Petrocephalus of Odzala offer insights into evolutionary patterns of signal diversification in the Mormyridae, a family of weakly electrogenic fishes from Africa

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1. Introduction

Weakly electric fishes exhibit novel evolutionary specializations for electrolocation as well as communication in the electrosensory modality (Bullock et al., 2005). Ever since the discovery that weakly electric fishes of Africa (superfamily Mormyroidea) produce weak electric organ discharges (EODs) as part of a specialized electrosensory communication and orientation system, sympatric species often express distinctive EODs, which may contribute to species recognition during mate choice in some lineages. Striking examples of interspecific EOD variation within assemblages have been reported for two monophyletic radiations: the Paramormyrops of Gabon and the Campylomormyrus of Lower Congo. Here, we describe a speciose assemblage of Petrocephalus in the Lékoli River system of Odzala National Park, Republic of Congo. This widespread genus comprises the subfamily (Petrocephalinae) that is the sister group to all other mormyrids (Mormyrinae). Eleven Petrocephalus species were collected in Odzala, five of which are not described taxonomically. We quantify EOD variation within this assemblage and show that all eleven species produce EOD waveforms of brief duration (species means range from 144 to 663 µs) compared to many other mormyrids. We also present reconstructed phylogenetic relationships among species based on cytochrome b sequences. Discovery of the Odzala assemblage greatly increases the number of Petrocephalus species for which EODs and DNA sequence data are available, permitting a first qualitative comparison between mormyrid sub-families of the divergence patterns that have been described within lineages. We find that the Petrocephalus assemblage in Odzala is not a monophyletic radiation. Genetic divergence among Petrocephalus species often appears higher than among Paramormyrops or Campylomormyrus species. In contrast, results of this study and others suggest that Petrocephalus may generally exhibit less interspecific EOD divergence, as well as smaller sex differences in EOD waveforms, compared to Paramormyrops and Campylomormyrus. We discuss possible causes and consequences of EOD diversification patterns observed within mormyrid subfamilies as a framework for future comparative studies of signal evolution using this emerging model system.

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and Bass, 1981; Moller and Serrier, 1986; Paintner and Kramer, 2003; Xu-Friedman and Hopkins, 1999). Among all groups of African electric fishes described to date, the Paramormyrops of Gabon (previously known as the ‘Gabon-clade Brineonomyrus’) and the Campylomormyrus of Lower Congo River are noteworthy because they contain the largest numbers of closely related species known to co-occur within assemblages (Feulner et al., 2007; Sullivan et al., 2004). Sympatric species of Paramormyrops or Campylomormyrus appear to exhibit distinctive, species-specific EOD waveforms (Arnegard et al., 2005; Arnegard and Hopkins, 2003; Feulner et al., 2006; Hopkins, 1999a; Sullivan et al., 2002). This pattern of signal variation – that is, relatively high stereotypy within species and divergence among sympatric species – suggests that EODs might act as ‘species markers’ during mate choice in some taxa. From an evolutionary viewpoint, EOD waveform variation deserves study, as it may be directly involved in the formation and maintenance of species boundaries within these rapidly radiating groups.

Systematic investigations using both morphological and molecular characters support the monophyly of the Mormyridae within the Osteoglossomorpha, as well as the recognition of two mormyriform families: the monotypic Gymnarchidae (Gymnarchus niloticus) and the speciose Mormyridae (Benveniste, 1994; Lavoué et al., 2000; Lavoué and Sullivan, 2004; Sullivan et al., 2000; Taverne, 1972). The family Mormyridae is, in turn, divided into two subfamilies: the monophyletic Mormyrinae with 19 genera and more than 180 species described thus far (Froese and Pauly, 2008); and the Petrocephalinae, comprising only one genus (Petrocephalus) in which about 25 of the 46 nominal species are currently considered valid (Bigorne and Pauly, 1991; Froese and Pauly, 2008; Gosse, 1984; Kramer and van der Bank, 2000; Lavoué et al., 2004). Following Lissmann’s discovery of the electroreceptive modality, most descriptive studies of electric fish assemblages in Africa have focused on members of the more diverse mormyrid subfamily, the Mormyrinae (Hopkins, 1999a; Kramer, 1990; Moller, 1995). Its sister group, the Petrocephalinae, is an important comparative reference for better understanding patterns of evolution of EODs and other traits within mormyrids as a whole, yet few comparative studies of the two mormyrid subfamilies have been made.

Relatively little is known about the ecology and systematics of Petrocephalus. The genus is distributed throughout large regions of tropical and subtropical Africa, where different species inhabit a variety of freshwater environments such as rivers, streams, and lakes. In some fish communities, Petrocephalus species are the most abundant electric fishes, or perhaps even the most abundant fish species present (personal observation). Like other mormyrids, Petrocephalus species are active at night. Few data on feeding ecology have been collected for species in this genus (Hyslop, 1986; Kouamélan et al., 2006; Matthes, 1964), and breeding has only been studied in captivity in a single species (Kirschbaum, 2006; Kirschbaum and Schugard, 2002). EOD waveforms were only known from nine Petrocephalus species prior to the present study (Bratton and Kramer, 1988; Hopkins et al., 2007; Kramer, 1997a; Kramer and van der Bank, 2000; Lavoué et al., 2004; Moritz et al., 2008; Paugy et al., 1994; Sullivan et al., 2000). While several morphological characters support the monophyly of Petrocephalus (Taverne, 1969), DNA samples from only four Petrocephalus species have been available previously to test and support this hypothesis with genetic evidence (Sullivan et al., 2000). Comparison of evolutionary patterns of electric signal diversification between Petrocephalus and other mormyrid groups has been hampered in the past by the low number of Petrocephalus species with recorded EOD waveforms and the lack of phyletogenic context for electric signal variation within this sister lineage to all other mormyrids.

During two recent expeditions to Odzala National Park in the Republic of Congo (Fig. 1), we encountered a surprising abundance and diversity of Petrocephalus species in the Lékoli River system (northwestern Congo basin). Our electrical recordings there double the number of described and operational Petrocephalus species with known EOD waveforms. Here, we quantify EOD variation within the Petrocephalus assemblage of Odzala. Electric organ anatomy is examined in several of the species. We generate complete sequences of the mitochondrial cytochrome b gene (cyt b) to estimate phylogenetic relationships within the Odzala assemblage and to genetically evaluate our operational taxonomic designations for five new Petrocephalus species. Within the framework of a resulting phylogenetic hypothesis for Petrocephalus, we begin to compare genetic divergence and EOD diversification patterns between this assemblage and two speciose mormyrid groups (Paramormyrops of Gabon and Campylomormyrus of Lower Congo) in which phylogenetic relationships were previously estimated using the same molecular marker.

2. Material and methods

2.1. Odzala field collections and taxonomic designations

We collected a total of 953 specimens of Petrocephalus from the Lékoli River and vicinity (Fig. 1) during two expeditions to Odzala National Park (Republic of Congo), which took place 5 August–5 September (in 2002) and 22 June–1 July (in 2006). These periods correspond to the long dry season in Odzala, which typically occurs from early June to late August each year. Most specimens were collected using fish traps baited with earthworms, dip nets (on hoops 1-m in diameter), or cast nets. Gill nets were used in a few instances. In the field, we designated each specimen as belonging to one of eleven operational taxonomic units (OTUs, referred to as Petrocephalus spp. 1–11) on the basis of body and fin shape, mouth position, and presence and placement of spots.

All of the Petrocephalus specimens collected from Odzala were deposited in the collection of the Cornell University Museum of Vertebrates (CUMV). Subsequent comparison of these specimens with type material and original morphological descriptions published for known Petrocephalus species allowed us to assign six of our OTUs to currently valid species: Petrocephalus sp. 1 to P. binoatus (Pellegrin, 1924); Petrocephalus sp. 4 to P. balayi (Sauvage, 1883); Petrocephalus sp. 5 to P. microphthalmus (Pellegrin, 1908); Petrocephalus sp. 7 to P. christyi (Boulenger, 1920); Petrocephalus sp. 8 to P. sauvagii (Boulenger, 1887); and Petrocephalus sp. 10 to P. grandoculis (Boulenger, 1920) (see Fig. 2). We did not find good morphological correspondence between any described species and five of our OTUs (Petrocephalus sp. 2, Petrocephalus sp. 3, Petrocephalus sp. 6, Petrocephalus sp. 9, and Petrocephalus sp. 11). The original numbering scheme for these five operational species is retained throughout the present paper. We do so to help future investigators compare results of this study to our field notes for 2002 and 2006, copies of which were also deposited in the CUMV.

Formal taxonomic descriptions of these five un-described species are beyond the scope of the present study, and they are, instead, being prepared for another paper. Morphometric and meristic measurements made on preserved specimens for this forthcoming paper indicate that all five un-described species can be distinguished morphologically from one another and from all other known Petrocephalus species (Lavoué et al., in preparation). This finding is especially strong evidence that these five OTUs represent good species, given that all are broadly sympatric within a very small region of the Lékoli River (shown in Fig. 1).

2.2. Electric organ discharge (EOD) recordings

While in the field, we recorded multiple EODs from each of 153 Petrocephalus specimens. Each individual was first transferred to a
plastic tank (ca. 80 cm long × 30 cm wide × 30 cm deep) filled with water from the location of capture. When measured, water conductivity at the collection sites ranged from 25 to 70 μS/cm. For EOD recording, we used silver/silver-chloride electrodes connected to a low-noise, differential amplifier (CWE Electronics, Inc. Bioamplifier) with frequency response 0.01 Hz–50 kHz. Electrodes were positioned at opposite ends of the tank. Recordings were monitored with a portable oscilloscope in order to avoid amplifier overload or signal distortion, and high sampling rates were used to capture fine waveform details. As the fish faced the positive electrode, signals were captured by digitizing EODs using an IOtech Wavebook sampling at 200 to 500 kHz (16-bit A/D converter; IOtech, Inc.). Waveforms were stored on a laptop computer using custom designed software. Ambient water temperature was measured during each recording (range = 22.5–29.2 °C; mean ± std. dev. = 26.0 ± 1.7 °C). Immediately after several EODs were recorded from each individual, we euthanized the fish with an overdose of the anesthetic MS222 and assigned it a unique specimen number corresponding to the digitized EODs. Specimens were then fixed in buffered 10% formalin for two weeks and transferred to 70% ethanol for subsequent storage. All collection and fish handling methods conformed to protocols approved by Cornell University’s Center for Research Animal Resources.

2.3. Examination of electric organ anatomy

EODs are generated by the summed activities of electrocytes making up the electric organ, which is contained in the caudal peduncle of the fish. Electric organs were examined histologically in the following Odzala species: *P. balayi* (specimen 5079, CU 87851); *P. microphthalmus* (specimen 5092, CU 87940); *P. sauvagii* (specimen 5206, CU 87864); and *P. sp. 9* (two individuals: specimen 5263, CU 88097; and an un-vouchered specimen from lot CU 89188). Some of the caudal peduncle was removed from these specimens after the whole fish had been previously fixed in the field and transferred

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**Fig. 1.** Map of Africa showing Odzala National Park (inset), which is located in the northwestern part of the Republic of Congo. An oval surrounds the region from which Petrocephalus specimens were collected in Odzala. The overall map of Africa also shows collection localities for the other Petrocephalus specimens used in this study (gray circles).
to 70% ethanol for storage. Electric organ samples were then infiltrated with JB-4 plastic embedding medium (Polysciences, Inc.), sectioned in parasagittal plane with a steel knife at 7–10 μm, and stained with toluidine blue. Mounted sections were examined under light microscope. Using a classification system previously established for mormyrids (Alves-Gomes and Hopkins, 1997), we characterized electrocyte ‘type’ with respect to anatomy of the stalk system and innervation pattern, which have important effects on the resulting EOD waveform (Bass, 1986b; Bennett, 1971).

2.4. Analysis of EOD waveform variation

Recorded EODs of all species were analyzed using custom software written for Matlab (Mathworks, Inc.). A single EOD waveform was arbitrarily selected among the multiple recordings for each individual. We used a method similar to that developed by Arnegard and Hopkins (2003) to measure waveform landmarks and EOD power spectra. To make results of our analysis comparable to published results for a recently-described Petrocephalus species, we employed some of the slight modifications to this method implemented by Lavoué et al. (2004). Total peak-to-peak amplitudes of all EODs were first normalized to a value of one. The time base of all EODs was then standardized to a uniform temperature of 25 °C to improve quantitative comparison of waveforms recorded at different ambient temperatures (T in °C). This was accomplished by adjusting the digitizing sampling rate from $R_T$ (the original sampling rate) to $R_{25 \text{°C}}$ (the sampling rate at 25 °C) using the temperature coefficient formula for rate functions:

$$R_{25 \text{°C}} = R_T \cdot \left(\frac{Q_{10}}{10}\right)^{(15 - T)/10}.$$
We used a $Q_{10}$ value of 1.6, which we established empirically for *Petrocephalus*. Given the modest variation in recording temperatures during our fieldwork in Odzala, however, changes to EOD waveforms resulting from this adjustment were minor. Although temperature corrections are applied to EOD measurements, we plot all EOD traces as raw, uncorrected waveforms to show signal variation under ambient conditions.

Fig. 3 provides abbreviations for all EOD measurements. The start ($T_1$) and end ($T_2$) of each EOD were taken as the first and last points, respectively, that deviated from baseline by at least 1.5% of total, peak-to-peak amplitude (Lavoué et al., 2004). EOD duration was calculated as the time difference between these two landmarks. We then determined the first ‘major’ peak of the EOD (i.e., the first peak with an amplitude at least as large as 5% of the waveform’s total, peak-to-peak swing, regardless of the sign of this peak’s voltage); this was labeled ‘$P_1$’. All other peaks at least as large as 1.5% of peak-to-peak amplitude were detected and numbered sequentially relative to $P_1$. Normalized voltages of all peaks were recorded. Durations of individual peaks were calculated between $T_1$ or $T_2$ and the nearest relevant zero crossing, or between two internal zero crossings, as appropriate (Fig. 3A). When a peak was apparent but its amplitude was less than 1.5%, we determined its normalized voltage for reporting purposes but set its duration to zero. This zeroing ensured that individual peak durations summed to the total duration calculated for each EOD. In addition to these time-domain waveform measurements, we also computed a fast Fourier transform (FFT) and peak spectral frequency of each EOD (Fig. 3B). A fourth EOD peak, ‘$P_4’ (Fig. 3C), was absent or less than 1.5% of peak-to-peak amplitude in all but three (of 153) individuals. Therefore, $P_4$ voltage and duration were excluded from the statistical analyses described below. In addition, a positive-going ‘minor’ initial peak (i.e., equal to or greater than 1.5%, but less than 5% of peak-to-peak amplitude) was found to occur before $P_1$ in all EODs of *Petrocephalus* sp. 9; this minor initial peak was labeled ‘$P^*_1’. No minor peaks were found to precede $P_1$ in the EODs of any other species. Due to the presence of $P^*_1$ in the EODs of *P*. sp. 9, duration of $P_1$ was calculated between two zero crossings for this species (Fig. 3D), whereas it was calculated between $T_1$ and the subsequent zero crossing for all other species (Fig. 3A). As with $P_4$, voltage and duration of $P_0$ were also excluded from ‘among-species’ statistical tests.

To the extent possible, we evaluated all measurements of EOD waveform variation for sex differences within species. To accomplish this, one investigator examined the body profile of each preserved specimen along the base of its anal fin in a ‘blind’ manner (i.e., without simultaneous consideration of EOD variation). In mormyrids, a strongly indented (as opposed to straight) anal fin base is an androgen-dependent character exhibited only by males (Herfeld and Moller, 1998; Pezzanite and Moller, 1998). This trait is often associated with sexual maturation and/or breeding activity. In the field, it is commonly observed in mature males of numerous mormyrid species (including *Petrocephalus* spp.) during both rainy and dry seasons (personal observation). On the basis of this trait, specimens were identified as ‘obvious males’ or ‘other individuals’. Only a subset of males can be identified by this method of evaluating sex. When studied, however, within-species divergence of male

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**Fig. 3.** Summary of eight characters quantified in all *Petrocephalus* EODs recorded in Odzala. (A) Example of an EOD recorded from *Petrocephalus* sp. 6 illustrating seven landmark-based measurements: normalized potentials and durations of peaks 1–3; and total EOD duration. Shown are landmarks describing the beginning ($T_1$) and end ($T_2$) of the waveform, as well as zero-crossings needed to calculate durations of various phases. $P_1$ is defined as the first ‘major’ waveform peak at least as large as 5% of total, peak-to-peak amplitude. All other peaks (detection threshold = 1.5% of total amplitude) are numbered relative to $P_1$. (B) Power spectrum of the same EOD showing peak spectral frequency, the eighth EOD character measured for all recorded individuals. (C) A final negative overshoot ($R_0$) is at least 1.5% of total peak-to-peak amplitude in only three individuals (here, *Petrocephalus christyi* specimen 5173). (D) Only *Petrocephalus* sp. 9 exhibits a first ‘major’ peak that is negative-going under the standard recording geometry. The EOD of this species is also unique in containing a ‘minor’ waveform peak ($P^*_0$) before $P_1$. Duration of $P_4$ is calculated between two zero crossings in *P*. sp. 9, whereas it is calculated between $T_1$ and the subsequent zero crossing in all other species. $P_4$ never attains 1.5% of the EOD’s total, peak-to-peak swing in *P*. sp. 9.
EOD waveforms has also been found to be androgen-dependent and is often associated with male maturation, social dominance, and/or breeding activity (Bass, 1986a; Carlson et al., 2000; Möller, 1995). Thus, morphological categorization of sex is a useful way to explore collections of recordings for potential sex differences in EOD waveforms. Fig. 4 gives the resulting sample sizes of the two categories of specimens collected for every species. Within the six species for which at least four individuals were collected in each category, waveform measurements were compared between ‘obvious males’ and ‘other individuals’ using two-sided t-tests (with separate variance estimates for the two categories, when appropriate). In these comparisons and all other statistical tests, arcsine transformations were used to improve the fit of peak voltage measurements (i.e., ratio data) to normal distributions. In order to keep these within-species t-tests as liberal as possible for the detection of sex differences, we did not correct the significance level to account for simultaneous examination of multiple measurements made on each EOD. When no evidence of a sex difference was found, we pooled all individuals within that species for subsequent statistical comparisons among species. One-way

![Fig. 4. Superimposed EODs of all recorded individuals for each Petrocephalus species in the Odzala assemblage. One EOD was arbitrarily selected from each individual for plotting and analysis. Waveforms were normalized to the same peak-to-peak voltage and plotted (head-positive up) on the same time scale as overlays centered on the largest positive peak of each waveform (scale bar = 0.2 msec). Shown for each plot are the numbers of ‘obvious males’ (see text) and the number of all ‘other individuals’. Within species, possible sex differences in the EODs were only apparent for P. binotatus. EODs of obvious males and all other individuals are superimposed together in the other species.]
2.5. PCR amplification and cyt b gene sequencing

All available DNA samples for the genus Petrocephalus were used for phylogenetic reconstruction based on cyt b sequences, a genetic marker that has been applied to the mormyrid family as a whole, as well as within the Paramormyrops and Cumphylomormyrus radiations. To root the trees, we used available sequences for three outgroup species belonging to the subfamily Mormyrininae (Myomurus macrops, Mormyrus nigricans, and Gnathonemus petersoni). New complete sequences of the cyt b gene (1140 base pairs) were generated for 30 Petrocephalus specimens from Odzala (Republic of Congo), 13 from Gabon, and five from Bénin. To this dataset we added sequences already published for five additional Petrocephalus specimens (Sullivan et al., 2000). Table 1 lists numbers of specimens by species, including museum catalogue numbers and field locality information.

DNA extraction from 90% ethanol-preserved fin clips or dorsal muscle, PCR amplification, and cyt b sequencing were as described by Sullivan et al. (2000). The entire mitochondrial cyt b gene was amplified and sequenced using two Petrocephalus-specific primers that we designed by comparing the complete mitochondrial sequences of Petrocephalus soudanensis and P. microphthalmus (unpublished data): forward L14740-pet (5'-CCG TTG TAT TCA ACT ACA GAA-3'); and reverse H15913-pet (5'-TGC ATC TCC GGA TTA CAC GAC CG-3'). All new DNA sequences generated in this study have been submitted to Genbank under accession numbers EU770154 to EU770201 (Table 1).

2.6. Phylogenetic analyses

Sequence electropherograms were edited using the Sequencher software package ver. 4.1.2 (Gene Codes Corp.). Sequences were aligned by eye using PAUP* ver. 4.1.10 (Sinauer Associates, Inc.) before being exported to phylogenetic software programs. Alignment was trivial and did not require any insertions or deletions. We measured pairwise genetic distances among cyt b haplotypes as the uncorrected p-distance, which is the number of aligned nucleotide differences between two haplotypes divided by the total number of nucleotides compared (1140 bp), expressed as a percentage. Using both Bayesian and maximum likelihood (ML) methods, phylogenetic trees were inferred for two different character matrices based on the cyt b data. The first matrix (dataset #1; 1140 nucleotide positions) included all positions and types of substitution. The second matrix (dataset #2) was the same as the first, except that fast evolving transitions at third codon positions were ignored (Phillips et al., 2004). This was accomplished by replacing all third codon position purines (A/G) with ‘R’, and all third codon position pyrimidines (C/T) with ‘Y’. The degenerate nucleotide codes ‘R’ and ‘Y’ were then arbitrarily re-coded as ‘A’ and ‘C’, respectively.

Partitioned Bayesian analyses were conducted with MrBayes ver. 3.1.2 (Husonbeek and Ronquist, 2001; Ronquist and Huelsenbeck, 2003; available at http://mrbayes.csit.fsu.edu/). Here, we used the general time reversible model with some sites assumed to be invariable and with variable sites assumed to follow a discrete gamma distribution [GTR+I+Γ; (Yang, 1994)]. Two independent Bayesian analyses were performed for each character matrix. The Markov chain Monte Carlo process was set so that four chains (three heated and one cold) ran simultaneously. Distributions of log-likelihood scores were examined to estimate average log-likelihood scores at stationarity. After reaching stationarity in the two independent runs, we continued the runs for 1,500,000 cycles (15,000 trees) to confirm lack of improvement in the likelihood scores. Parameter values and trees were sampled every 100 generations. For each character matrix (i.e., datasets #1 and #2), 50% majority-rule consensus trees were calculated from the 30,000 trees pooled from the two runs.

We performed ML analyses using the software package GARLI ver. 0.951 (Zwickl, 2006; available at http://www.bio.utexas.edu/faculty/antisense/garli/Garli.html). Heuristic phylogenetic searches were conducted under a GTR + I + Γ model, which is the default model implemented by GARLI. Twenty individual runs were performed using default search settings (5,000,000 generations) and termination criteria with random starting topologies. To evaluate robustness of internal branches, 100 bootstrap replications (BP) were performed in GARLI for each character matrix.

3. Results

3.1. Electric organ anatomy and EOD waveform variation

Species in the Petrocephalus assemblage of Odzala National Park exhibit similarity in electric organ anatomy. Stalks arise from the posterior electroyte face and receive innervation on the posterior side of each electroyte in all five species examined (P. balayi, P. microphthalmus, P. sp. 6, P. sauvagii, and P. sp. 9). In no case do stalks penetrate through the electroyte. Therefore, like three previously-examined Petrocephalus species from elsewhere in Africa (Alves-Gomes and Hopkins, 1997; Lavoué et al., 2004; Sullivan et al., 2000), Petrocephalus species in the Odzala assemblage share the 'NPp' type of electroyte anatomy (i.e., Non-Penetrating, posterior innervation). Anatomy of the stalk system in electroytes of P. sp. 9 is shown in Fig. 5.

EOD variation in the Petrocephalus assemblage of Odzala is illustrated in Fig. 4, in which amplitude-normalized waveforms (one EOD per individual) are superimposed within species. Overall, a relatively high degree of similarity in EOD wave shape is also apparent among several of these species. Without exception, EODs of all eleven species begin with a head-positive voltage excursion away from baseline. This event is thought to correspond to a potential arising from the electroyte stalk system on the posterior electroyte face and/or due to firing of the posterior face itself (e.g., see Fig. 6 of Sullivan et al. (2000)). Although positive-going, the first peak in the EOD of Petrocephalus sp. 9 (labeled P1') is exceptionally small compared to all other species (see Fig. 3D for an amplified trace).

Across many of the Odzala species, EODs exhibit two principal peaks -- a major positive peak (P1) followed by an even larger negative peak (P2) -- giving the waveforms a mostly biphasic overall appearance. This pattern of discharge is similar to most other species of mormyrids that have NPp electroytes (Alves-Gomes and Hopkins, 1997; Sullivan et al., 2000). The two main phases to the EOD waveform, and the corresponding peaks, P1 and P2, are believed to be caused by the sequence of action potentials activating posterior and anterior faces of the electroytes, respectively (Bennett, 1965). Notably, however, the ordering of these positive and negative 'major' peaks is reversed in EODs of Petrocephalus sp. 9, imparting an 'inverted' appearance to this waveform relative...
to the other species. We realize that, for any species of mormyrid, a significant head-negative peak in the EOD is produced when an action potential causes a synchronized inward current surge across the posterior faces of the electrocyte membranes, while a head-positive peak is caused when an action potential causes an inward current surge across the anterior faces. The normal posterior face – anterior face sequence is apparently reversed in Petrocephalus simus. A negative-going fourth peak (P4) was only found in EODs of Petrocephalus sp. 9 (Fig. 5), the physiological basis for this waveform reversal is unexplained.

In EODs of many of the Petrocephalus species from Odzala, a third peak (P3) often follows the first two principal peaks. However, P3 is usually much smaller in amplitude than P2 or P1. When present, P3 is head-positive in all but Petrocephalus sp. 9. A negative-going fourth peak (P4) was only found in EODs of Petrocephalus christyi and Petrocephalus sp. 11 at the 1.5% threshold (used for automated
peak detection in this study). Among the large number of new Petrocephalus recordings we made in Odzala, we consistently found overall duration of each electric organ discharge to be extremely brief relative to many species of Mormyrinae. Mean EOD duration within Petrocephalus species at Odzala ranges from only 0.144 to 0.663 msec. Among all 153 individuals recorded there, the total range of EOD durations is 0.144-1.022 msec. Table 2 provides summary statistics for this and several other waveform measurements.

In comparing EODs between ‘obvious males’ and all ‘other individuals’ using t-tests within species, evidence of possible sex differences only emerged for three waveform characteristics measured in a single species: Petrocephalus. Based on these tests, average duration of EODs produced by definitive males of Petrocephalus appeared to be significantly greater than average EOD duration for all other conspecifics (P = 0.0025; see Table 2 for means, standard deviations, and ranges of each category of individuals). EODs of obvious males also showed a tendency to have a larger relative P1 amplitude than did all other individuals of this species (P = 0.0233; Table 2). Relative amplitude at P2 was also found to be smaller in definitive males (P = 0.0233; Table 2), as normalized P1 and P2 voltages are perfectly correlated whenever these are the largest two peaks in the waveform (e.g., the EOD of Petrocephalus sp. 11 is an exception to this). Aside from Petrocephalus, no evidence of any EOD sex difference was found within the other species (P > 0.05 in all t-tests; individual results not shown). Therefore, conspecifics were pooled in all other species for subsequent analyses of EOD variation among species.

Despite the overall similarity of EOD waveforms among a number of the Petrocephalus species in Odzala (Fig. 4), many differences were also detected. When comparing EODs (e.g., between species), it is important to use recordings made under a standard geometry of electrode placements, as we did for this study. A known recording geometry is essential, for example, when relating waveform variation in senders to mechanistic variation in the anatomy of their electrocytes (Bass, 1986b; Bennett, 1971). Given a single recording geometry, EODs of Petrocephalus species are clearly the most divergent among all of Odzala’s Petrocephalus species. Measured potentials at first and second ‘major’ peaks (as well as all subsequent peaks) in EODs of Petrocephalus species are opposite in sign compared to the respective peaks in EODs of other species. Statistical testing is unnecessary for estimating the significance of this major difference. However, EODs become inverted in polarity as soon as a mormyrid sender reverses its orientation and faces the opposite direction. From the viewpoint of receivers, adherence to a known recording geometry may also be behaviorally relevant. Often, mormyrids receiving EODs of other individuals (even in the dark) seem to have access to somatosensory information on sender orientation due to the close body contact that commonly occurs between sender and receiver during anti-parallel displays and courtship bouts (Bell et al., 1974; Crawford et al., 1986; Moller, 1995; Wong and Hopkins, 2007). Information on sender orientation likely becomes ambiguous rapidly as the distance between sender and receiver increases. Therefore, for the purpose of statistically testing waveform variation among species, we invert EODs of Petrocephalus species such that the ordering of ‘major’ positive and negative peaks matches the typical pattern seen in EODs of other Petrocephalus species. This is equivalent to comparing absolute values of normalized peak amplitudes among species. In Table 2, however, all summary statistics correspond to original EOD recordings made in the standard manner.

Given sample size limitations inherent when making collections from unknown faunas in remote locations, we were only able to statistically test for EOD variation among six Petrocephalus species (listed in Table 3) for which at least eight individuals each were recorded. Four of these species have not yet been described taxonomically. In the case of one of the described species (Petrocephalus), identifiable males were kept as a separate group in this analysis for two reasons. (1) In some mormyrids, sex differences in EOD waveforms can be as large as differences among sympatric species (e.g., Arnegard and Hopkins, 2003); and (2) the possibility of sex differences in the EODs of Petrocephalus was evident from earlier t-tests in this species. All eight waveform characters examined vary significantly among the seven groups of EODs (ANOVA P < 0.0001 for each character). Results of post-hoc comparisons between species are given in Table 3. Multiple waveform characters differ significantly between species at the conservative Bonferroni-adjusted threshold in all pairwise combinations of taxa. This result is congruent with the delineation of Petrocephalus sp. 2, sp. 3, sp. 6, and sp. 9 as new species on morphological grounds (Lavoué et al., in preparation). Although we were only able to digitally capture EODs from one specimen of Petrocephalus sp. 11 (the fifth newly discovered species), the duration of its EOD is the shortest, and peak spectral frequency the highest, of all Petrocephalus specimens recorded in Odzala. Despite the many quantitative differences in EOD waveforms that are evident between species, however, the magnitudes of these differences are rather small in many cases (Table 2). Moreover, the potential sex differences found earlier in the EODs of Petrocephalus no longer appear significant in these more conservative, post-hoc comparisons.

3.2. Genetic analysis

The aligned matrix of cyt b sequences contains 30 specimens among eleven Petrocephalus species of Odzala (Table 1). The matrix also includes cyt b sequences from 23 specimens of Petrocephalus belonging to six species collected in different regions of Africa. The complete Petrocephalus dataset includes 48 different cyt b
Table 2
Summary of EOD characters measured for the eleven Petrocephalus species of Odzala National Park. The top four rows are counts of individuals. Rows below these give means ± one standard deviation and ranges (min., max.) for each of the measurements (units listed at the left). Standard deviations are only provided for sample sizes of three or more. All statistics are based on EODs corrected to 25 °C using an empirically-determined Q10 function for Petrocephalus (see text). Peak 'voltages' reported here are based on EODs that have been normalized to a total peak-to-peak amplitude of one, but they have not been otherwise transformed.

<table>
<thead>
<tr>
<th>Sample size (N)</th>
<th>Number of individuals with 2 peaks &gt; 1.5%</th>
<th>Number of individuals with only 3 peaks &gt; 1.5%</th>
<th>Number of individuals with 4 peaks &gt; 1.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. binotatus, obvious males</td>
<td>21</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>P. binotatus, other individuals</td>
<td>16</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>P. sp. 2</td>
<td>43</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>P. sp. 3</td>
<td>12</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>P. balayi</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>P. microphilus</td>
<td>15</td>
<td>10</td>
<td>5</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>vP0: voltage of peak 0</th>
<th>P0 not present</th>
<th>P0 not present</th>
<th>P0 not present</th>
<th>P0 not present</th>
<th>P0 not present</th>
<th>P0 not present</th>
</tr>
</thead>
<tbody>
<tr>
<td>vP1: voltage of peak 1</td>
<td>0.261 ± 0.100 (0.139, 0.516)</td>
<td>0.203 ± 0.042 (0.134, 0.292)</td>
<td>0.285 ± 0.031 (0.228, 0.358)</td>
<td>0.195 ± 0.027 (0.151, 0.235)</td>
<td>0.315 (0.291, 0.339)</td>
<td>0.200 ± 0.047 (0.120, 0.289)</td>
</tr>
<tr>
<td>vP2: voltage of peak 2</td>
<td>−0.739 ± 0.100 (−0.861, −0.484)</td>
<td>−0.797 ± 0.042 (−0.866, −0.708)</td>
<td>−0.715 ± 0.031 (−0.772, −0.642)</td>
<td>−0.805 ± 0.027 (−0.849, −0.765)</td>
<td>−0.685 (−0.800, −0.711)</td>
<td></td>
</tr>
<tr>
<td>vP3: voltage of peak 3</td>
<td>0.016 ± 0.021 (0.000, 0.068)</td>
<td>0.013 ± 0.027 (0.000, 0.082)</td>
<td>0.069 ± 0.026 (0.012, 0.136)</td>
<td>0.035 ± 0.011 (0.025, 0.060)</td>
<td>0.043 (0.042, 0.045)</td>
<td>0.014 ± 0.020 (0.000, 0.049)</td>
</tr>
<tr>
<td>vP4: voltage of peak 4</td>
<td>P4 not present</td>
<td>P4 not present</td>
<td>−0.002 ± 0.002 (−0.006, 0.000)</td>
<td>P4 not present</td>
<td>P4 not present</td>
<td>−0.003 ± 0.004 (−0.012, 0.000)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>durP0: duration of peak 0 (msec)</th>
<th>P0 not present</th>
<th>P0 not present</th>
<th>P0 not present</th>
<th>P0 not present</th>
<th>P0 not present</th>
<th>P0 not present</th>
</tr>
</thead>
<tbody>
<tr>
<td>durP1: duration of peak 1 (msec)</td>
<td>0.179 ± 0.037 (0.099, 0.242)</td>
<td>0.159 ± 0.032 (0.089, 0.207)</td>
<td>0.116 ± 0.016 (0.089, 0.150)</td>
<td>0.110 ± 0.009 (0.095, 0.126)</td>
<td>0.131 (0.126, 0.135)</td>
<td>0.293 ± 0.054 (0.214, 0.415)</td>
</tr>
<tr>
<td>durP2: duration of peak 2 (msec)</td>
<td>0.109 ± 0.057 (0.048, 0.293)</td>
<td>0.095 ± 0.023 (0.058, 0.135)</td>
<td>0.039 ± 0.007 (0.031, 0.058)</td>
<td>0.086 ± 0.024 (0.059, 0.132)</td>
<td>0.061 (0.057, 0.064)</td>
<td>0.065 ± 0.039 (0.036, 0.152)</td>
</tr>
<tr>
<td>durP3: duration of peak 3 (msec)</td>
<td>0.042 ± 0.056 (0.000, 0.166)</td>
<td>0.016 ± 0.045 (0.000, 0.138)</td>
<td>0.053 ± 0.012 (0.000, 0.085)</td>
<td>0.467 ± 0.138 (0.351, 0.789)</td>
<td>0.134 (0.129, 0.140)</td>
<td>0.004 ± 0.006 (0.000, 0.016)</td>
</tr>
<tr>
<td>durP4: duration of peak 4 (msec)</td>
<td>P4 not present</td>
<td>P4 not present</td>
<td>durP4 never calculated</td>
<td>P4 not present</td>
<td>P4 not present</td>
<td>durP4 never calculated</td>
</tr>
</tbody>
</table>

| Tdur: total EOD duration (msec) | 0.330 ± 0.074 (0.240, 0.534) | 0.270 ± 0.033 (0.205, 0.318) | 0.208 ± 0.024 (0.164, 0.281) | 0.663 ± 0.163 (0.520, 1.022) | 0.326 (0.324, 0.329) | 0.362 ± 0.073 (0.252, 0.511) |

<table>
<thead>
<tr>
<th>P. sp. 6</th>
<th>P. christyi</th>
<th>P. savagii</th>
<th>P. sp. 9</th>
<th>P. granadolinus</th>
<th>P. sp. 11</th>
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<td>29</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Number of individuals with 2 peaks &gt; 1.5%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Number of individuals with only 3 peaks &gt; 1.5%</td>
<td>29</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Number of individuals with 4 peaks &gt; 1.5%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>
haplotypes. Of these, 43 are each found in only a single individual. The remaining five haplotypes are each shared by only two speci-
mens of the same species or OTU (i.e., P. sp. 6 specimens 5147 and
5148; P. christyi specimens 5173 and 5261; P. sp. 9 specimens 5227
and 5263; P. simus specimens 2035 and 2270; and P. pallidomacu-
latus specimens 23 and 24). Importantly, no haplotypes are shared
by any of the Petrocephalus species. Intraspecific genetic divergence
among haplotypes of the Odzala species ranges from zero to ten
substitutions (i.e., maximum p-distance = 0.9% in P. sauvagii). Cyto-
chrome b sequence divergence among Odzala species ranges from
1.5% (P. sauvagii vs. P. sp. 9) to as high as 14.5% (P. microphthalmus
vs. the rest of the assemblage). The latter value represents the
maximum genetic distance observed among any Petrocephalus spe-
cies included in our study (from any region of Africa). By compar-
ison, highest within-group divergence for the Paramormyrops and
Campylomormyrus radiations are 7% and 8%, respectively (Feulner
et al., 2006; Sullivan et al., 2002, 2004). Maximum cyt b p-distance
within the entire subfamily Mormyrinae is estimated to be about
17% among 37 species (Sullivan et al., 2000), which is only moder-
ately larger than the maximum sequence divergence we find
among the 16 Petrocephalus species listed in Table 1.

Using M. macrops, M. nigricans, and G. petesi (all in the subfamily
Mormyrinae) to root the estimated phylogenetic trees, ML and
Bayesian analyses of datasets #1 and #2 resulted in similar tree
topologies in which the genus Petrocephalus (i.e., the subfamily
Petrocephalinae) appears monophyletic. Fig. 6 shows the 50%
majority-rule consensus Bayesian tree (dataset #1) with branch
lengths proportional to number of substitutions per site. In this
tree, each Petrocephalus species is recovered as a monophyletic
group strongly supported by Bayesian posterior probabilities (PP)
and bootstrap proportions (BP). The only exception is P. viviani,
which is endemic to the Lower Guinean province. The Petrocepha-
lus assemblage of Odzala does not form a monophyletic group rel-
ative to other Petrocephalus species from different regions of Africa
(Fig. 6). This lack of regional monophyly is also true of the Petroce-
phalus species from Gabon (e.g., see the Ivindo River specimens
in Fig. 6). Only the Petrocephalus species of the Nilo-Sudanian prov-
ince are recovered as a monophyletic group, although with low
support (PP = 0.63, BP < 50%). Petrocephalus microphthalmus of Odz-
ala (two specimens operationally assigned in the field to ‘species
5’) and all four specimens of P. microphthalmus from the Ogooué
River system of Gabon form a well-supported monophyletic group
(PP = 1.0, BP = 100%). This P. microphthalmus clade appears to be
the deeply-divergent sister group to all remaining Petrocephalus
species, although monophyly of the latter group is only moderately
supported (PP = 0.69, BP < 50%).

4. Discussion

Our discovery of a speciose assemblage of Petrocephalus in
Odzala National Park offers an expanded view of electric signal var-
ation and phylogenetic relationships within the Petrocephalinae,
thereby establishing a better comparative framework for asking
questions about mormyrid evolution. We describe EODs produced
by four Petrocephalus species for which electrical recordings were
unavailable prior to this study (P. binotatus, P. christyi, P. grandocu-
lis, and P. sauvagii), as well as for five previously-unknown species
(Petrocephalus spp. 2, 3, 6, 9, and 11). Given the broad sympathy of
Petrocephalus species collected in Odzala, patterns of variation in
their electric signals and cyt b sequences are consistent with the
hypothesis that these operational taxa are reproductively isolated
from one another. Four of the five new species produce EODs that
differ statistically from those of all other sympatric Petrocephalus.
One of them (P. sp. 9) produces the most divergent species—typical
EOD known for the genus – a waveform that appears ‘inverted’
compared to those of all other species. We were only able to record
one individual of the fifth new species (P. sp. 11, specimen 6183),
yet measurable features of its EOD fall outside the ranges we esti-
imated for all other congeners. Moreover, the cyt b haplotype of
species 6183 is not shared by any other species. Each of the other
four operational taxa appears to be exclusively monophyletic in
terms of cyt b sequences sampled from multiple individuals. These
results support our recognition of five novel Petrocephalus species
on the basis of robust morphological diagnoses (Lavoué et al., in
preparation). In addition, by examining a larger number of species,
we found even stronger genetic evidence than previously available
(Sullivan et al., 2000) for the monophyly of Petrocephalus and,
therefore, the Petrocephalinae. Although it looks as if P. microph-
thalmus may be the sister group to the rest of the subfamily, this
apparent relationship may change as more taxa are sampled with
wider geographic coverage and additional markers are used to ex-
plore phylogeny.

Our recordings in Odzala suggest three generalizations about
EOD variation in Petrocephalus, all of which appear fully congruent
with published recordings for other species and populations re-
duced elsewhere in Africa (Braiton and Kramer, 1988; Hopkins
et al., 2007; Kramer, 1997a; Kramer and van der Bank, 2000; Lav-
oué et al., 2004; Moritz et al., 2008; Paugy et al., 1994; Sullivan
et al., 2000). First, all known Petrocephalus species produce pulses
of extremely brief duration resulting from rapid firing of electro-
cytes during each discharge of the electric organ. The time course
of each EOD is completed in less than 700 microseconds by the vast
majority of individuals recorded so far.

Second, sex differences in EOD waveforms may be generally
smaller in the Petrocephalinae than in the Mormyrinae. We found
weak evidence of such a sex difference in the population of P. bi-
otatus at Odzala. In this case, magnitudes of the apparent
waveform differences were rather small. No EOD sex differences
were evident among all other Petrocephalus specimens sampled
in Odzala, although we observed signs of breeding activity in
mormyrids collected from the Lékoli River during both the 2002
and 2006 expeditions. However, we had little to no power to de-
tect sex differences in all but the three Petrocephalus species for
which we recorded at least 18 definitive males each. Neverthe-
less, it is our impression that divergent male EODs are more com-
monly encountered among species of Mormyrinae, given similar
numbers of recordings made during the same seasonal period
in this region of Africa. When found, magnitudes of EOD sex
differences are often greater in the Mormyrinae (personal obser-
vation). Although the generality of this contrast between mormy-
Table 3

Results of post-hoc multiple comparisons of EOD measurements in pairwise tests among groups defined by species (and, in one case, sex). All EODs were recorded in Odzala. Measurement abbreviations are defined in Fig. 3 and Table 2. *P*-values for comparisons of peak durations or for total EOD duration are given above the diagonal, and *P*-values for comparisons of normalized voltages at different waveform peaks or for peak spectral frequency are given below the diagonal. Significant *P*-values (at the Bonferroni-corrected threshold) are shown in bold.

<table>
<thead>
<tr>
<th></th>
<th>P. biornatus, obvious males (N = 2)</th>
<th>P. biornatus, all other individuals (N = 16)</th>
<th>P. sp. 2 (N = 43)</th>
<th>P. sp. 3 (N = 12)</th>
<th>P. microphthalmus (N = 15)</th>
<th>P. sp. 6 (N = 20)</th>
<th>P. sp. 9 (N = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P. biornatus, obvious males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>durP&lt;sub&gt;v&lt;/sub&gt;</td>
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</tr>
<tr>
<td><strong>P. biornatus, all other individuals</strong></td>
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<td></td>
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<td></td>
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<tr>
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<tr>
<td><strong>P. microphthalmus</strong></td>
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</tbody>
</table>

* For statistical comparisons (presented here) of waveform peaks between *P. microphthalmus* sp. 9 and all other species, normalized voltages measured in the EODs of *P. sp. 9* have been made opposite in sign (see text for rationale).
Fig. 6. Inferred phylogeny for Petrocephalus (53 specimens) estimated as the 50% majority-rule consensus of 30,000 pooled trees from two independent Bayesian analyses of complete cytochrome b sequences (dataset #1). Myomyrus macrops, Mormyrops nigricans, and Gnathonemus petersii are used as outgroups to root the tree. The overall topology shown here is congruent with that derived by maximum likelihood (ML). Numbers at internal branches are Bayesian posterior probabilities (PP), followed by bootstrap proportions (%) from the ML analysis (BP, shown only if they exceed 50%). Asterisks (*) indicate the most strongly supported relationships (PP = 1.0 and BP = 100%). Specimen voucher numbers (in bold) follow operational taxonomic designations assigned to individuals in the field. Black vertical bars indicate groups of specimens (by species) sampled from Odzala, and gray bars indicate specimens or published sequences from other regions of Africa. To the immediate right of these bars are currently valid species names assigned to specimens on the basis of morphology. Overlay plots of EODs recorded from specimens in the tree are shown to the far right (plot construction as in Fig. 4; scale bar = 0.2 msec).

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rid subfamilies remains somewhat tentative at present, it appears to be supported by the literature. Sex differences in EOD waveforms have only ever been published for two other Petrocephalus species. Magnitudes of the waveform differences described in these cases were small (Kramer, 1997a; Kramer and van der Bank, 2000) compared to the dramatic sex differences that have been described for numerous species of Mormyrinae (Arnegard and Hopkins, 2003; Arnegard et al., 2006; Bass, 1986a; Bass and Hopkins, 1983; Feulner et al., 2006; Herfeld and Møller, 1998; Hopkins, 1999a; Kramer, 1997a; Landsman et al., 1990; Sullivan and Hopkins, 2004; Sullivan et al., 2002). In a single report, Kramer (1997b) provides illustrative examples of the degrees of sex differences observed in the two mormyrid subfamilies. Our hypothesis that the Petrocephalinae generally exhibits smaller sexual signal dimorphism in EOD waveforms requires testing with hormone manipulations in Petrocephalus, which have not previously been published for any species in this group.

Third, while EODs certainly vary quantitatively among Petrocephalus species in Odzala (and elsewhere; e.g., Lavoué et al., 2008)...

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**Fig. 7.** Interspecific EOD variation in the *Paramormyrops* ‘species flock’ of Gabon. Entire assemblages of all sympatric species and morphs are shown for (A) the Okano River near Mitzig and (B) Mouvanga Creek (see map provided by Arnegard et al., 2005). (C) Only four of eleven species/morphs are shown for the Ivindo River assemblage at Loa-Loa Rapids. Photographs (by the authors and D. Reid) are of living specimens (scale bar = 20 mm), all of which are adults or large sub-adults except for the juvenile of *Paramormyrops* sp. ‘TEN’ from Loa-Loa. A representative EOD is plotted for each species/morph (scale bar = 1.0 msec). Taxon codes for un-described species/morphs follow Sullivan et al. (2004) and Arnegard et al. (2005). Operational taxa formerly known as ‘CAB’ (Okano River) and ‘BP1’ (Mouvanga Creek) were recently identified as *Paramormyrops kingsleyae* (Hopkins et al., 2007). It has not yet been determined whether co-occurring morphs of the ‘magnostipes’ complex are incipient or nascent species, or if their divergent EODs reflect relatively-stable signal dimorphisms in the different populations (Arnegard et al., 2005).
flying EOD variation is useful for identifying specific differences in measurable waveform features, such as total duration or amplitudes of identifiable peaks (e.g., Table 2). However, not every landmark can be identified in the diverse array of waveforms expressed across moromyrid groups, and physiological correspondence (something akin to physiological ‘homology’) can be difficult or impossible to establish for many of the landmarks that appear to be shared. The ‘inverted’ EOD of Petrocephalus sp. 9 illustrates this problem. Such issues hinder the application of a landmark-based PCA approach to the full range of EODs seen in both moromyrid subfamilies. A more general method is desirable for comparative studies of EOD divergence patterns. One candidate is wavelet analysis. To date, this approach has only been applied to EOD variation among South America’s gymnotiform fishes (Crampton et al., this issue). Presently, the only preliminary quantitative comparison of signal divergence patterns we have been able to make between the Petrocephalus assemblage of Odzala and the two well-studied assemblages of Mormyrinae is the interspecific diversity of EOD durations, relative to the intraspecific variation in waveform duration is functionally relevant to species recognition in these fishes (Arnegard et al., 2006; Hopkins and Bass, 1981; Xu-Friedman and Hopkins, 1999), although other waveform features are clearly relevant as well.

Like the Petrocephalus of Odzala, cyt b sequence variation has also been investigated in Paramormyrops and Campylomormyrus, allowing signal divergence within all three groups to be compared in light of patterns of genetic variation. Table 4 compares some of the general characteristics that have been described for all three groups. The Petrocephalus species of Odzala National Park do not, by themselves, compose a monophyletic lineage that has radiated within a single region of Africa. In contrast, an extensive, monophyletic radiation of Paramormyrops is largely endemic to the Ogooué River system and neighboring drainages. Based on its monophyly and restricted geographic distribution, Sullivan et al. (2002, 2004) describe this group as a ‘riverine species flock’. From our ongoing work on Paramormyrops, we now conservatively estimate that we have detected and electrically recorded 22 different species within this radiation, of which only seven are currently described. The geographic coverage of our collecting and recording efforts in Gabon has been the most extensive so far for moromyrids inhabiting any similarly-sized region, or system of adjacent river systems, in Africa (e.g., Fig. 2 of Arnegard et al., 2006). Because of this, it is feasible that possible areas within and bordering Gabon have not yet been surveyed, however, we still expect the discovery of several additional Paramormyrops species in the future. Similarly, Feulner et al. (this issue) show that five to eight species of Campylomormyrus may have arisen within the Lower Congo River, of which only five are currently described taxonomically. They further suggest that this group may be an example of an ‘adaptive radiation’, owing to the presumed ecological significance of snout shape variation among species (Feulner et al., 2007). The number of known species in this radiation will almost certainly grow as the geographic coverage of sampling is expanded throughout the extensive Congo River basin. No species flocks or adaptive radiations of Petrocephalus have yet been suggested.

Notably, the Petrocephalus of Odzala exhibit higher levels of genetic divergence (p-distances based on cyt b sequences range from 1.5 to 14.5% among species) than do Campylomormyrus (0–8%) or Paramormyrops (0–7%). In fact, maximal cyt b divergence within Petrocephalus approaches that found throughout the entire subfamily Mormyrinae (Sullivan et al., 2000). Moreover, each species of Petrocephalus from Odzala is recovered as a monophyletic group in our analyses of cyt b sequences. In phylogenetic trees estimated with the same mitochondrial gene, the majority of Paramormyrops species and some of the Campylomormyrus species do not appear to
Coefficient of variation (CV) among uncertainty about their status as conspecifics morphs

Pattern of interspecific variation in

Range of known EOD durations

Coefficient of variation (CV) among ‘within-species’ means of EOD duration for co-occurring taxa

Table 4

Patterns of genetic divergence and electric signal variation described for three speciose assemblages of mormyrid electric fishes: the Petrocephalus of Odzala National Park (Lekoli River system, Congo basin), Paramormyrops* of Gabon (Ogooué River basin and vicinity), and Campylomormyrops of Lower Congo River.

<table>
<thead>
<tr>
<th></th>
<th>Petrocephalus of Odzala</th>
<th>Paramormyrops* of Gabon</th>
<th>Campylomormyrops of Lower Congo River</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated number of species detected to date (subset un-described)</td>
<td>11 (5)</td>
<td>22 (15) in the entire ‘species flock’, 11 species/morphs co-occur at the most species-rich locality (Loa-Loa Rapids on the Ivindo River); of these, 6 from this site are not yet described taxonomically (including both morphs).</td>
<td>5–8 (0–3)*</td>
</tr>
<tr>
<td>Interspecific genetic divergence: range of pairwise p-distances between species calculated for cyt b</td>
<td>1.5–14.5%</td>
<td>0–7%</td>
<td>0–8%</td>
</tr>
<tr>
<td>Degree of monophyly and endemism</td>
<td>Non-monophyletic and non-endemic; some of the resident species are widely distributed in Africa.</td>
<td>Monophyletic and largely endemic to the Ogooué River system (similar to a ‘species flock’), with some species also occurring in other coastal drainages of Gabon or in the neighboring Ntem River.</td>
<td>May be monophyletic and largely endemic to the Lower Congo River, although some of the assigned species are known from regions of the Congo basin outside the Lower Congo region; much more geographic coverage of sampling is needed throughout the extensive Congo River basin to better evaluate the monophyly and endemism of Campylomormyrops species in this putative Lower Congo radiation.</td>
</tr>
<tr>
<td>Pattern of interspecific variation in electric signal waveforms (i.e., EODs)</td>
<td>Comparatively low variation in EOD waveshape.</td>
<td>High interspecific waveform variation.</td>
<td>High interspecific waveform variation.</td>
</tr>
<tr>
<td>Range of known EOD durations</td>
<td>0.14–1.0 msec</td>
<td>0.2–15 msec</td>
<td>0.2–30 msec</td>
</tr>
<tr>
<td>Coefficient of variation (CV) among ‘within-species’ means of EOD duration for co-occurring taxa</td>
<td>41%</td>
<td>95%**</td>
<td>Original EODs not available for calculation of CV.</td>
</tr>
<tr>
<td>References</td>
<td>This study.</td>
<td>Arnegard et al. (2005); Arnegard and Hopkins (2003); Hopkins et al. (2007); Sullivan et al. (2002, 2004).</td>
<td>Feulner et al. (2006, 2007, this issue); see also Hopkins et al. (1999a).</td>
</tr>
</tbody>
</table>

** Previously referred to as the Brienomyrus ‘species flock’ of Gabon [see Sullivan et al. (2000), Lavoué et al. (2003), and Hopkins et al. (2007) for justification of the generic reassignment to Paramormyrops].

Counts exclude Campylomormyrops tamanrassae [i.e., ‘clade F’ of Feulner et al. (this issue)]; due to the widespread distribution of C. tamanrassae outside the Congo River basin, it is quite possible that this species did not originate in the Lower Congo. Specimen K15 and ‘clade E’ possibly correspond to three un-described species. Because species status remains difficult to evaluate in these cases (owing to the small number of specimens collected), we give the potential range of un-described species discovered so far as 0–3 for the Campylomormyrops radiation of Lower Congo.

Type 1 and type 2 morphs of the ‘magniotropis’ complex are excluded from this calculation of CV for the sympatric assemblage of Paramormyrops at Loa-Loa Rapids due to uncertainty about their status as conspecifics morphs versus reproductively-isolated species (see text).

be exclusively monophyletic with respect to other species in these groups (Table 4 gives the relevant references). The lack of phylogenetic congruence between cyt b and nuclear markers in Paramormyrops, for example, has been attributed to incomplete genetic lineage sorting (in cyt b) and/or introgressive hybridization, which are hallmarks of rapid and recent evolutionary radiations (Sullivan et al., 2004). Because sister groups are of equal age, the Petrocephalus lineage is necessarily as old as that of the morphologically much more diverse and much more speciose Mormyrinae. Thus, it should perhaps not be surprising to see such phylogenetically deep divisions within Petrocephalus and mitochondrial monophyly of species (indicating long periods of separate identity) in contrast to the species flock phenomena we see in the recently diversified clades of Paramormyrops and Campylomormyrops. Compared to the more geographically circumscribed evolutionary theaters in which Lower Guinea Paramormyrops and Lower Congo Campylomormyrops seem to have speciated, assembly of the Petrocephalus community in Odzala likely took place by the accumulation of species that had evolved in different contexts and different areas of the Congo River Basin, and perhaps elsewhere, over a long period of time. Indeed, much of the speciation that has occurred during the radiations of Paramormyrops and Campylomormyrops has likely happened more rapidly, more recently, and across smaller spatial scales (i.e., regionally, within large drainage basins) than has generally occurred in Petrocephalus.

In light of the overall differences in cyt b divergence among species, contrasting patterns of EOD diversification raise questions about signal function and the evolutionary processes shaping signal variation. For instance, have divergent selection pressures on EOD waveforms generally been stronger in the Mormyrinae than in the Petrocephalinae? To what extent do distinct EODs act as ‘species markers’ (or ‘badges of species identity’) in the two mormyrid subfamilies? What are the roles of other signals or cues during mate choice in these groups, particularly in assemblages showing low EOD variation among co-occurring species? We propose that the Petrocephalinae tends to exhibit smaller sex differences in EOD waveforms, in general, compared to the Mormyrinae. Sexual dimorphism in signaling traits is commonly linked to sexual selection (e.g., Andersson, 1994; Chenoweth and Blows, 2003; Dunn et al., 2001; Gemner and Turner, 2005), suggesting that the targets and/or strengths of sexual selection may differ in significant ways between certain mormyrid lineages. Given the relatively modest degree of EOD variation seen among many Petrocephalus species (e.g., Fig. 6), other kinds of species markers might be particularly important for species recognition in this lineage. Sequences of pulse intervals (SPIs) have received little scrutiny in the context of species recognition in any mormyrid group (Carlson, 2002). Thus, it remains possible that the SPI component of electrical communication might contribute to this function in some Petrocephalus species, as well as in other mormyrid groups exhibiting low EOD waveform variation.

Other sensory modalities should not be overlooked when studying species recognition and mate choice in weakly electric fishes (Crawford et al., 1986; Moller, 2002). In contrast to many other groups of mormyrids, distinctive melanin markings (or spots) have proven particularly useful to us in taxonomically diagnosing
Petrocephalus species of the Odzala assemblage (Lavoué et al., in preparation; see Fig. 2). Is it possible that these spots function as species markers during courtship? Petrocephalus species do tend to have large eyes relative to many groups of Mormyrinae. Kirschbaum (2006) observed spawning at night in F. poudemansi under captive conditions. However, he also observed courtship behaviors during the day and pair formation in this species, suggesting that there may be opportunity for visual assessment of mating cues in Petrocephalus. In Odzala National Park, some streams draining from patches of open savanna into the Lékoli River are tea colored, yet relatively free of suspended particulate matter and, therefore, rather transparent (personal observation). Other possible functions of the characteristic melanin patterns seen in Petrocephalus include predation avoidance or the promotion of group cohesion during diurnal schooling.

Weakly electric fishes are promising models for answering many general questions about signal evolution, owing to a valuable history of behavioral and neurobiological research (Bullock et al., 2005; Möller, 1995; Rose, 2004; Zakon, 2003) as well as unique properties of the electrosensory communication channel (Hopkins, 1999b). In addition to raising questions about signal function and ultimate evolutionary mechanisms, a contrasting pattern of EOD variation in the two mormyrid subfamilies motivates inquiry into proximate mechanisms of signal diversification. Some important leads to understanding these mechanisms have already been achieved. For example, the evolutionary innovation of ‘penetrating’ electrocyte stalks early in the radiation of the Mormyrinae is known to have contributed to waveform variation within this subfamily (Bass, 1986b; Bennett, 1971; Sullivan et al., 2000). At the level of electrocyte anatomy, Petrocephalus lacks this important mode of EOD diversification. Nevertheless, the genus still exhibits measurable EOD divergence among species, the most notable example of which is the ‘inverted’ waveform of Petrocephalus sp. 9. In all other known cases of polarity reversal in the EOD waveforms of mormyrids – such as in Mormyrops zanclirostris, some individuals of Mormyrops anguilloides, and perhaps Petrocephalus cabbalis (see Hopkins et al., 2007) – the electrocytes themselves appear to be anatomically reversed in terms of side of innervation. Evidently, rather than these kinds of changes in electrocyte anatomy, physiological changes in electrical excitability are the primary mechanisms of EOD divergence in Petrocephalus. In the case of Petrocephalus sp. 9, for example, we suspect that part of the mechanism for waveform ‘inversion’ might have been changes in firing threshold for one or both electrocyte faces, although we have yet to test this possibility. At the level of ion channels, Zakon et al. (2006, 2008) show that a sodium channel gene expressed specifically in electric organ has experienced greatly enhanced diversifying selection during two independent radiations of weakly electric fishes (including radiation of the Mormyroidae in Africa). Their intriguing findings at the molecular level have not yet been related physiologically to variation in EOD waveforms. Finally, based on work that has only been done in the Mormyrinae, Xu-Friedman and Hopkins (1999) review what is known about electrosensory time-coding of EOD waveforms by the Knollenorgan pathway. It is reasonable to predict that fundamental differences will be found in this electrosensory pathway between lineages exhibiting widely varying degrees of EOD diversity. In this line of research and others, comparative studies between the Petrocephalinae and the Mormyrinae should prove illuminating when answering mechanistic questions about signal evolution using the mormyrid model.

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