values differed between the sexes of *M. caudisquamatus* in a similar way to *M. pongolensis*: mean Namp was smaller, but all other character values were greater in males than females. However, with only three *M. caudisquamatus* males (compared to 10 females) statistical difference testing for significance was not possible.

TABLE 2. Comparison of characters of the electric organ discharge (EOD) in males of South African *Marcusenius* species from different South African locations to *M. devosi* from Kenya, using Multivariate and Univariate Analyses of Variance (MANOVA, ANOVA). *Marcusenius krameri* from Mokolo River, Limpopo System; *M. pongolensis* (Sabie) from Sabie River; *M. pongolensis* T, from Pongola = Type River; *M. pongolensis* (Kosi Bay), from Swamanzani and Siyadla rivers, Kosi Bay; *M. caudisquamatus*, from Mhlatuze River; *M. devosi*, from Tana River, Kenya. ANOVA *p* values in the body of the table not shown when >0.05. For explanation of EOD waveform characters, see Figure 2.

MALES	Namp (V)	Pdur (µs)	Ndur (µs)	PNsep (µs)	Parea (V×µs)	Narea (V×µs)
MANOVA				$<10^{-4}$		
ANOVA	$<10^{-4}$					
post tests						
caudisquamatus, devosi						
caudisquamatus, pongolensis (Kosi Bay)						
caudisquamatus,krameri						
caudisquamatus, pongolensis (Sabie)						
caudisquamatus, pongolensis (Type)						
devosi, pongolensis (Kosi Bay)	<0.01					
devosi,krameri	<0.01	<0.05			<0.05	
devosi,pongolensis (Sabie)	<0.01					
devosi,pongolensis (Type)	<0.01					
Pongolensis (Kosi Bay), *krameri*	<0.05					
pongolensis(KosiBay),*pongolensis*(Sabie)						
pongolensis(KosiBay),*pongolensis*(Type)						
krameri, pongolensis (Sabie)	<0.01					
krameri, pongolensis (Type)	<0.01					
pongolensis(Sabie),*pongolensis*(Type)						

Comparisons among samples. The first hypothesis addressed was: the EODs of all samples of the same sex are identical among groups, that is, the EODs do not reflect differences among the species or allopatric groups. The precaution of sex-specific testing was indicated because in *M. pongolensis* (originating from the Sabie and the Crocodile rivers of the Incomati System) a sex difference in EOD waveform had been discovered (Kramer 1997, Kramer *et al*. 2007).

Specimen numbers in males/females (plus juveniles if present) that were available for inferential statistical analysis were as follows: *M. krameri*, $N = 5/17$; *M. pongolensis,* $N = 10/35$ (Sabie River), $N = 5/5$ (Pongola River), $N = 8/13$ (Kosi System); *M. caudisquamatus* $N = 3/10$ (Mhlatuze System), and *M. devosi* $N = 5/17$ (Tana River). The sample of $N = 3$ for the *M. caudisquamatus* males was excluded from the analysis, but descriptive statistics are given.

The comparison of female EODs amongst species or groups yielded a MANOVA $F_{\geq 6,90} \geq 7.094$, $p < 0.0001$; and for males $F_{\geq 6,29} \geq 3.327$, $p<0.0001$ (MANOVA p-value: same for Wilks' Lambda, Roy's Greatest Root, Hotelling Lawley Trace, Pillai Trace tests). In both sexes, the null hypothesis of no EOD waveform difference among groups was, therefore, rejected.

In the ANOVAs subsequent to MANOVA, the EOD character Namp proved to contribute most to the variation observed in males (Table 2). As shown by Games-Howell, post hoc tests to find out which group pairings underlie the variation observed, three *M. pongolensis* groups all differed significantly from *M. devosi* and *M. krameri* by smaller Namp values (Table 2). *M. krameri* EODs also showed smaller Pdur and Parea values than *M. devosi*.

TABLE 3. Comparison of characters of the electric organ discharge (EOD) in females of South African *Marcusenius* species from different South African locations to *M. devosi* from Kenya, using Multivariate and Univariate Analyses of Variance (MANOVA, ANOVA). *Marcusenius krameri* from Mokolo River, Limpopo System; *M. pongolensis* (Sabie) from Sabie River; *M. pongolensis* T, from Pongola = Type River; *M. pongolensis* (Kosi Bay), from Swamanzani and Siyadla rivers, Kosi Bay; *M. caudisquamatus*, from Mhlatuze River; *M. devosi*, from Tana River, Kenya. ANOVA p values in the body of the table not shown when >0.05. For explanation of EOD waveform characters, see Figure 2.

FEMALES	Namp (V)	Pdur (µs)	Ndur (µs)	PNsep (µs)	Parea (V×µs)	Narea (V×µs)
MANOVA				$<10^{-4}$		
ANOVA	$<10^{-4}$	$<10^{-4}$			$<10^{-4}$	0.003
post tests						
caudisquamatus, devosi	<0.01	<0.01			<0.01	
caudisquamatus, pongolensis (Kosi Bay)						
caudisquamatus, krameri	<0.01	<0.01				
caudisquamatus, pongolensis (Sabie)						
caudisquamatus, pongolensis (Type)						
devosi, pongolensis (Kosi Bay)	<0.01	<0.01			<0.01	
devosi, krameri		<0.01			<0.01	
devosi, pongolensis (Sabie)	<0.01	<0.01			<0.01	
devosi, pongolensis (Type)	<0.01	<0.05				
Pongolensis (Kosi Bay), *krameri*	<0.05					
pongolensis(KosiBay),*pongolensis*(Sabie)		<0.01				
pongolensis(KosiBay),*pongolensis*(Type)						
krameri, pongolensis (Sabie)	<0.01	<0.05				<0.05
krameri, pongolensis (Type)	<0.01					
Pongolensis (Sabie), *pongolensis* (Type)	<0.05					

In females, four EOD characters contributed significantly to the variation among groups: Namp, Pdur, Parea and—only in one species pairing—Narea (Table 3). Namp was the most variable character, as it differed significantly in nine species (group) pairings at $p<0.05$. Thus, the two species with the strongest N phase amplitudes, *M. devosi* and *M. krameri*, proved significantly different from most other groups. The next diverse character was Pdur where there were 8 species (or group) pairings with significant differences at $p<0.05$. In four of these pairings significant differences occurred also in the character Parea which is correlated with Pdur.

In order to test more closely for multivariate differentiation among samples and species, we conducted Discriminant Analyses as an extension of MANOVA. In both sexes, the specimens of two species showed non-overlapping 95% confidence circles, independent of any other group: *M. devosi* of course, but also *M. krameri* (Figures 4 and 5). This is support for the identity of *M. krameri* as a separate species.

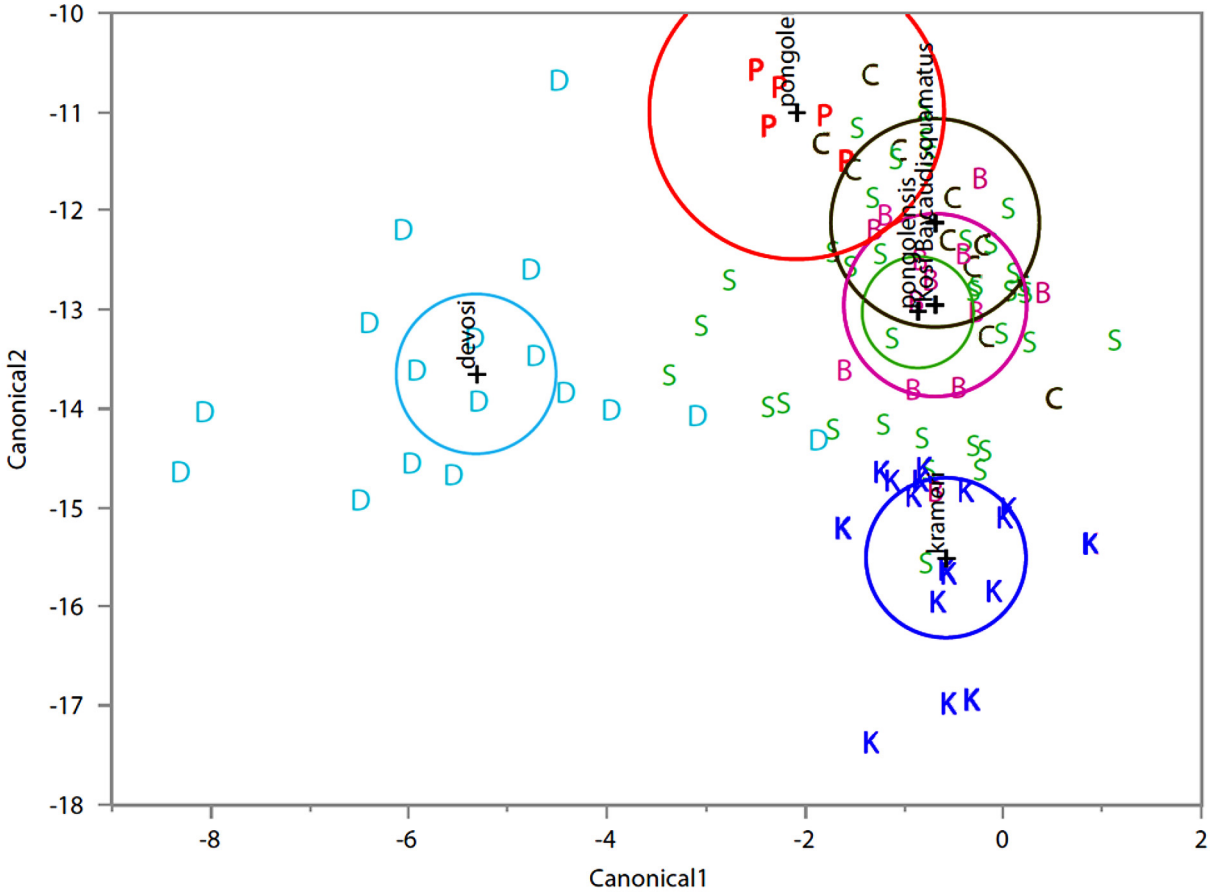

FIGURE 4. Female. Discriminant analysis (DA) of characters of the electric organ discharge (EOD) waveform of five female plus juvenile samples of South African *Marcusenius* species, compared to *M. devosi* specimens from Kenya (D symbols). B, S and P symbols, *M. pongolensis* specimens from different South African locations; K symbols, *M. krameri*. EOD waveform characters that were included in DA as given in Table 3, but PNsep excluded because of irrelevance in the female sample.

The circles for the Sabie and Kosi system samples of *M. pongolensis* were separate from those for the previous two species in both sexes. For females, the confidence circle for the Kosi System sample overlapped the Sabie sample circle completely (Figure 4), and nearly so for males (Figure 5). This close association supports the notion that the Kosi System and the Sabie River bulldogs are the same species. The circle for the samples from the Pongola River was well separated (significantly different) from that for the Sabie System, and separated at least at borderline significance from the circle for the Kosi System individuals in both sexes. This is a surprise for members of the same species and needs more sampling and verification.

The EOD circle for female *M. caudisquamatus* partially overlapped that of the three *M. pongolensis* female samples (specimen numbers were insufficient to test the males). Therefore, *M. caudisquamatus* female EODs do not appear to be clearly differentiated from any of the other *M. pongolensis* samples. In both discriminant analyses (Figs 4 and 5) the lowest number of misclassifications (best model fit) was found by stepwise variable selection,

identifying and excluding weak or redundant discriminators. Thus, Ndur was excluded from the analysis for males, and PNsep for females.

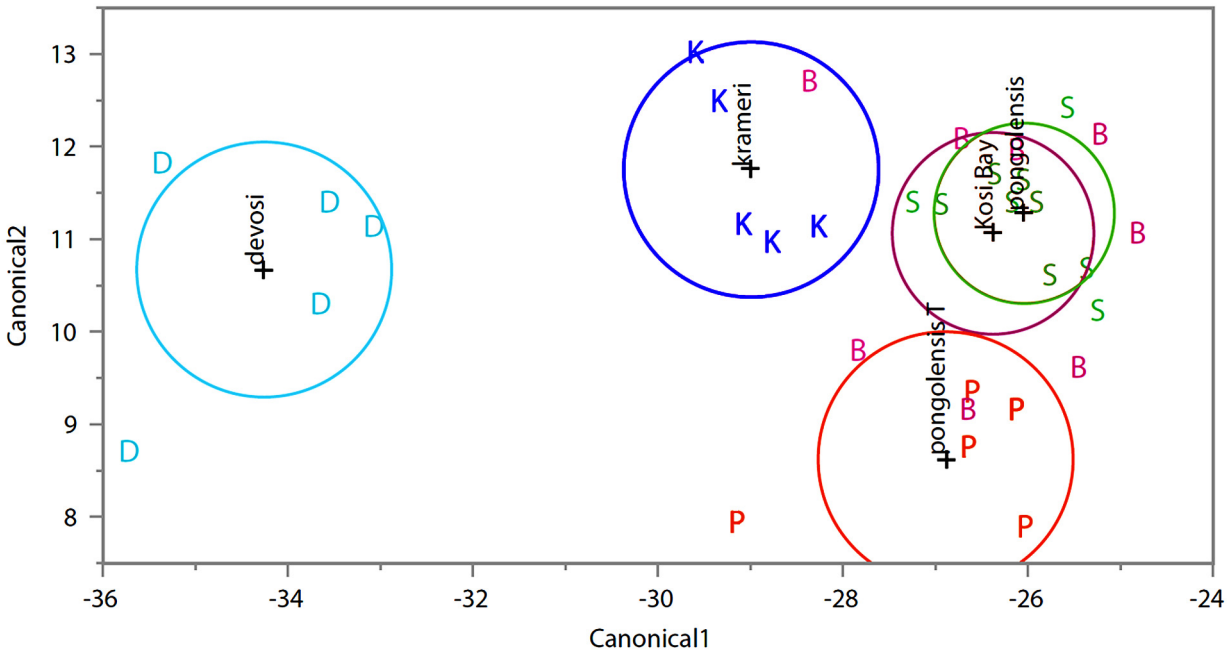

FIGURE 5. Male. Discriminant analysis (DA) of characters of the electric organ discharge (EOD) waveform of five male samples of South African *Marcusenius* species, compared to *M. devosi* specimens from Kenya (D symbols). B, S and P symbols, *M. pongolensis* specimens from different South African locations; K symbols, *M. krameri*. EOD waveform characters that were included in DA as given in Table 2, but Ndur excluded because of irrelevance in the male sample.

Discussion

The electric organ discharges (EODs) of *M. devosi* and *M. krameri* differed significantly from each other, and from all other South African groups tested by their lack of an obvious difference between sexes (Table 1, Figure 3). EOD waveforms differed between the sexes in all other samples of *M. pongolensis* and *M. caudisquamatus* from different localities in this study, confirming the conclusions of Kramer *et al*. (2007) for the populations they studied. Furthermore, *M. krameri*'s EOD waveform was supported as an entity distinctly separate from the EODs of all other South African bulldog fish species in comparisons of the two sexes. This is in agreement with Maake's *et al*. (2014) morpho-genetic study that designated species status for *M. krameri*.

Maake *et al*. (2014) also reported intraspecific morphological differentiation in *M. pongolensis*: specimens from the independent Kosi System clustered separately from the other South African *pongolensis* origins, because of significant differences in five morphological characters (Maake *et al*. 2014: Figure 5b, Table 4). This study on EOD waveform characteristics also found differences between Kosi System samples and *M. pongolensis* (Pongola River) samples; however, it added Sabie System samples the EODs of which were also different. In Discriminant Analysis, the EODs of Incomati (Sabie) System samples were indistinguishable from Kosi System samples (independently for both sexes), in spite of the Incomati System being separated from the Kosi System by the Pongola System, that is, there is no continuous Sabie-Kosi population. Both Sabie and Kosi system EOD samples were significantly differentiated from Pongola System samples (no overlap of 95% confidence circles), again independently for both sexes. The present EOD study implies that both Sabie and Kosi *M. pongolensis* samples are differentiated from *M. pongolensis* of the Pongola System. This contrasts with the genetic/morphological results of Maake *et al*. (2014) in which the Kosi samples were differentiated from Sabie/Pongola samples. Arguably, the latter view is in better agreement with the observation that the Kosi System is an independent coastal system with slower water flow and more lake-like inundation events, whereas the other two systems arise in the Drakensberg where strong currents and intermittent violent inundations are rather common (LBK, personal observation).

Therefore, different morphological adaptations are expected and have in fact been pointed out by Maake *et al*. (2014). The intriguing variation in *M. pongolensis* between type river samples and samples from the neighbouring systems result in a polymorphism that should be studied in more detail. Explanations for this, though hypothetical, could be the existence of unknown taxa in the neighbouring systems, or incomplete lineage sorting.

In a comparison of *M. pongolensis* and *M. caudisquamatus*, EOD differentiation from each other could not be conclusively shown by the present study. This was unexpected, following Maake *et al*.'s (2014) conclusive study showing that *M. pongolensis* is significantly divergent from *M. krameri* and *M. caudisquamatus* (2.0–5.8% divergence), but closest to *M. macrolepidotus*, based on phylogenetic analyses of the mitochondrial cytochrome *b* gene and comparisons of morphological characters.

One reason for this lack of differentiation could be that *M. caudisquamatus* represents the only snoutfish species in this extremely southern habitat for a mormyrid, where there is no competition for the use of the electric communication channel from other species, hence little pressure for further evolution; certainly not for evolution driven by character displacement, as may be assumed for the snoutfish species in rivers farther north. There, *Petrocephalus wesselsi* Kramer & van der Bank, 2000 and, depending on the system, one additional *Marcusenius* species also communicate by EOD. Still, verification by additional sampling of *M. caudisquamatus* would be helpful.

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