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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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ABSTRACT

Electric signals of mormyrid fishes have recently been described from several regions of Africa. Members of the Mormyridae 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 subfamilies 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.

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 and sense electric organ discharges (EODs) for these purposes (Lissmann, 1951, 1958) researchers have suspected a central role for the electrosensory system in the evolutionary diversification of this group. Owing to the improvement of portable recording technology, EODs have recently been described for mormyroid species inhabiting an increasing number of regions across sub-Saharan Africa (Arnegard and Carlson, 2005; Feulner et al., this issue; Hopkins et al., 2007; Kramer et al., 2003, 2004, 2007; Lavoué et al., 2004, 2008; Moritz et al., 2008; Sullivan and Hopkins, 2004). This recent work has greatly expanded the catalogue of known EODs, in which an impressive diversity of electrical waveforms has been described among mormyroid species.

Behavioral and neuroethological studies in this group indicate that discrimination of EOD waveform differences by many African electric fishes often contributes to species- and/or sex-recognition and, in some cases, individual discrimination (Arnegard et al., 2006; Graff and Kramer, 1992; Hanika and Kramer, 2005; Hopkins

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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 *Brienomyrus*') 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-typical 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 Mormyroidea within the Osteoglossomorpha, as well as the recognition of two mormyroid 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 Paugy, 1991; Froese and Pauly, 2008; Gosse, 1984; Kramer and van der Bank, 2000; Lavoué et al., 2004). Following Lissmann's discovery of the electrosensory 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 of all 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 Schugardt, 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 phylogenetic 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. binotatus* (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

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).

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.* sp. 6 (specimen 5150, CU 88049); *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

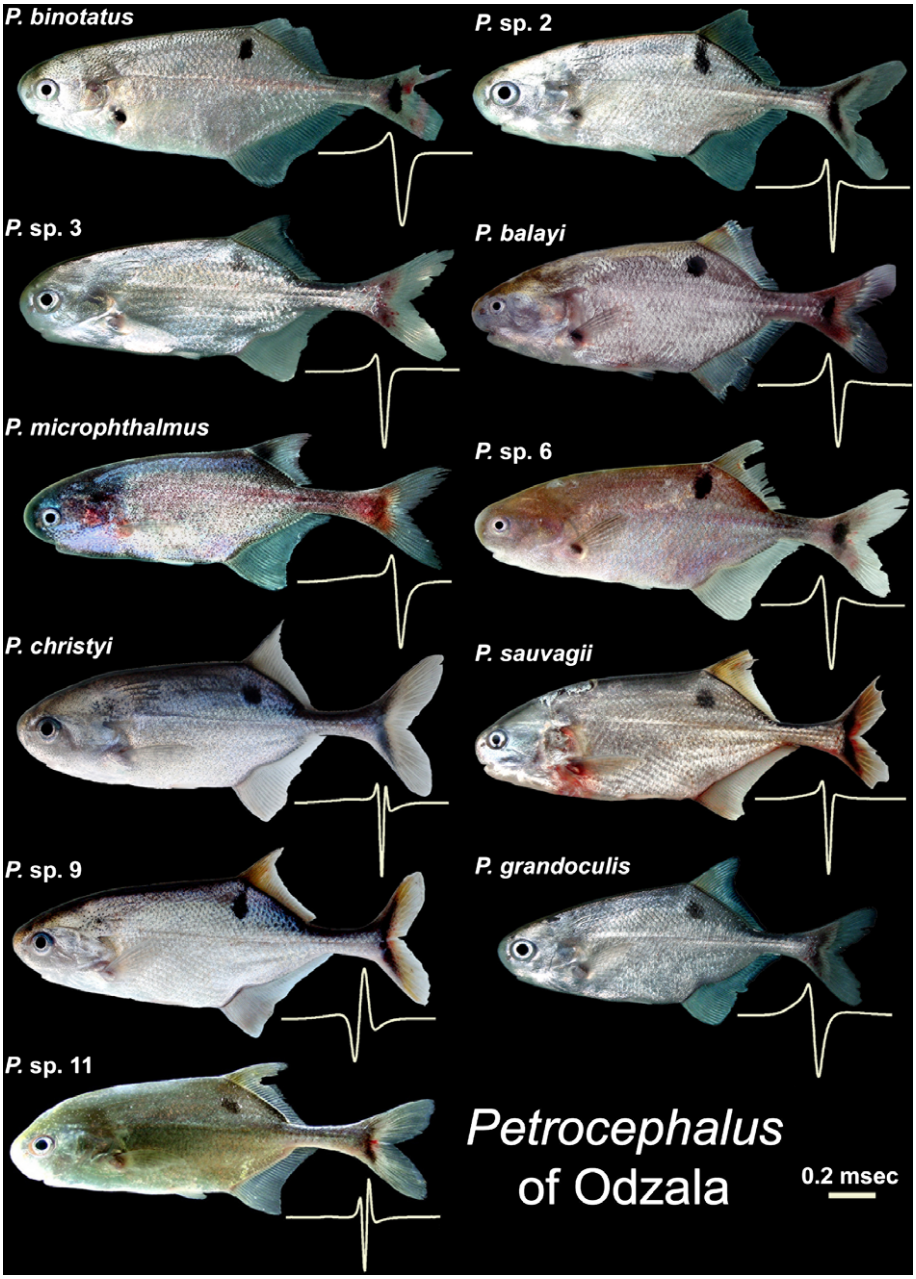

Fig. 2. Photographs of living specimens of the eleven *Petrocephalus* species collected in Odzala National Park in 2002 and 2006. Names are provided for taxa which were subsequently assigned to currently valid species, and numbers are used for un-described species. A representative EOD is shown for each species. All EODs are plotted on the same time scale, and head-positive is up in each voltage trace (scale bar = 0.2 msec). Instead of being scaled by specimen size, fish images have been enlarged as much as possible to best illustrate differences in shape and melanin patterning among species. Standard lengths of the eleven specimens shown here range from *ca.* 5.6–14.5 cm.

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 $R_{25\,^\circ C}$ (the sampling rate at 25 °C) using the temperature coefficient formula for rate functions:

$$R_{25\,^\circ C} = R_T \cdot [Q_{10}]^{((25\,^\circ C - T)/10)}.$$

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_0'. No minor peaks were found to precede P_1 in the EODs of any other species. Due to the presence of P_0 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

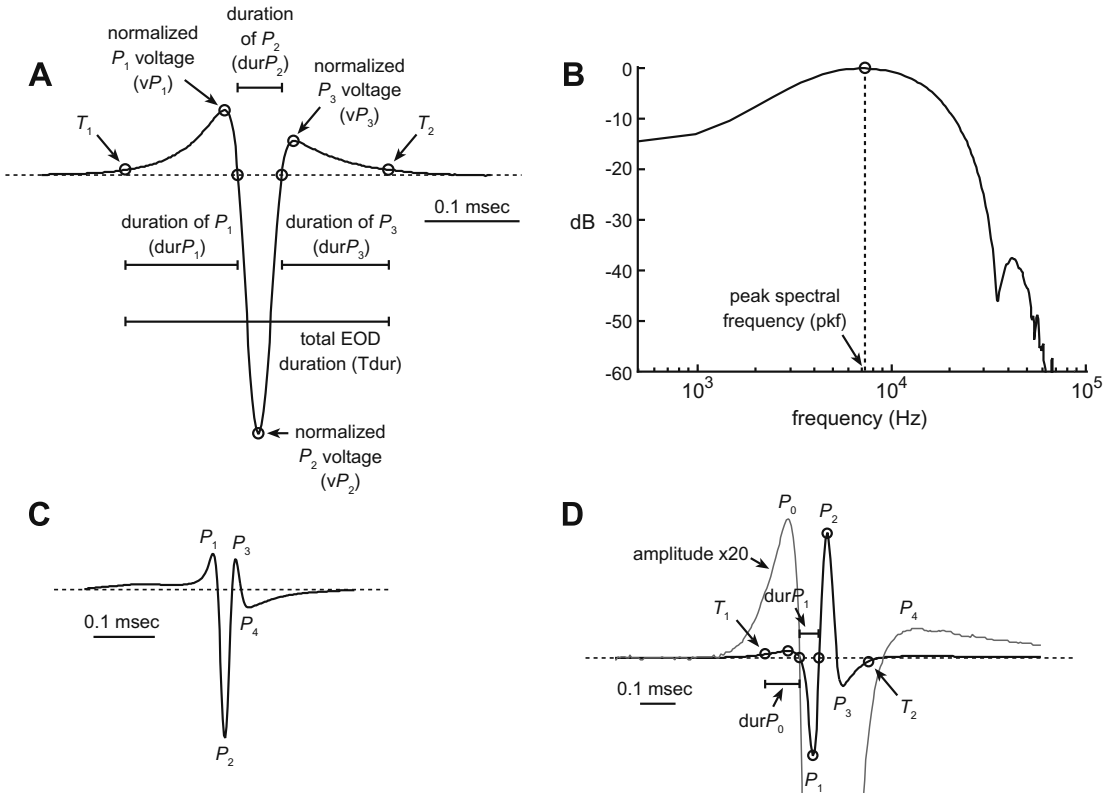

Fig. 3. Summary of eight characters quantified in all *Petrocephalus* EODs recorded in Odzala. (A) Example of an EOD recorded from *Petrocephalus* sp. 6 illustrating seven landmark-based measurements: normalized potentials and durations of peaks 1–3; and total EOD duration. Shown are landmarks describing 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 (P_4) 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_1 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; Moller, 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

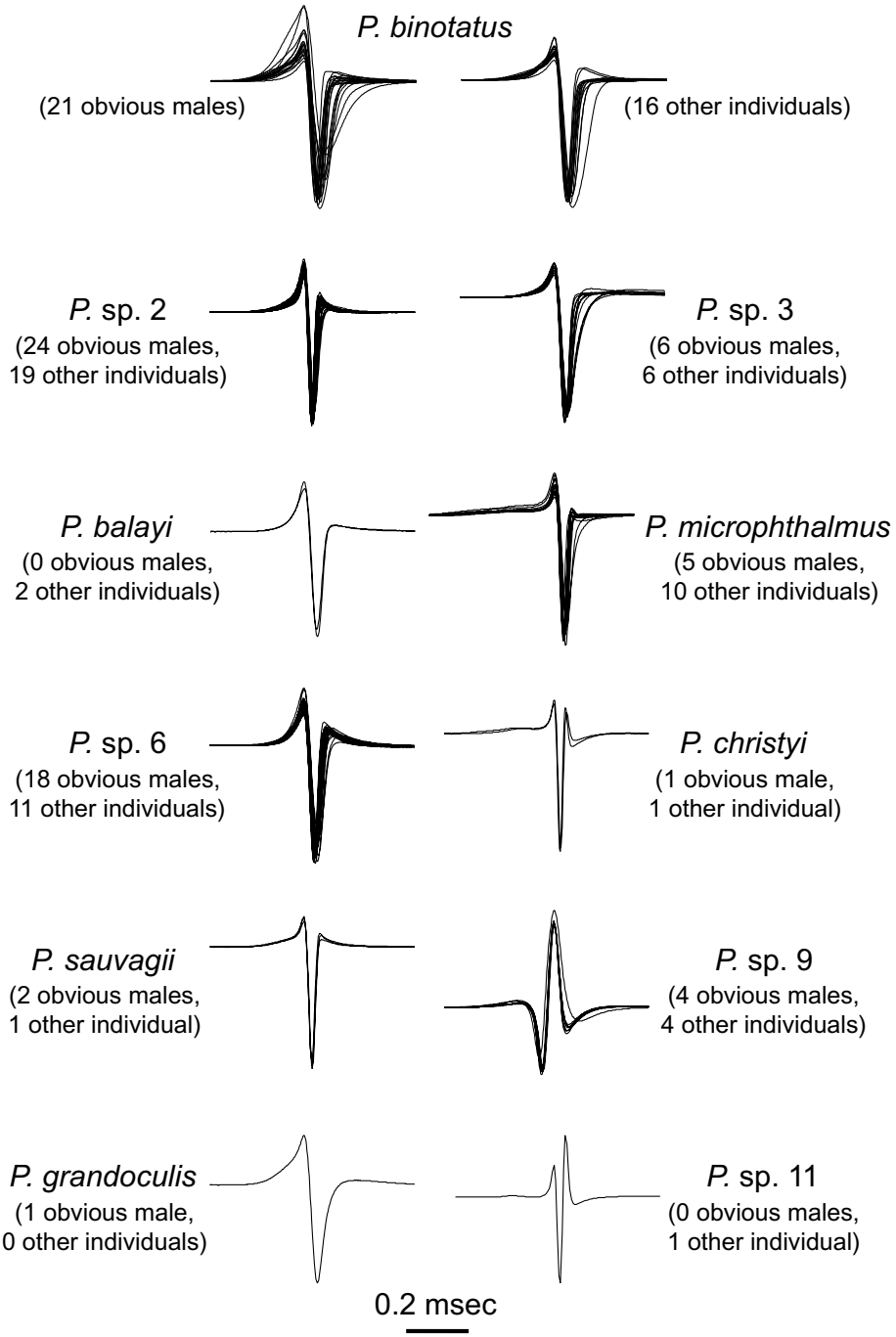

Fig. 4. Superimposed EODs of all recorded individuals for each *Petrocephalus* species in the Odzala assemblage. One EOD was arbitrarily selected from each individual for plotting and analysis. Waveforms were normalized to the same peak-to-peak voltage and plotted (head-positive up) on the same time 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.

ANOVA was used to evaluate variation in each of eight waveform measurements among groups of EODs defined by species (keeping 'obvious males' as separate groups when appropriate). Only groups containing five or more samples were included in this analysis. Post-hoc multiple comparisons were then made among group means using the T′-method, which is a generalization of Tukey's test for the case of unequal samples sizes (Sokal and Rohlf, 1998). Here, we adhered to a conservative Bonferroni-adjusted threshold ($P < 0.00625$) when determining statistical significance of post-hoc comparisons across the eight measured waveform characters. All statistical analyses were performed using Statistica ver. 6.1 (StatSoft, Inc.).

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 *Campylomormyrus* radiations. To root the trees, we used available sequences for three outgroup species belonging to the subfamily Mormyrinae (*Myomyrus macrops*, *Mormyrops nigricans*, and *Gnathonemus petersii*). 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′-TCG ATC TCC GGA TTA CAA 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 (Huelsenbeck 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 electrocyte face and receive innervation on the posterior side of each electrocyte 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 electrocyte. 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 electrocyte anatomy (i.e., Non-Penetrating, posterior innervation). Anatomy of the stalk system in electrocytes 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 waveshape 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 electrocyte stalk system on the posterior electrocyte 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 'P_0') 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 (P_1) followed by an even larger negative peak (P_2) – giving the waveforms a mostly biphasic overall appearance. This pattern of discharge is similar to most other species of mormyrids that have NPp electrocytes (Alves-Gomes and Hopkins, 1997; Sullivan et al., 2000). The two main phases to the EOD waveform, and the corresponding peaks, P_1 and P_2, are believed to be caused by the sequence of action potentials activating posterior and anterior faces of the electrocytes, 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

Table 1
Catalogue of 56 specimens used for phylogenetic analysis. Shown for each is a valid species name (when applicable) or our operational species designation, specimen voucher number, Genbank accession number for the complete cyt *b* sequence, collection locality ('CAR' = Central African Republic), and museum catalogue number. Museum acronyms: CU, Cornell University Museum of Vertebrates (Ithaca, NY, USA); MNHN, Museum National d'Histoire Naturelle (Paris); AMNH, American Museum of Natural History (New York City).

Species	Specimen no.	Genbank no.	Locality	Museum catalogue no.
Petrocephalus binotatus	5001	EU770164	Odzala, Congo basin	CU 88064
	5002	EU770165	Odzala, Congo basin	CU 88065
	6133	EU770166	Odzala, Congo basin	CU 92390
	6134	EU770167	Odzala, Congo basin	CU 92390
Petrocephalus sp. 2	5049	EU770168	Odzala, Congo basin	CU 88070
	5091	EU770169	Odzala, Congo basin	CU 87837
	6132	EU770170	Odzala, Congo basin	CU 92391
	6201	EU770171	Odzala, Congo basin	CU 92388
Petrocephalus sp. 3	5175	EU770181	Odzala, Congo basin	CU 88058
	5361	EU770182	Odzala, Congo basin	CU 88118
Petrocephalus balayi	5079	EU770191	Odzala, Congo basin	CU 87851
	5314	EU770192	Odzala, Congo basin	CU 88111
Petrocephalus microphthalmus	5092	EU770188	Odzala, Congo basin	CU 87940
	5108	EU770187	Odzala, Congo basin	CU 87939
Petrocephalus sp. 6	5148	EU770156	Odzala, Congo basin	CU 87852
	5147	EU770157	Odzala, Congo basin	CU 88047
	6095	EU770158	Odzala, Congo basin	CU 92392
	6139	EU770159	Odzala, Congo basin	CU 92392
Petrocephalus christyi	5173	EU770184	Odzala, Congo basin	CU 88057
	5261	EU770183	Odzala, Congo basin	CU 88095
Petrocephalus sauvagii	5206	EU770160	Odzala, Congo basin	CU 87864
	6162	EU770161	Odzala, Congo basin	CU 92387
	6166	EU770162	Odzala, Congo basin	CU 92387
Petrocephalus sp. 9	5213	EU770172	Odzala, Congo basin	CU 88085
	5227	EU770173	Odzala, Congo basin	CU 87839
	5263	EU770174	Odzala, Congo basin	CU 88097
	6167	EU770175	Odzala, Congo basin	CU 92386
Petrocephalus grandoculis	5362	EU770154	Odzala, Congo basin	CU 88119
	6181	EU770155	Odzala, Congo basin	CU 92385
Petrocephalus sp. 11	6183	EU770163	Odzala, Congo basin	CU 92389
Petrocephalus sullivani	2039	EU770177	Makokou, Ivindo R., Gabon	CU 79700
	2046	EU770176	Makokou, Ivindo R., Gabon	CU 89397
	2037	EU770178	Makokou, Ivindo R., Gabon	CU 79700
	6214	EU770180	Makokou, Ivindo R., Gabon	CU 92345
	2038	AF201606[a]	Makokou, Ivindo R., Gabon	CU 79700
	3961	EU770179	Ayengbe, Ntem R., Gabon	CU 80714
Petrocephalus simus	2270	AF201604[a][b]	Makokou, Ivindo R., Gabon	CU 89400
	2035	AF201604[a][b]	Makokou, Ivindo R., Gabon	CU 79701
	3743	EU770193	Franceville, Ogooué R., Gabon	CU 80483
	3986	EU770194	Mioung, Ntem R., Gabon	CU 80699
	6293	EU770195	Makokou, Ivindo R., Gabon	CU 92263
	6294	EU770196	Makokou, Ivindo R., Gabon	CU 92263
Petrocephalus microphthalmus	2080	EU770186	Makokou, Ivindo R., Gabon	CU 82205
	2199	EU770185	Makokou, Ivindo R., Gabon	CU 82208
	6283	EU770189	Makokou, Ivindo R., Gabon	CU 92354
	6284	EU770190	Makokou, Ivindo R., Gabon	CU 92354
Petrocephalus pallidomaculatus	Pes1	EU770197	Ouémé R., Bénin	MNHN[c]
	19	EU770198	Ouémé R., Bénin	MNHN[c]
	20	EU770201	Ouémé R., Bénin	MNHN[c]
	23	EU770200	Ouémé R., Bénin	MNHN[c]
	24	EU770199	Ouémé R., Bénin	MNHN[c]
Petrocephalus bovei	1124	AF201605[a]	Niger R., Mali	MNHN 1999-614
Petrocephalus soudanensis	–	AF201607[a]	Volta R., Ghana	MNHN 1999-279
Myomyrus macrops	2524	AF201602[a]	Ubangui R., CAR	AMNH 228166
Gnathonemus petersii	2453	AF201585[a]	Sangha R., CAR	AMNH 228157
Mormyrops nigricans	–	AF201598[a]	Ogooué R., Gabon	CU 79745

[a] Sequences determined by Sullivan et al. (2000).
[b] Specimens 2035 and 2270 of *Petrocephalus simus* share the same cytochrome *b* haplotype; these sequences have been deposited in Genbank under the same accession number.
[c] Specimens awaiting registry in the catalogue of the MNHN.

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 anterior faces of the electrocyte membranes, while a head-positive peak is caused when an action potential causes an inward current surge across the posterior faces. The normal posterior face – anterior face sequence is apparently reversed in *P.* sp. 9. Since electrocytes are innervated on the posterior side and have non-penetrating stalks in *P.* 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 (P_3) often follows the first two principal peaks. However, P_3 is usually much smaller in amplitude than P_2 or P_1. When present, P_3 is head-positive in all but *Petrocephalus* sp. 9. A negative-going fourth peak (P_4) was only found in EODs of *P. christyi* and *Petrocephalus* sp. 11 at the 1.5% threshold (used for automated

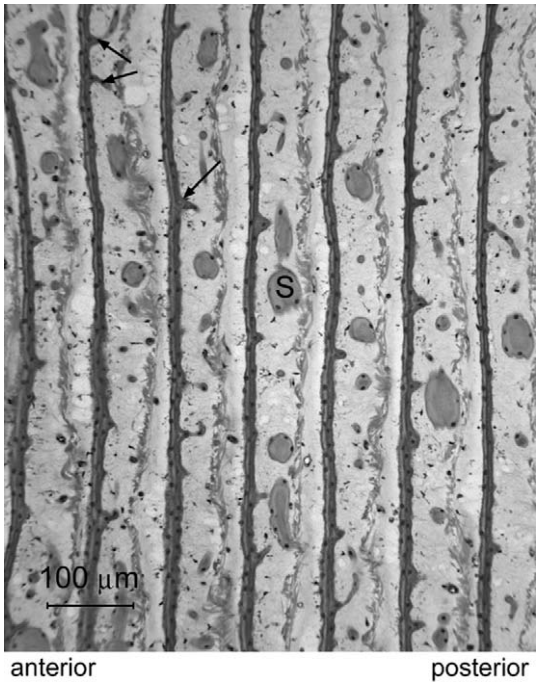

Fig. 5. Electric organ of *Petrocephalus* species 9 (specimen 5263; cat. no. CU 88097). The photomicrograph shows portions of seven disk-shaped electrocytes cut in mid-sagittal plane (plastic section; 7 µm thick; stained with toluidine blue; anterior to the left; scale bar = 100 µm). Electrocytes are approximately 15 µm thick and separated from one another by 80 µm. Numerous stalklets (e.g., arrows) emerge from the posterior face of each electrocyte disk and eventually merge with larger and larger tube-like stalks (e.g., S), which remain on the posterior side of the electrocyte. Innervation is also on the posterior side of each electrocyte (observed but not indicated in this photo).

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: *P. binotatus*. Based on these tests, average duration of EODs produced by definitive males of *P. binotatus* appeared to be significantly greater than average EOD duration for all other conspecifics individuals ($P = 0.0025$; 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 P_1 amplitude than did all the other individuals of this species ($P = 0.0233$; Table 2). Relative amplitude at P_2 was also found to be smaller in definitive males ($P = 0.0233$; Table 2), as normalized P_1 and P_2 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 *P. binotatus*, 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 *P.* sp. 9 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 *P.* sp. 9 are opposite in sign compared to the respective peaks in EODs of all 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 *P.* sp. 9 such that the ordering of 'major' positive and negative peaks matches the typical pattern seen in EODs of all 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 (*P. binotatus*), 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 *P. binotatus* 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 *P. binotatus* 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 Q_{10} 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.

	P. binotatus, obvious males	*P. binotatus*, other individuals	*P.* sp. 2	*P.* sp. 3	*P. balayi*	*P. microphthalmus*
Sample size (*N*)	21	16	43	12	2	15
Number of individuals with only 2 peaks ⩾ 1.5%	12	14	1	0	0	10
Number of individuals with only 3 peaks ⩾ 1.5%	9	2	42	12	2	5
Number of individuals with 4 peaks ⩾ 1.5%	0	0	0	0	0	0
vP_0: voltage of peak 0	P_0 not present	P_0 not present	P_0 not present	P_0 not present	P_0 not present	P_0 not present
vP_1: voltage of peak 1	0.261 ± 0.100 (0.139, 0.516)	0.203 ± 0.042 (0.134, 0.292)	0.285 ± 0.031 (0.228, 0.358)	0.195 ± 0.027 (0.151, 0.235)	0.315 (0.291, 0.339)	0.200 ± 0.047 (0.120, 0.289)
vP_2: voltage of peak 2	−0.739 ± 0.100 (−0.861, −0.484)	−0.797 ± 0.042 (−0.866, −0.708)	−0.715 ± 0.031 (−0.772, −0.642)	−0.805 ± 0.027 (−0.849, −0.765)	−0.685 (−0.709, −0.661)	−0.800 ± 0.047 (−0.880, −0.711)
vP_3: voltage of peak 3	0.016 ± 0.021 (0.000, 0.068)	0.013 ± 0.027 (0.000, 0.082)	0.069 ± 0.026 (0.012, 0.136)	0.035 ± 0.011 (0.025, 0.060)	0.043 (0.042, 0.045)	0.014 ± 0.020 (0.000, 0.049)
vP_4: voltage of peak 4	P_4 not present	P_4 not present	−0.002 ± 0.002 (−0.006, 0.000)	P_4 not present	P_4 not present	−0.003 ± 0.004 (−0.012, 0.000)
durP_0: duration of peak 0 (msec)	P_0 not present	P_0 not present	P_0 not present	P_0 not present	P_0 not present	P_0 not present
durP_1: duration of peak 1 (msec)	0.179 ± 0.037 (0.099, 0.242)	0.159 ± 0.032 (0.089, 0.207)	0.116 ± 0.016 (0.089, 0.150)	0.110 ± 0.009 (0.095, 0.126)	0.131 (0.126, 0.135)	0.293 ± 0.054 (0.214, 0.415)
durP_2: duration of peak 2 (msec)	0.109 ± 0.057 (0.048, 0.293)	0.095 ± 0.023 (0.058, 0.135)	0.039 ± 0.007 (0.031, 0.058)	0.086 ± 0.024 (0.059, 0.132)	0.061 (0.057, 0.064)	0.065 ± 0.039 (0.036, 0.152)
durP_3: duration of peak 3 (msec)	0.042 ± 0.056 (0.000, 0.166)	0.016 ± 0.045 (0.000, 0.138)	0.053 ± 0.012 (0.000, 0.085)	0.467 ± 0.138 (0.351, 0.789)	0.134 (0.129, 0.140)	0.004 ± 0.006 (0.000, 0.016)
durP_4: duration of peak 4 (msec)	P_4 not present	P_4 not present	durP_4 never calculated because vP_4 always < 1.5%	P_4 not present	P_4 not present	durP_4 never calculated because vP_4 always < 1.5%
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)
pkf: peak spectral frequency (kHz)	3.886 ± 1.005 (2.171, 6.522)	3.922 ± 1.075 (1.647, 6.107)	10.408 ± 1.641 (6.646, 14.185)	4.658 ± 0.768 (3.358, 5.630)	6.301 (6.164, 6.438)	8.162 ± 3.088 (1.981, 12.817)
	P. sp. 6	*P. christyi*	*P. sauvagii*	*P.* sp. 9	*P. grandoculis*	*P.* sp. 11
Sample size (*N*)	29	2	3	8	1	1
Number of individuals with only 2 peaks ⩾ 1.5%	0	0	0	0	0	0
Number of individuals with only 3 peaks ⩾ 1.5%	29	0	3	0	1	0
Number of individuals with 4 peaks ⩾ 1.5%	0	2	0	8	0	1
vP_0: voltage of peak 0	P_0 not present	P_0 not present	P_0 not present	0.034 ± 0.009 (0.025, 0.047)	P_0 not present	P_0 not present
vP_1: voltage of peak 1	0.274 ± 0.042 (0.200, 0.379)	0.217 (0.205, 0.228)	0.190 ± 0.020 (0.170, 0.208)	−0.420 ± 0.034 (−0.456, −0.344)	0.338	0.207
vP_2: voltage of peak 2	−0.726 ± 0.042 (−0.800, −0.621)	−0.783 (−0.795, −0.772)	−0.810 ± 0.020 (−0.830, −0.792)	0.580 ± 0.034 (0.544, 0.656)	−0.662	−0.590
vP_3: voltage of peak 3	0.099 ± 0.022 (0.054, 0.160)	0.161 (0.146, 0.176)	0.078 ± 0.024 (0.051, 0.096)	−0.137 ± 0.028 (−0.179, −0.092)	0.027	0.410
vP_4: voltage of peak 4	−0.007 ± 0.002 (−0.013, −0.003)	−0.066 (−0.086, −0.045)	P_4 not present	0.007 ± 0.003 (0.000, 0.011)	−0.009	−0.057
durP_0: duration of peak 0 (msec)	P_0 not present	P_0 not present	P_0 not present	0.107 ± 0.018 (0.084, 0.135)	P_0 not present	P_0 not present
durP_1: duration of peak 1 (msec)	0.115 ± 0.014 (0.097, 0.146)	0.219 (0.179, 0.258)	0.128 ± 0.006 (0.121, 0.131)	0.054 ± 0.004 (0.049, 0.059)	0.154	0.023
durP_2: duration of peak 2 (msec)	0.052 ± 0.009 (0.041, 0.082)	0.026 (0.025, 0.026)	0.036 ± 0.002 (0.035, 0.038)	0.050 ± 0.011 (0.040, 0.077)	0.094	0.023
durP_3: duration of peak 3 (msec)	0.114 ± 0.022 (0.082, 0.161)	0.017 (0.017, 0.018)	0.083 ± 0.022 (0.063, 0.106)	0.100 ± 0.021 (0.086, 0.150)	0.132	0.023

	P. sp. 6	*P. christyi*	*P. sauvagii*	*P.* sp. 9	*P. grandoculis*	*P.* sp. 11
durP_4: durationof peak 4(msec)	durP_4 never calculated because vP_4 always < 1.5%	0.076 (0.062, 0.089)	P_4 not present	durP_4 never calculated because vP_4 always < 1.5%	durP_4 not calculated because vP_4 < 1.5%	0.075
Tdur: total EODduration(msec)	0.281 ± 0.032(0.231, 0.339)	0.338(0.284, 0.390)	0.247 ± 0.022(0.232, 0.273)	0.311 ± 0.045(0.270, 0.418)	0.380	0.144
pkf: peak spectral frequency (kHz)	7.107 ± 1.008 (4.578, 9.178)	10.418 (2.254, 18.583)[†]	10.222 ± 0.867 (9.674, 11.222)	9.074 ± 1.176 (6.288, 10.109)	4.584	22.111

[†] Bimodal power spectrum; peak spectral frequency detected in lower mode in one individual and in higher mode in the other individual.

haplotypes. Of these, 43 are each found in only a single individual. The remaining five haplotypes are each shared by only two specimens 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. pallidomaculatus* specimens 23 and 24). Importantly, no haplotypes are shared by any two *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*). Cytochrome *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* species included in our study (from any region of Africa). By comparison, 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 moderately larger than the maximum sequence divergence we find among the 16 *Petrocephalus* species listed in Table 1.

Using *M. macrops*, *M. nigricans*, and *G. petersii* (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. sullivani*, which is endemic to the Lower Guinean province. The *Petrocephalus* assemblage of Odzala does not form a monophyletic group relative to other *Petrocephalus* species from different regions of Africa (Fig. 6). This lack of regional monophyly is also true of the *Petrocephalus* species from Gabon (e.g., see the Ivindo River specimens in Fig. 6). Only the *Petrocephalus* species of the Nilo-Sudanian province are recovered as a monophyletic group, although with low support (PP = 0.63, BP < 50%). *Petrocephalus microphthalmus* of Odzala (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 variation 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. grandoculis*, and *P. sauvagii*), as well as for five previously-unknown species (*Petrocephalus* spp. 2, 3, 6, 9, and 11). Given the broad sympatry 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 estimated for all other congeners. Moreover, the cyt *b* haplotype of specimen 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. microphthalmus* 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 explore 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 recorded elsewhere in Africa (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). First, all known *Petrocephalus* species produce pulses of extremely brief duration resulting from rapid firing of electrocytes 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. binotatus* 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 detect sex differences in all but the three *Petrocephalus* species for which we recorded at least 18 definitive males each. Nevertheless, it is our impression that divergent male EODs are more commonly 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 observation). 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**.

	P. binotatus, obvious males ($N = 21$)	*P. binotatus*, all other individuals ($N = 16$)	*P.* sp. 2 ($N = 43$)	*P.* sp. 3 ($N = 12$)	*P. microphthalmus* ($N = 15$)	*P.* sp. 6 ($N = 29$)	*P.* sp. 9 ($N = 8$)
P. binotatus, obvious males		$durP_1$: $P = 0.3941$ $durP_2$: $P = 0.7543$ $durP_3$: $P = 0.7388$ Tdur: $P = 0.1114$	**$durP_1$: $P < 0.0001$** **$durP_2$: $P < 0.0001$** $durP_3$: $P = 0.9921$ **Tdur: $P < 0.0001$**	**$durP_1$: $P < 0.0001$** $durP_2$: $P = 0.3714$ **$durP_3$: $P < 0.0001$** **Tdur: $P < 0.0001$**	**$durP_1$: $P < 0.0001$** **$durP_2$: $P = 0.0002$** $durP_3$: $P = 0.3197$ Tdur: $P = 0.8215$	**$durP_1$: $P < 0.0001$** **$durP_2$: $P < 0.0001$** **$durP_3$: $P < 0.0001$** Tdur: $P = 0.1676$	**$durP_1$: $P < 0.0001$** **$durP_2$: $P = 0.0004$** $durP_3$: $P = 0.2063$ Tdur: $P = 0.9974$
P. binotatus, all other individuals	vP_1: $P = 0.0252$ vP_2: $P = 0.0252$ vP_3: $P = 0.9249$ pkf: $P = 0.9999$		**$durP_1$: $P = 0.0001$** **$durP_2$: $P < 0.0001$** $durP_3$: $P = 0.3350$ Tdur: $P = 0.0814$	**$durP_1$: $P = 0.0002$** $durP_2$: $P = 0.9866$ **$durP_3$: $P < 0.0001$** **Tdur: $P < 0.0001$**	**$durP_1$: $P < 0.0001$** $durP_2$: $P = 0.0468$ $durP_3$: $P = 0.9930$ **Tdur: $P = 0.0017$**	**$durP_1$: $P = 0.0001$** **$durP_2$: $P = 0.0003$** **$durP_3$: $P < 0.0001$** Tdur: $P = 0.9990$	**$durP_1$: $P < 0.0001$** $durP_2$: $P = 0.0216$ $durP_3$: $P = 0.0101$ Tdur: $P = 0.8550$
P. sp. 2	vP_1: $P = 0.5934$ vP_2: $P = 0.5934$ **vP_3: $P < 0.0001$** **pkf: $P < 0.0001$**	**vP_1: $P = 0.0001$** **vP_2: $P = 0.0001$** **vP_3: $P < 0.0001$** **pkf: $P < 0.0001$**		$durP_1$: $P = 0.9991$ **$durP_2$: $P = 0.0006$** **$durP_3$: $P < 0.0001$** **Tdur: $P < 0.0001$**	**$durP_1$: $P < 0.0001$** $durP_2$: $P = 0.1395$ $durP_3$: $P = 0.0849$ **Tdur: $P < 0.0001$**	$durP_1$: $P = 0.9999$ $durP_2$: $P = 0.5122$ **$durP_3$: $P < 0.0001$** **Tdur: $P = 0.0002$**	**$durP_1$: $P = 0.0001$** $durP_2$: $P = 0.9836$ $durP_3$: $P = 0.4514$ Tdur: $P = 0.0195$
P. sp. 3	vP_1: $P = 0.0267$ vP_2: $P = 0.0267$ vP_3: $P = 0.0138$ pkf: $P = 0.8863$	vP_1: $P = 0.9996$ vP_2: $P = 0.9996$ **vP_3: $P = 0.0003$** pkf: $P = 0.9076$	**vP_1: $P = 0.0002$** **vP_2: $P = 0.0002$** vP_3: $P = 0.0856$ **pkf: $P < 0.0001$**		**$durP_1$: $P < 0.0001$** $durP_2$: $P = 0.4974$ **$durP_3$: $P < 0.0001$** **Tdur: $P < 0.0001$**	$durP_1$: $P = 0.9997$ $durP_2$: $P = 0.0470$ **$durP_3$: $P < 0.0001$** **Tdur: $P < 0.0001$**	**$durP_1$: $P = 0.0005$** $durP_2$: $P = 0.1308$ **$durP_3$: $P < 0.0001$** **Tdur: $P < 0.0001$**
P. microphthalmus	vP_1: $P = 0.0178$ vP_2: $P = 0.0178$ vP_3: $P = 0.9626$ **pkf: $P < 0.0001$**	vP_1: $P = 0.9999$ vP_2: $P = 0.9999$ vP_3: $P = 0.9999$ **pkf: $P < 0.0001$**	**vP_1: $P = 0.0001$** **vP_2: $P = 0.0001$** **vP_3: $P < 0.0001$** **pkf: $P = 0.0014$**	vP_1: $P = 0.9999$ vP_2: $P = 0.9999$ **vP_3: $P = 0.0005$** **pkf: $P < 0.0001$**		**$durP_1$: $P < 0.0001$** $durP_2$: $P = 0.8849$ **$durP_3$: $P < 0.0001$** Tdur: $P = 0.0099$	**$durP_1$: $P < 0.0001$** $durP_2$: $P = 0.9413$ **$durP_3$: $P = 0.0015$** Tdur: $P = 0.6984$
P. sp. 6	vP_1: $P = 0.9420$ vP_2: $P = 0.9420$ **vP_3: $P < 0.0001$** **pkf: $P < 0.0001$**	**vP_1: $P = 0.0008$** **vP_2: $P = 0.0008$** **vP_3: $P < 0.0001$** **pkf: $P < 0.0001$**	vP_1: $P = 0.9848$ vP_2: $P = 0.9848$ vP_3: $P = 0.0186$ **pkf: $P < 0.0001$**	**vP_1: $P = 0.0015$** **vP_2: $P = 0.0015$** **vP_3: $P < 0.0001$** **pkf: $P = 0.0021$**	**vP_1: $P = 0.0006$** **vP_2: $P = 0.0006$** **vP_3: $P < 0.0001$** pkf: $P = 0.5033$**		**$durP_1$: $P = 0.0001$** $durP_2$: $P = 0.9999$ $durP_3$: $P = 0.9975$ Tdur: $P = 0.9644$
P. sp. 9	**vP_1: $P < 0.0001$** (†) **vP_2: $P < 0.0001$** (†) **vP_3: $P < 0.0001$** (†) **pkf: $P < 0.0001$**	**vP_1: $P < 0.0001$** (†) **vP_2: $P < 0.0001$** (†) **vP_3: $P < 0.0001$** (†) **pkf: $P < 0.0001$**	**vP_1: $P < 0.0001$** (†) **vP_2: $P < 0.0001$** (†) vP_3: $P = 0.0091$ (†) pkf: $P = 0.6019$	**vP_1: $P < 0.0001$** (†) **vP_2: $P < 0.0001$** (†) **vP_3: $P < 0.0001$** (†) **pkf: $P < 0.0001$**	**vP_1: $P < 0.0001$** (†) **vP_2: $P < 0.0001$** (†) **vP_3: $P < 0.0001$** (†) pkf: $P = 0.9027$	**vP_1: $P < 0.0001$** (†) **vP_2: $P < 0.0001$** (†) vP_3: $P = 0.5744$ (†) pkf: $P = 0.1450$	

† For statistical comparisons (presented here) of waveform peaks between *Petrocephalus* 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).

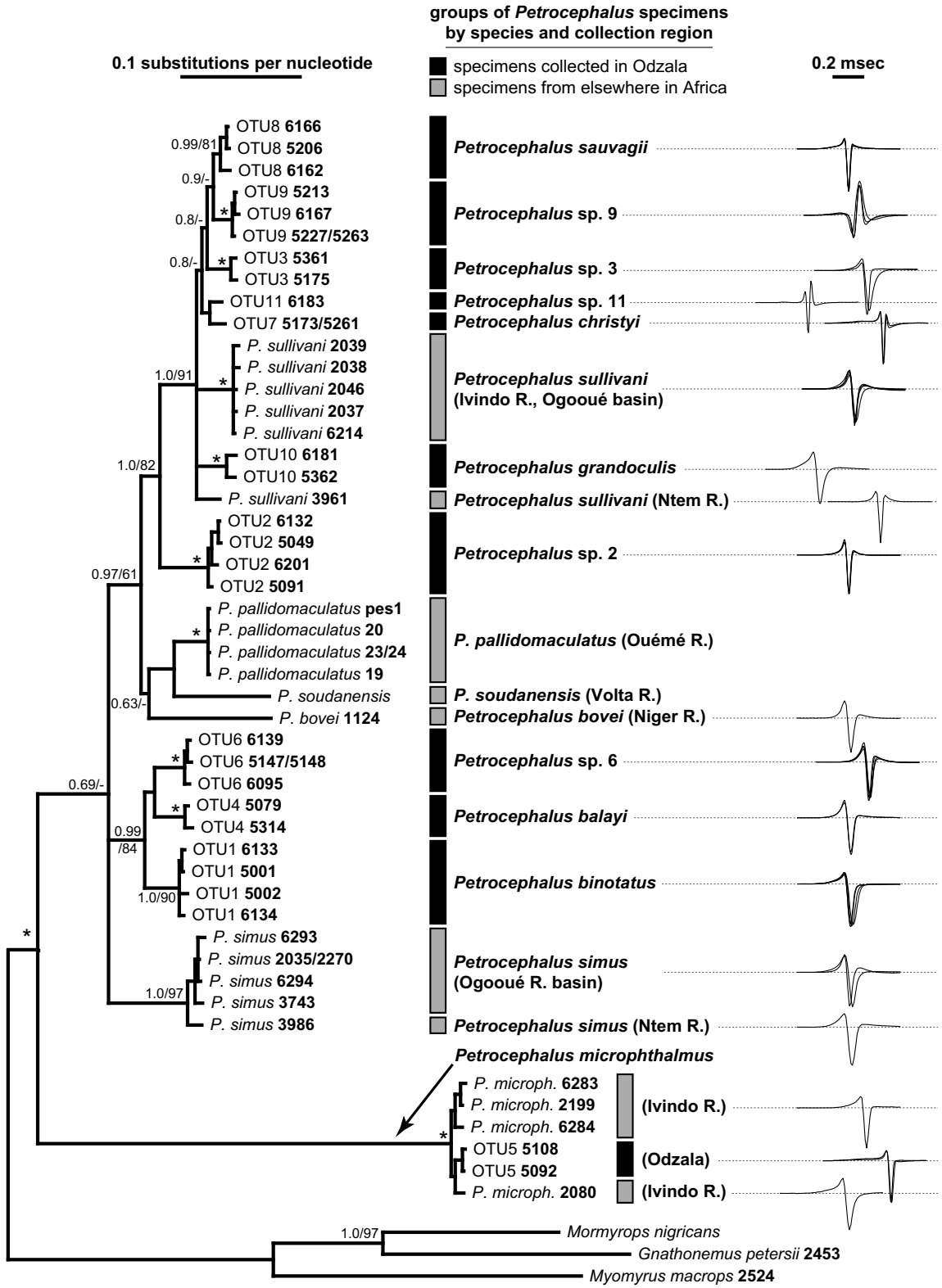

Fig. 6. Inferred phylogeny for *Petrocephalus* (53 specimens) estimated as the 50% majority-rule consensus of 30,000 pooled trees from two independent Bayesian analyses of complete cytochrome *b* sequences (dataset #1). *Myomyrus macrops*, *Mormyrops nigricans*, and *Gnathonemus petersii* are used as 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).

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 Moller, 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.,

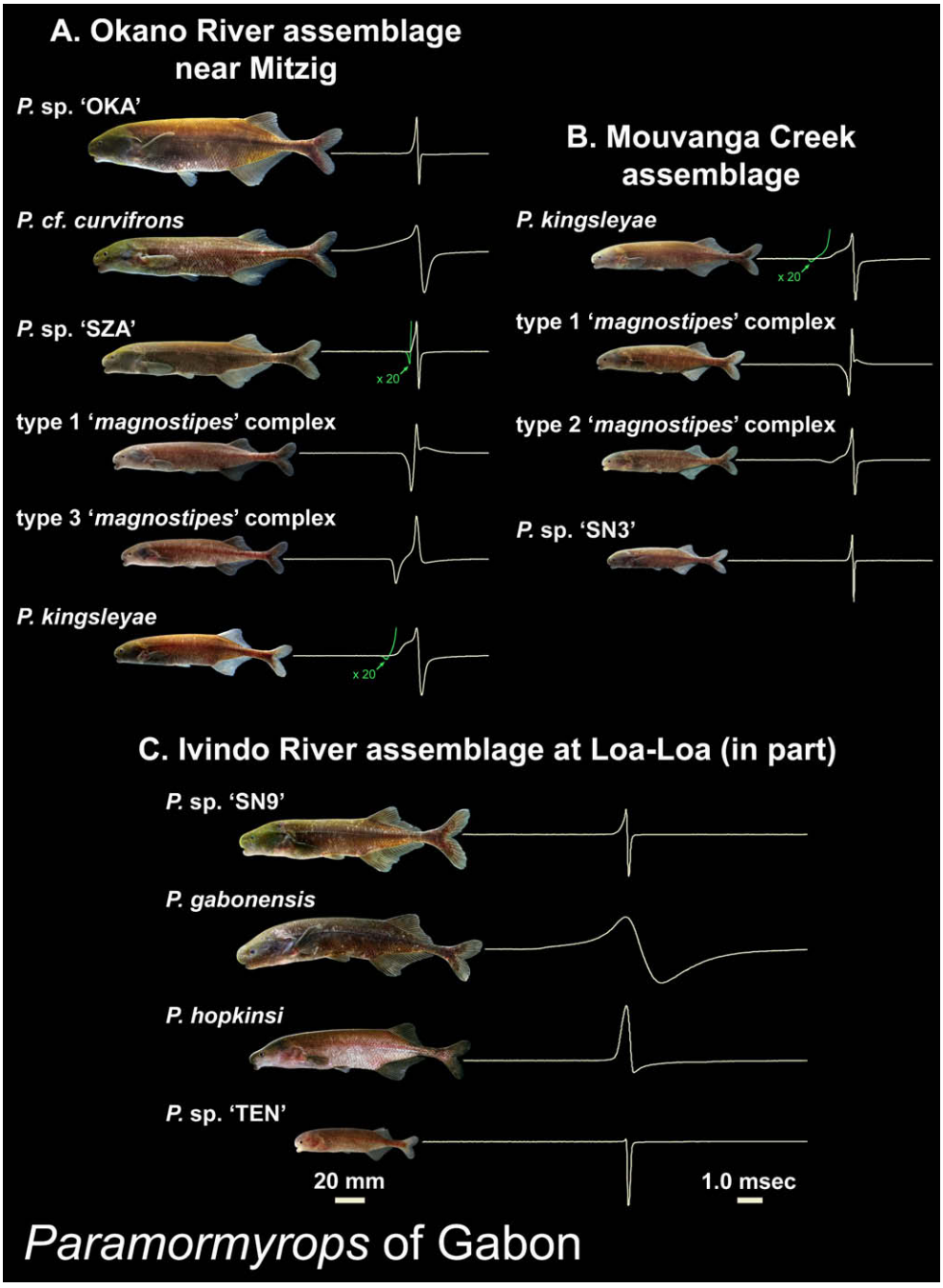

Fig. 7. Interspecific EOD variation in the *Paramormyrops* 'species flock' of Gabon. Entire assemblages of all sympatric species and morphs are shown for (A) the Okano River near Mitzig and (B) Mouvanga Creek (see map provided by Arnegard et al., 2005). (C) Only four of eleven species/morphs are shown 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).

2004), the degree of waveform variation appears slight, in general, relative to the interspecific differences observed within a number of groups of Mormyrinae (Hopkins, 1980, 1999a; Hopkins et al., 2007). The overall pattern of modest EOD divergence among many of the sympatric *Petrocephalus* species in Odzala is particularly evident in comparison to two speciose assemblages of mormyrids which have attracted much attention recently: the *Paramormyrops* of Gabon (Lower Guinean province) and the *Campylomormyrus* of Lower Congo. Striking patterns of signal divergence among sympatric species have been described in these groups of Mormyrinae (Arnegard et al., 2005; Arnegard and Hopkins, 2003; Feulner et al., 2006; Hopkins, 1999a; Sullivan et al., 2002).

In Fig. 7, we show examples of interspecific waveform variation among co-occurring *Paramormyrops* species in three electric fish communities found in the Ogooué River system of Gabon. Waveform variation among *Campylomormyrus* species of the Lower Congo River is illustrated by Feulner et al. (this issue). In each group, EODs of several species are highly-divergent in waveshape. For instance, species with nearly monophasic EODs are found in both groups. Among *Paramormyrops*, EOD duration ranges from as little as 0.2 msec in *Paramormyrops* sp. 'BN2' to as much as 15 msec in *Paramormyrops gabonensis*, based on 1.5% deviations from baseline as in the present study (personal observation). In Lower Congo, EOD durations range from about 0.2 msec to 30 msec among sympatric *Campylomormyrus* species (P.G.D. Feulner, personal communication). A much smaller range of EOD durations is seen among the *Petrocephalus* species in Odzala National Park (Table 2). Accounting for this compressed range, we also find smaller dispersion among mean EOD durations (i.e., among the 'within-species' means) for the sympatric *Petrocephalus* of Odzala, compared to a sympatric assemblage of *Paramormyrops* at Loa-Loa Rapids (on the Ivindo branch of the Ogooué River system). Species richness is similar in both electric fish communities. From our extensive work at Loa-Loa Rapids over a number of field seasons, we know that eleven species/morphs of *Paramormyrops* co-occur at this site. Only two of the eleven are currently considered morphs, for which species status remains unclear due to their morphological and genetic similarity (see Arnegard et al., 2005). As in Table 2, we have calculated the within-species (or within-morph) means for EOD durations in all eleven species/morphs at Loa-Loa (data not shown). These species averages range from 0.29 msec to 7.1 msec. The coefficient of variation among these values is 102% at Loa-Loa, whereas it is only 41% for the eleven *Petrocephalus* species of Odzala (based on the within-species means shown in Table 2, with all 37 individuals of *P. binotatus* combined for calculation of the within-species average). Excluding the two sympatric morphs of *Paramormyrops* (types 1 and 2 of the '*magnostipes*' complex), the coefficient of variation among mean EOD durations at Loa-Loa is still 95%, as reported in Table 4. Thus, the community at Loa-Loa Rapids exhibits both greater dispersion of within-species mean durations, relative to *Petrocephalus*, and a greatly expanded overall range of EOD durations among sympatric *Paramormyrops* species.

We recognize that our suggestion of greater EOD diversification within *Paramormyrops* and *Campylomormyrus*, relative to the *Petrocephalus* assemblage of Odzala, is largely qualitative at this point. Much more rigorous quantitative comparisons of waveform divergence patterns – in sound phylogenetic contexts – are certainly still needed. These sorts of studies should prove quite fruitful for better understanding signal evolution in the mormyrid model system. Principal components analysis of multiple waveform landmarks has already been used to quantify overall waveform variation in subsets of *Paramormyrops* species (Arnegard et al., 2005; Arnegard and Hopkins, 2003). However, the biological relevance of composite measures of EOD variation, such as this, remains somewhat unclear with respect to sensory coding and signal discrimination. Indeed, a landmark-based approach to quantifying 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 mormyrid 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 mormyrid 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 exhibited by these groups. Total duration is only a single univariate measure of the EOD waveform. Nevertheless, neuroethological studies of EOD time-coding in mormyrids suggest that interspecific 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 mormyrids inhabiting any similarly-sized region, or system of adjacent river systems, in Africa (e.g., see Fig. 2 of Arnegard et al., 2006). Because many inaccessible 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

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 (Lékoli River system, Congo basin), *Paramormyrops*[*] of Gabon (Ogooué River basin and vicinity), and *Campylomormyrus* of Lower Congo River.

	Petrocephalus of Odzala	*Paramormyrops*[*] of Gabon	*Campylomormyrus* of Lower Congo River
Estimated number of species detected to date (subset un-described)	11 (5)	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).	5–8 (0–3)[**]
Interspecific genetic divergence: range of pairwise *p*-distances between species calculated for cyt *b*	1.5–14.5%	0–7%	0–8%
Degree of monophyly and endemism	Non-monophyletic and non-endemic; some of the resident species are widely distributed in Africa.	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.	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 *Campylomormyrus* species in this putative Lower Congo radiation.
Pattern of interspecific variation in electric signal waveforms (i.e., EODs)	Comparatively low variation in EOD waveshape.	High interspecific waveform variation.	High interspecific waveform variation.
Range of known EOD durations	0.14–1.0 msec	0.2–15 msec	0.2–30 msec
Coefficient of variation (CV) among 'within-species' means of EOD duration for co-occurring taxa[***]	41%	95%[***]	Original EODs not available for calculation of CV.
References	This study.	Arnegard et al. (2005); Arnegard and Hopkins (2003); Hopkins et al. (2007); Sullivan et al. (2002, 2004).	Feulner et al. (2006, 2007, this issue); see also Hopkins et al. (1999a).

[*] 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 *Campylomormyrus tamandua* [i.e., 'clade F' of Feulner et al. (this issue)]: due to the widespread distribution of *C. tamandua* 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 *Campylomormyrus* radiation of Lower Congo.

[***] Type 1 and type 2 morphs of the '*magnostipes*' 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 *Campylomormyrus*. Compared to the more geographically circumscribed evolutionary theaters in which Lower Guinea *Paramormyrops* and Lower Congo *Campylomormyrus* 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 *Campylomormyrus* 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; Genner 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 *P. soudanensis* 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 predator 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; Moller, 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 *Mormyrops caballus* (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 not yet been able 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 Mormyroidea 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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