Differentiation of courtship songs in parapatric sibling species of dwarf stonebashers from southern Africa (Mormyridae, Teleostei)

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Summary

We describe the nocturnal courtship songs of male dwarf stonebashers, *Pollimyrus castelnaui*, from the Okavango River and its inland delta. We examined the question of whether the songs are sufficiently differentiated from those of its parapatric sibling species, the only recently discovered *P. marianne* from the Upper Zambezi River, to form a potential cue for mate choice. Both species vocalised two sound types in courtship, the moan and the grunt, which they combined into long songs in similar fashion. However, one sound type was clearly differentiated: while *P. castelnaui* moans were of a husky quality and composed of three or four broadband formants, *P. marianne* moans were more tonal, with a single spectral line dominating the first and any higher formants (and a smaller bandwidth $\mathrm{BW}_{-10\ \mathrm{dB}}$ for the dominant frequency of the first formant). Moan and Grunt Duration and the moan Pulse Group Period (mPGP) were longer, and the latter more variable, in *P. castelnaui* compared to *P. marianne* (range of mPGP: 10-30 ms in *P. castelnaui*, 7-16.7 ms in *P. marianne*). *P. castelnaui* grunts were of longer duration and composed of more pulses than those of *P. marianne*. A single male from the contact zone between the Okavango and the Zambezi, the lower Kwando River, resembled *P. castelnaui* in moan $\mathrm{BW}_{-10\ \mathrm{dB}}$ but *P. marianne* in Moan Duration and mPGP. Both southern African species thus vocalise in a species-specific fashion. Since in both species several characteristics of both moans and grunts show high between- and low within-male variability, mate choice may be selective for individual high-quality males characterised by acoustic features.

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Abbreviations: BD, Body Depth; CV, coefficient of variation; EOD, Electric Organ Discharge; gD, Grunt Duration; gIPI, Grunt Inter-Pulse Interval; gPAF, Grunt Peak-Amplitude Frequency; gPD, Grunt Pulse Duration; gPRR, Grunt Pulse Repetition Rate; IGI, Inter-Grunt Interval; jnd, just noticeable difference; |M|, absolute value of the mean; mD, Moan Duration; $\mathrm{mBW}_{-10\ \mathrm{dB}}$, bandwidth of moan at -10 dB of Peak-Amplitude Frequency; mgD, Duration of Moan-Grunt bout; mPAF1, Peak-Amplitude Frequency of the first formant of a moan; mPGP, Moan Pulse-Group Period; SD, standard deviation; SE, standard error; SL, Standard Length; SPL, Sound Pressure Level.

Introduction

The dwarf stonebasher *Pollimyrus castelnaui* (Boulenger, 1911) inhabits the Okavango River and Delta, whereas its recently discovered sister species, *Pollimyrus marianne*, inhabits the Upper Zambezi River (Kramer et al., 2003). Both mega systems are sporadically linked by a tenuous water connection via the intermediate Kwando/Linyanti System, a tributary of the Zambezi River (for details, see Kramer et al., 2003). Although they cannot be distinguished by the naked eye, both species are well differentiated in morphology, genetics, and the waveform of their electric organ discharges (EODs; Kramer et al., 2003; Markowski et al., subm.), and are regarded as vicariant sibling species.

In addition to their electrical signalling by EODs, the males of *Pollimyrus marianne* vocalise mating calls during courtship and spawning (Lamml & Kramer, 2005), as also known for two *Pollimyrus* species from West Africa (review, Crawford, 1997). While the song elements are basically similar among all *Pollimyrus* species studied so far, their complexity and sequence in a song may differ. *Pollimyrus marianne* males produce single tonal moans, or, when a female approaches the nest site, a long-lasting moan that is superimposed by several pulsatile grunts (Lamml & Kramer, 2005). *Pollimyrus adspersus* from the West African Niger River generates long courtship songs in which moans alternate with pulsatile grunts, which are often terminated by a long growl, whereas its sibling species *Pollimyrus isidori* vocalises a single grunt followed by several short tonal moans (Crawford et al., 1986; Bratton & Kramer, 1989; Crawford et al., 1997a, 1997b).

We here describe the mating songs of *P. castelnaui* for the first time. In contrast to the two West African *Pollimyrus* species, EOD waveform is well differentiated between the two southern African species, thus qualifying for a cue in Paterson's Specific Mate Recognition System (Paterson, 1985). It was unknown whether the mating songs of the two southern African species were sufficiently differentiated to fulfill a similar function. Therefore, we critically compared *P. castelnaui*'s mating song characteristics with those of *P. marianne*. Additionally, we studied whether inter-individual variability in sound characteristics would potentially support selective mate choice or individual recognition, in view of the acoustic discrimination capabilities found in West African *Pollimyrus* species (Marvit & Crawford, 2000a, 2000b; Fletcher & Crawford, 2001).

Methods

Animal collection and care

The *P. castelnaui* (Boulenger, 1911) were caught in March 2002 in the Okavango delta near Makwena, Sepopa Nguma lagoon, near the panhandle (19°03′45.3″S, 22°23′24.3″E; coll.: F.H. van der Bank; Figure 1A). One male had been caught already in August 2000 (18°57′30.6″S, 22°23′12.0″E; coll.: R. Bills). Three *P. marianne* Kramer et al., 2003 males were caught on 22 August 1999 in a side-channel of the Upper Zambezi River near Kalimbeza/Lisikili, downstream of Katima Mulilo (17°32′31.9″S, 24°26′17.7″E; coll.: F.H. van der Bank & B. Kramer; Figure 1B). One *P. marianne* male (no. 04) was reared in captivity (2002). An additional *P. marianne* male originated from the Kwando River (caught in August 2004 at Kongola bridge; 17°47′26.7″S, 23°20′40.0″E; coll.: F.H. van der Bank & B. Kramer). The species identity of all animals was confirmed by their characteristic EOD waveform. The males' sex was apparent from an indentation of the anal fin base (see Kramer et al., 2003).

Either male-female pairs, or one to three males (in the largest aquaria) were kept together with several females in aquaria of different size (150-780 litres). These were well equipped with stones and roots as shelters and planted with *Vesicularia dubyana* and *Cryptocoryne affinis*. The aquarium bottom was covered with sand. The L:D cycle was 12:12 hours. Animals were fed on chironomid larvae five to six times per week.

Behavioural observations

We observed a reproductive male's (focal animal) nocturnal behaviour from the early stages of a male's reproductive cycle on, when a male started to build a nest in its territory. The fish showed reproductive behaviour under environmental conditions that were allowed to vary between 50-200 μS cm^{-1} water conductivity and 23-26°C temperature. We conducted nocturnal video recordings using infrared illumination and an infrared-sensitive camera (model Panasonic FK 6990-IQ; videotape recorder, Panasonic AG-7330, Hifi, S-VHS). We placed a hydrophone in a male's territory close to its nest and recorded all acoustic signals continuously from 10 *P. castelnaui* males (SL, 5.9 ± SD 0.29 cm), four *P. marianne* males (SL, 6.0 ± SD 0.38 cm), and one male from the Kwando River (SL, 5.2 cm; only moans). Ten males that vocalised courtship songs (Moans and Moan-Grunt bouts) also reproduced successfully, and reared larvae in their nests. Five males, among them the single Kwando male, did not reproduce. Two of the non-reproducing males had vocalised only moans, the remaining three had vocalised Moan-Grunt bouts in addition to pure moans.

Analysis of acoustic signals

All sounds were monitored with a hydrophone (Brüel & Kjær, Model 8101, 1 Hz-125 kHz frequency response), amplified with a measuring amplifier (Brüel & Kjær 2610, 2 Hz-200 kHz frequency response) and tape-recorded on the first audio-track of the video-recorder. To reduce low-frequency noise, the amplified hydrophone output was bandpass-filtered (40 Hz-10 kHz passband, 24 dB attenuation / octave, linear phase response) using an electronic filter (Wavetek Rockland Inc., Model 452). Vocalisations were digitised from videotape recordings at 48 kHz sampling rate and 16 bit vertical resolution using the standard soundcard of an IBM-compatible computer (Pentium IV, 1.7 GHz), controlled by the software Avisoft-SASLabPro Version 4.33 (Avisoft Bioacoustics, Berlin, Germany). For analysis we only used sounds we were able to assign to a specific individual, based on relative sound pressure level and fish distance from the hydrophone. Sounds with good signal-to-noise ratio were typically generated within 20 cm of the hydrophone.

Table 1 summarises all sound characteristics and specifies the measuring methodology. Temporal parameters, such as Moan-Grunt bout Duration

Table 1. Summary of estimated sound characteristics and how they were determined.

Sound type	Sound characteristic (Abbr.)	Description of sound characteristic	Used data source and measuring methodology	Illustrated in Figure	Table no.
Moan	Moan Duration (mD)	Time from onset to end of sound.	Oscillogram.	2A	2
	Moan PAF1 (mPAF1)	Average frequency at peak amplitude of the first formant in the amplitude spectrum.	Amplitude spectrum. Whole sound or, when the moan was longer than 2 s, a section of 2 s duration where moan showed lowest frequency modulation.	2C	2
	Moan $BW_{-10\ dB}$ ($mBW_{-10\ dB}$)	Bandwidth of moan at −10 dB of peak-amplitude frequency.	Separation (in Hz) of two points where the amplitude spectrum first dropped by 10 dB relative to the peak.	2C	2
	Moan $PAF1_{min}$ ($MPAF1_{min}$)	Minimum frequency of the moan PAF1.	Sonogram. Minimum of PAF1 in the sonogram of the whole sound after resampling at 6 kHz (frequency resolution, 5 Hz).	2B	2
	Moan $PAF1_{max}$ ($mPAF1_{max}$)	Maximum frequency of the moan PAF1.	Sonogram. Maximum of PAF1 in the sonogram of the whole sound after resampling at 6 kHz (frequency resolution, 5 Hz).	2B	2
	Pulse Group Period (mPGP)	Time Period between the main peaks of pulse groups.	Oscillogram and high temporal resolution sonogram (temporal resolution, 0.33 ms). Mean value of 15 pulse group periods.	$2A'–2A''$	2
Grunt	Moan-grunt Bout Duration (mgD)	Time from onset to end of moan-grunt bout.	Oscillogram. Duration of the moan during a moan-grunt bout.	3A	3
	Inter-Grunt Interval (IGI)	Time interval between the onset of consecutive grunts.	Oscillogram.	3B	3

Table 1. (Continued).

Sound type	Sound characteristic (Abbr.)	Description of sound characteristic	Used data source and measuring methodology	Illustrated in Figure	Table no.
	Grunt Duration (gD)	Time from peak of the first grunt pulse to peak of the last grunt pulse.	Oscillogram (temporal resolution, 40 μs).	3C+D	3
	Pulse Number	Number of pulses per grunt.	Cursor function of the zoomed oscillogram.		3
	Grunt Pulse Duration (gPD)	Time from onset to end of one grunt pulse.	Cursor function of the zoomed oscillogram. See text for further information.	3C$''$	3
	Grunt Inter-Pulse Interval (gIPI)	Time interval between the consecutive peaks of grunt pulses.	Mean value of all grunt pulse intervals as measured from oscillograms.	3C$'$	3
	Grunt Inter-Pulse Interval 3-13 ($gIPI_{3\text{-}13}$)	Average of 10 grunts for the third to thirteenth inter-pulse interval.	Grunt pulse intervals as measured from oscillograms.		3
	Grunt Pulse Repetition Rate (gPRR)	Number of grunt pulses per second.	Reciprocal of the mean grunt inter-pulse interval.		3
	Grunt Peak-Amplitude Frequency (gPAF)	Average frequency at the peak amplitude of the amplitude spectrum.	Amplitude spectrum. Whole grunt.	3E	3
	Grunt Frequency of mPAF1 (gPAF1)	Average frequency of the moan PAF1 during the grunt.	Amplitude spectrum. Whole grunt.	3E	3

(mgD) and Moan Duration (mD), were measured from sound pressure oscillograms, with grunt Pulse Duration (gPD) and grunt Inter-Pulse Intervals (gIPI) estimated visually from zoomed oscillograms with Avisoft's cursor function. Grunt Pulse Duration was defined as the time period during which the pulse sound wave clearly emerged from the superimposed moan oscillations. Grunt Inter-Pulse Intervals were measured by visually estimating each time interval between adjacent peaks of grunt pulses. Moan Pulse-Group Period (mPGP) describes the time period between the maxima of adjacent pulse groups of a moan. Zoomed oscillograms and a concurrent sonogram representation of high temporal resolution (0.33 ms) were used to determine the pulse of highest intensity within a single pulse group. We averaged 15 mPGPs from the middle section of a moan, where it showed the lowest frequency modulation. Mean gPD was calculated by averaging the duration of 15 consecutive grunt pulses. The mean gIPI was defined as the average of all grunt IPIs.

After resampling the digitized vocalisations at 6 kHz, using an anti-aliasing filter (Avisoft), we determined the Peak-Amplitude Frequency (PAF) and the moan bandwidth at -10 dB relative to the peak amplitude ($\mathrm{mBW}_{-10\ \mathrm{dB}}$) by FFT amplitude spectra which were usually generated based on 2048 to 16384 time data points. Therefore, FFT amplitude spectra comprised between 1024 to 8192 frequency data points (resolution: 0.366-2.93 Hz). To determine the absolute SPL of vocalisations generated at about 10 cm from the hydrophone we read the relative SPL off the measuring amplifier, and calculated the absolute values (dB re: 1 μPa) based on the sensitivity of the hydrophone.

In order to know whether the temporal fine structure (IPI) of a sound was affected by the distance of the sound source from the hydrophone we made test measurements with single-cycle sine-wave pulses of 1 ms duration, repeated at an IPI of 5, 10, 15, 20 and 25 ms, that were generated by a function generator (Hewlett-Packard model 3314A). The output of this device was connected to an underwater loudspeaker (model UW-30 Diatran, University Sound, Oklahoma City) via a power amplifier (model AD5250B; Sherwood Europe GmbH). Using one of the recording aquaria in which several males of both species had produced their vocalisations (dimensions, $240 \times 65 \times 50$ cm high), we recorded the test signals at 1-55 cm distance from the loudspeaker, and did not measure any distance-dependent effect on the temporal structure

(IPI) of the recorded sounds. In the near field, signal intensity fell off dramatically with distance: -15 dB re: 1 μPa between 1 and 10 cm distance, and -13.5 dB re: 1 μPa between 10 and 30 cm, but only -1.5 dB re: 1 μPa between 30 and 55 cm distance. The absolute SPL of the signal was 115 dB re: 1 μPa at 1 cm, 100 dB re: 1 μPa at 10 cm, 86.5 dB re: 1 μPa at 30 cm and 85 dB re: 1 μPa at 55 cm distance from the loudspeaker.

Statistical analysis of vocalisations

Statistical analyses were performed with the software packages StatView version 5.0 (SAS Institute Inc.) and Prism version 3.0 (GraphPad Inc.). Because sound characteristics may depend on the size of a resonant structure rather than on SL, we roughly calculated an individual's body size as the product of Standard Length (SL) times Body Depth (BD). SL is defined as the distance between tip of snout and midbase of caudal fin. BD is the greatest vertical distance across the body (see Figure 3 in Kramer et al., 2003). We measured SL and BD from photographs taken within a few weeks before or after the sound recordings.

The vocalisations of *P. castelnaui* and *P. marianne* were recorded at a mean water temperature of 25°C, whereas those of *P. adspersus* and *P. isidori* (Crawford et al., 1997b) were recorded at about 28°C. To compare the vocalisations of West and southern African stonebashers, temperature compensation to 28°C was applied, using Q_{10} values of 1.06 for mD, 1.8 for mPAFl, 1.99 for gD and 2.02 for gPRR, as determined from a male *P. marianne* (Lamml & Kramer, 2005).

Coefficients of variation (CV) were calculated as $CV = SD / |M| \times 100$.

Results

The courtship songs of P. castelnaui compared with those of P. marianne

The males of both sibling species, *P. castelnaui* and *P. marianne*, vocalised basically the same two categorically different sound types, moans, composed of pulse groups that were repeated hundreds of time (Figures 1, 2), and grunts that consisted of short sequences of strong acoustic pulses (Figure 3). The sequence of sound patterns in a courtship song was similar between the two species. Moans and grunts were generated ever more frequently when a female started visiting a male's territory during the prespawning nights.

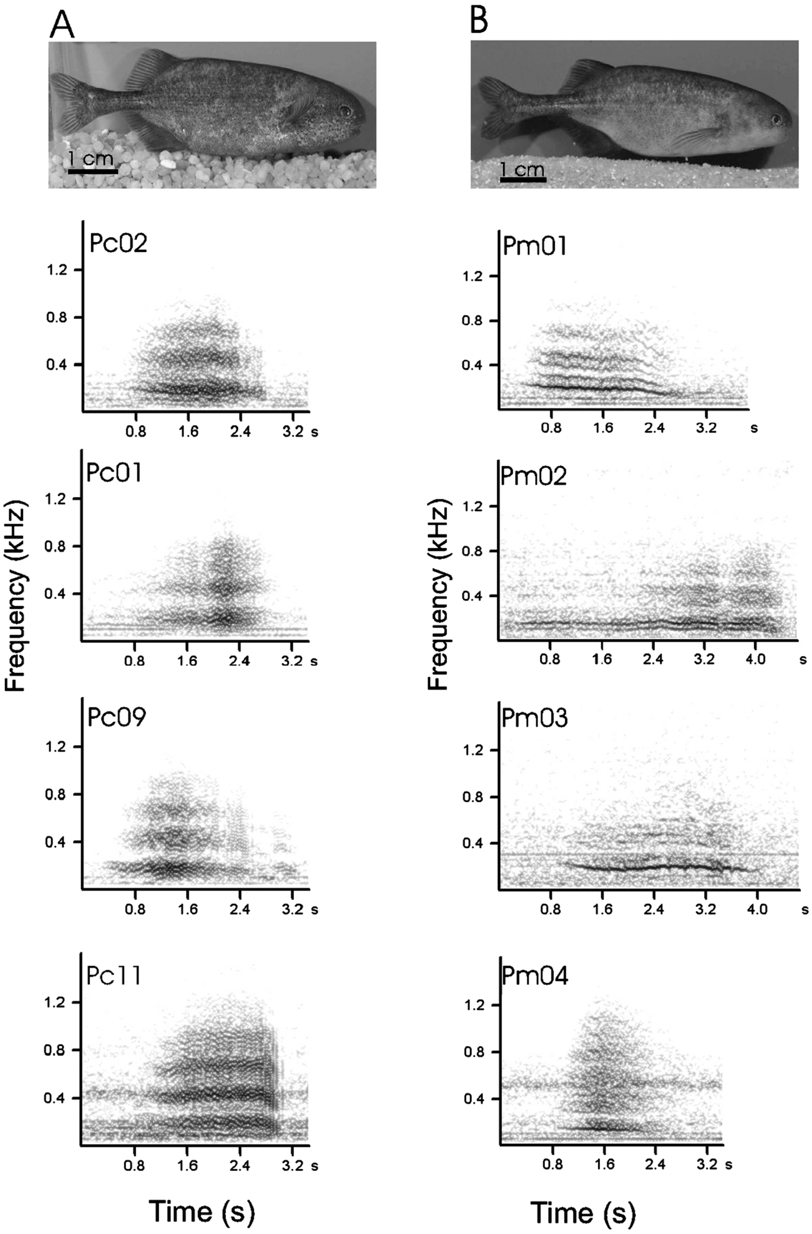

Figure 1. (A) A *Pollimyrus castelnaui* male from the Okavango; (B) a *Pollimyrus marianne* male from the Upper Zambezi River. Note indented anal fin base. Below, moans recorded from four *P. castelnaui* (left) and four *P. marianne* individuals (right), shown as sonograms (frequency resolution, 5 Hz; temporal resolution, 10.7 ms).

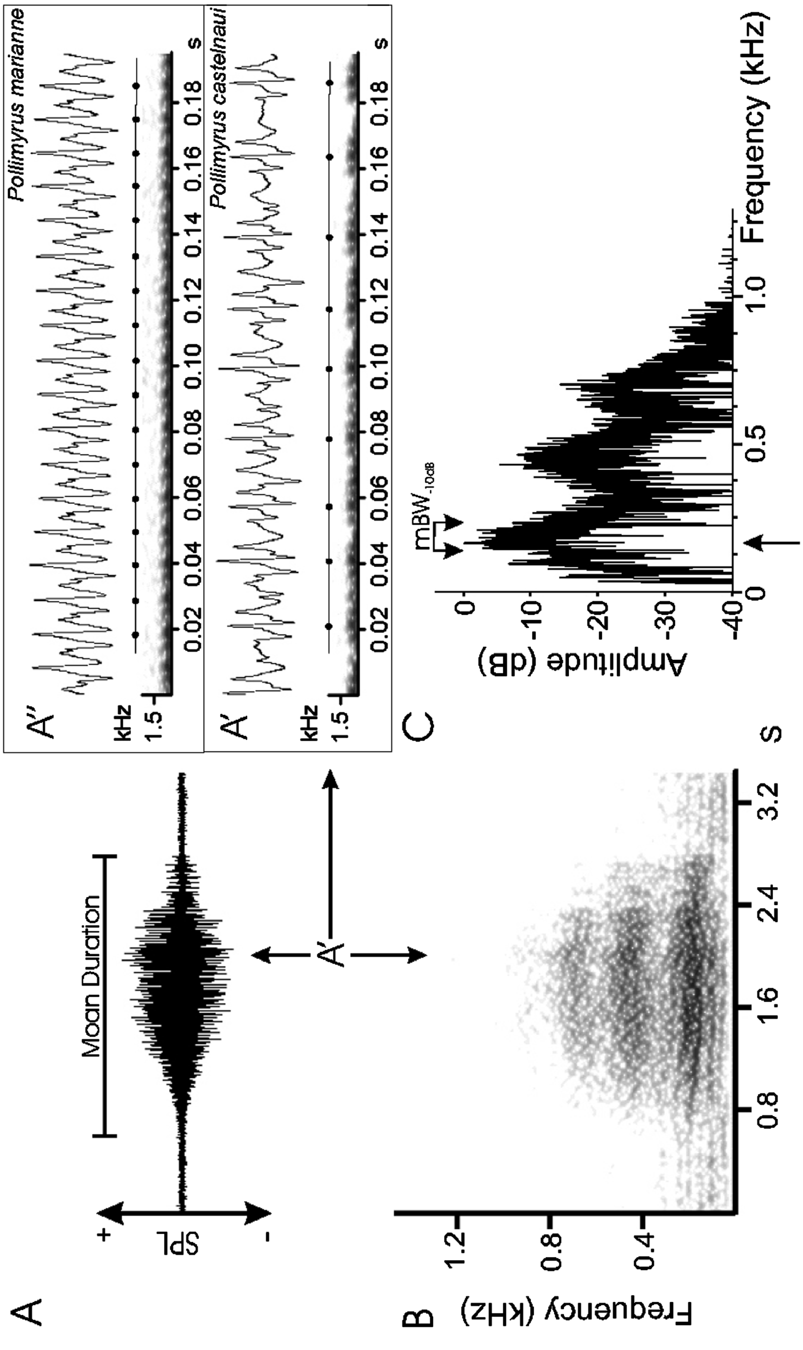
A
SPL
+
-
Moan Duration
A'
B
Frequency (kHz)
1.2
0.8
0.4
0.8
1.6
2.4
3.2
s
A''
Pollimyrus marianne
kHz
1.5
0.02 0.04 0.06 0.08 0.10 0.12 0.14 0.16 0.18
s
A'
Pollimyrus castelnaui
kHz
1.5
0.02 0.04 0.06 0.08 0.10 0.12 0.14 0.16 0.18
s
C
Amplitude (dB)
0
-10
-20
-30
-40
mBW-10dB
mPAF1
0
0.5
1.0
Frequency (kHz)

Figure 2.

A male ready to reproduce used to moan while patrolling his territory and nest site. When, however, a female was visiting the territory or the nest site, the male generated long-lasting moans superimposed by grunts (Moan-Grunt bout, Figure 3A, B). A male did not vocalise when directly engaged in a spawning posture with a female (in which the male was tilted sideways and underneath the female, with anal fins mutually tightly linked). Immediately after separation of the pair, the female used to leave the territory and the male resumed vocalising moans. The courtship behaviour of *P. castelnaui* was very similar to that reported for *P. marianne* (Lamml & Kramer, 2005).

Some moan characteristics indicate differences between the two species (Figure 1). *Pollimyrus castelnaui* moans are composed of three, sometimes four 'noisy' or broad-band components (formants) the peaks of which were only poorly harmonically related amongst each other, in contrast to the more

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Figure 2. Acoustic properties of a *P. castelnaui* moan. (A) Oscillogram, (B) sonogram and (C) amplitude spectrum. (A′, A″) A moan is composed of a sequence of many pulse groups: A′ for *P. castelnaui*, A″ for *P. marianne*, shown at high temporal resolution. The main pulse within each pulse group is indicated by a dot, determined by (1) where strongest amplitudes in the oscillogram were found, and (2) by location of blackest portions of concurrent sonogram, lower panels (temporal resolution, 0.33 ms). (B) The sonogram reveals three or four broad bands of high intensity, with most energy at about 160 Hz (first formant) that corresponds to the fourth harmonic of a fundamental frequency close to 40 Hz, determined by a mean mPGP of 24 ms. Amplitude spectra reveal a broadband mPAFl at 161 Hz and two weaker peaks of higher frequencies (corresponding to the central frequencies of the second and third formants). The sonogram is based on 1024 point FFTs with a frame size of 50%, 93.75% frame overlap and Hamming Window applied. Frequency resolution, 5 Hz; temporal resolution, 10.7 ms; passband filter 40 Hz-10 kHz.

Figure 3. Moan-Grunt bout of *P. castelnaui*. (A) Oscillogram, (B) sonogram. (C-C″) Oscillograms of a grunt at increasingly higher resolution. A sequence of 43 grunt pulses with a mean gIPI of 34 ms is composed of acoustic pulses with a mean duration of 3.5 ms. (C′) Between single grunt pulses, sinusoidal oscillations of lower amplitude representing the superimposed moan are visible. (E) The grunt Peak-Amplitude Frequency (of 1051 Hz) was not present in the moan. The oscillations of the superimposed moan show a frequency increase of gPAFl to about 200 Hz that recedes to about 180 Hz after termination of the grunt. (F, G) IPI diagrams for 10 grunts of a *P. castelnaui* and *P. marianne* male superimposed (last interval not shown when longer than 60 ms). Abscissa, interval number in the order of occurrence; ordinate, IPI duration. (B) The sonogram is based on 1024-point FFTs, with a frame size of 50%, 75% frame overlap (frequency resolution, 11 Hz; temporal resolution, 21.3 ms), or (D) 93.75% frame overlap (frequency resolution, 11 Hz; temporal resolution, 5.33 ms) with a Hamming Window applied; passband filter, 40 Hz-10 kHz.

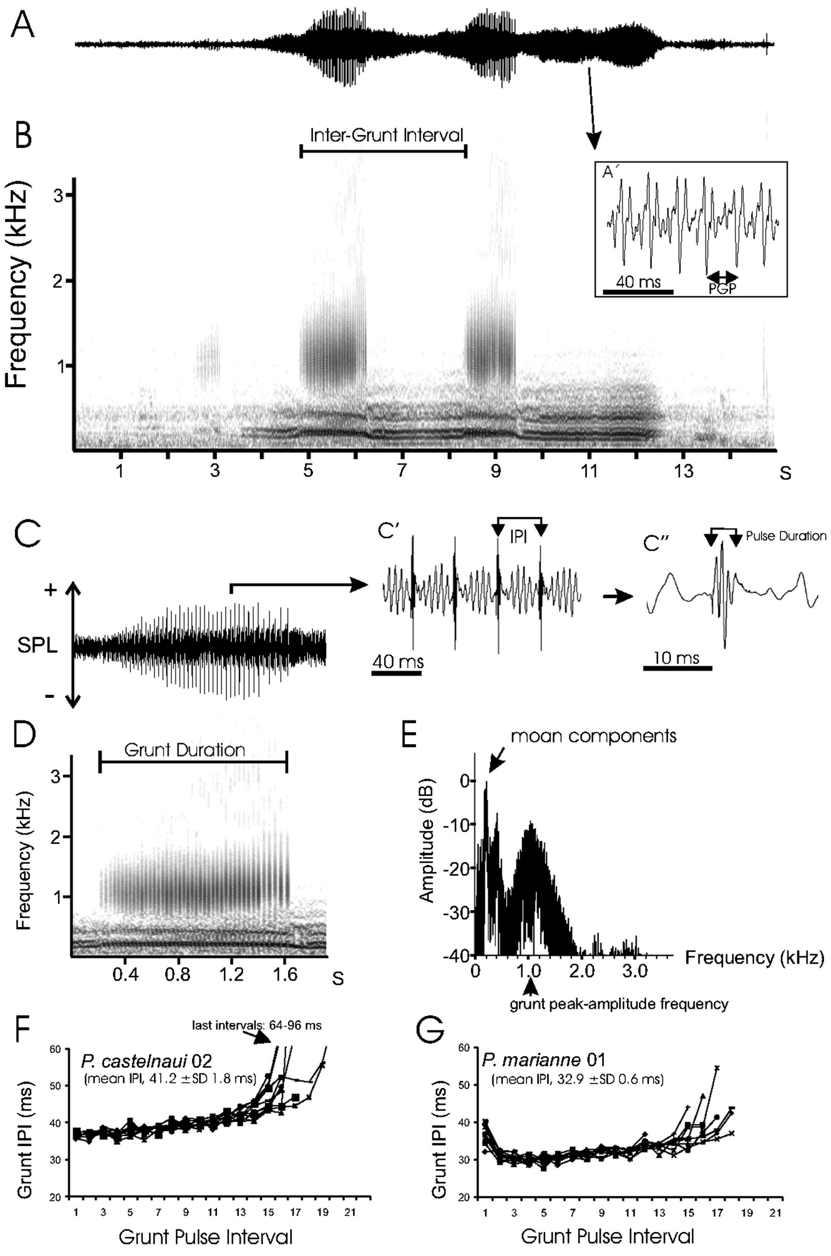
A
A´
40 ms
PGP
B
Inter-Grunt Interval
Frequency (kHz)
3
2
1
1
3
5
7
9
11
13
s
C
C′
IPI
C″
Pulse Duration
+
SPL
-
40 ms
10 ms
D
Grunt Duration
Frequency (kHz)
3
2
1
0.4
0.8
1.2
1.6
s
E
moan components
Amplitude (dB)
0
-10
-20
-30
-40
0
1.0
2.0
3.0
Frequency (kHz)
grunt peak-amplitude frequency
F
last intervals: 64-96 ms
P. castelnaui 02
(mean IPI, 41.2 ±SD 1.8 ms)
Grunt IPI (ms)
60
50
40
30
20
1
3
5
7
9
11
13
15
17
19
21
Grunt Pulse Interval
G
P. marianne 01
(mean IPI, 32.9 ±SD 0.6 ms)
Grunt IPI (ms)
60
50
40
30
20
1
3
5
7
9
11
13
15
17
19
21
Grunt Pulse Interval

Figure 3.

Table 2. Moan characteristics for *Pollimyrus castelnaui* and *Pollimyrus marianne*.

	Sound characteristic	mD$^{2)}$ (s)	mPAF1$^{2)}$ (Hz)	mPAF1$_{\min}$$^{2)}$ (Hz)	mPAF1$_{\max}$$^{2)}$ (Hz)	mPGP$^{2)}$ (ms)	mBW$_{-10\ \mathrm{dB}}$$^{2)}$ (Hz)
P. castelnaui	Grand Mean$^{1)}$	3.0	174.0	149.8	194.0	20.2	47.0
(Okavango)	SD	1.18	14.93	9.06	15.68	6.09	23.18
$N = 10$	SE	0.37	4.55	2.86	4.96	1.93	7.33
	Range	1.6-4.6	151-196	137-143	163-218	10.3-29.5	14.9-84.7
	number of individuals	10	10	10	10	10	10
	ANOVA $F_{9,86}$	**3.606**	**8.742**	**4.457**	**12.087**	**17.057**	**11.661**
	ANOVA p	**0.0008**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
P. marianne	Grand Mean$^{1)}$	2.0	153.5	127.0	175.5	13.2	17.6
(U. Zambezi)	SD	0.79	18.08	13.29	22.69	4.10	4.01
$N = 4$	SE	0.39	9.04	6.64	11.35	2.05	2.01
	Range	1.4-3.2	142-180	116-145	160-209	7.3-16.7	12.3-20.9
	number of individuals	4	4	4	4	4	4
P. marianne	Mean$^{1)}$	1.9	187.5	156.4	220.2	13.9	40.6
(Kwando)	SD	1.19	3.92	11.17	17.91	2.11	16.17
$N = 1$	SE	0.37	1.24	3.53	5.66	0.80	5.11
	Range	0.6-4.7	181-194	141-180	190-249	11.3-16.9	18-68

Water temperature, 25.0 ± SD 0.28°C (*P. castelnaui*), 24.9 ± SD 0.34°C (*P. marianne*) and 25.3°C (Kwando animal). All sounds were passband-filtered at 40 Hz-10 kHz.

1) The grand mean were calculated by averaging the individual means of 10 *P. castelnaui* specimens and of four *P. marianne* specimens. At least 7 moans were averaged to determine an individual's mean.

2) Sound characteristics used in MANOVA and univariate ANOVAs.

tonal *P. marianne* moans that are characterised by a distinctly narrowband strongest component, which corresponds best to the centre frequency of the first formant in *P. castelnaui*, and that is here termed Peak-Amplitude Frequency of the first formant, mPAFl (details, Table 2). Even in *P. castelnaui*, additional spectral lines within the broad-band formants were detectable. The $\mathrm{mBW}_{-10\ \mathrm{dB}}$ around the usually dominant spectral component, mPAF1, was much wider in *P. castelnaui* (15-85 Hz, Figure 2) than in *P. marianne* (12-21 Hz, Table 2). In *P. castelnaui*, in addition to an mPAFl of about 160 Hz, high energy occurred at about 430 Hz in the second, and at about 700 Hz in the third formant (Figure 2B, C) which, together with additional spectral components within the first formant, suggest a fundamental frequency of about 40 Hz. In some *P. castelnaui* moans the second formant at about 400 Hz was stronger than the first (see *P. castelnaui* 11 in Figure 1A).

The relatively weak harmonic structure of a moan was determined by the fine structure within a pulse group, mPGP, and the precision at which it was repeated. At high temporal resolution, the oscillogram of *P. castelnaui* moans showed identifiable pulse groups repeated hundreds of time. A pulse group consisted of one strong pulse that was followed by a few smaller oscillations. The mPGP varied moderately within the centre part of a moan. Compared to *P. marianne*, *P. castelnaui* individuals generated moans with very long mPGPs (mean mPGP up to 30 ms) in the centre part of a moan (Figures 2A′, A″), with even longer mPGPs in the terminal section of some moans. For better comparison, we analysed mPGPs of the centre parts, only, where the moans showed the lowest frequency modulation. Seven of ten *P. castelnaui* generated longer mPGPs (*P. castelnaui*: range, 10-30 ms; mean, 20.2 ms) than the *P. marianne* male with the longest mean mPGP of 16.7 ms (*P. marianne*: range, 7-16.7 ms; mean, 13.2 ms).

Moreover, *P. castelnaui* moans were often louder than *P. marianne* moans at similar SL and distance from the hydrophone. The absolute SPL for the ‘best’ moans (and average grunts) of *P. castelnaui* was 110 dB re: 1 μPa at about 10 cm from the hydrophone, in contrast to *P. marianne* moans with an SPL of about 104 dB re: 1 μPa at about the same distance. *P. marianne* seemed to concentrate their energy of softer moans in a narrow-band spectral component (caused by more constant mPGPs), whereas *P. castelnaui* vocalised louder and more broad-band moans (caused by more variable mPGPs).

The moans of one specimen of *P. marianne* from the Kwando River, a presumed hybridisation zone (Kramer et al., 2003), were similar in their mean mPGP of 13.9 ms to that of *P. marianne* from the Upper Zambezi (mean mPGP, 13.2 ms), but not to *P. castelnaui*'s (mean mPGP, 20.2 ms). However, the Kwando male's $\mathrm{mBW}_{-10\ \mathrm{dB}}$ was 40 Hz compared to 17 Hz in *P. marianne* and 47 Hz in *P. castelnaui* (Table 2); thus in this sound characteristic, the Kwando male resembled *P. castelnaui*.

The males of both species also gave moans and rarely grunts during or shortly after what appeared to be purely agonistic interactions directed against both males and females. In *P. castelnaui*, 'agonistic moans' were often very brief, and of a higher frequency than courtship moans (e.g., $\mathrm{mPAF}_{1\,\mathrm{max}}$, 200 ± SD 16 Hz rather than 188 ± SD 8 Hz, $\geqslant 7$ agonistic moans recorded from Pc 09, Student's t-test, $p = 0.0667$). *Pollimyrus castelnaui*'s agonistic moans resembled those of *P. marianne* in their shorter mD and higher mPAF1 when compared to courtship moans (Lamml & Kramer, 2005). For the present species comparison of courtship sounds, we used only vocalisations that were not directly accompanied by overt aggression, such as circling while giving bites or butts.

Pollimyrus castelnaui Moan-Grunt bouts comprised one or many grunts with a highly variable Inter-Grunt Interval (IGI) of 2.8 to 8.8 s (Figure 3 A, B, Table 3). The duration of a Moan-Grunt bout varied considerably depending on the presence of a gravid female in the male's territory. When a female was present, Moan-Grunt bouts could last up to 58 s, during which the vocalising male did not attack the visiting female. However, the male often terminated a female's visit with a well-aimed bash against the female (in total darkness). The mean duration of Moan-Grunt bouts did not differ between the two species. The IGI was higher in *P. castelnaui* because of one male's exceptionally long mean IGI of 8.8 s.

A grunt is composed of a short sequence of strong acoustic pulses. The *P. castelnaui* as a group generated grunts that were composed of more pulses than in *P. marianne* (ranges of the individuals' means: *P. castelnaui*, 15-34 pulses; *P. marianne*, 13-18 pulses). Accordingly, the mean gD of *P. castelnaui* (674 ms) was longer than in *P. marianne* (546 ms), but the *P. marianne* values were still within the range of *P. castelnaui*.

The *P. castelnaui* grunt in Figure 3C consists of 43 pulses. Grunt pulses always began with a short negative inflection that was followed by a few major oscillations (Figure 3C″). The mean gPD in *P. marianne* was longer

Table 3. Grunt characteristics for *Pollimyrus castelnaui* and *Pollimyrus marianne* (also moan characteristics when in combination with grunts).

	Sound characteristic	mgD (s)	IGI (s)	gD$^{3)}$ (ms)	# Pulses / Grunt$^{3)}$	gPD$^{3)}$ (ms)	gIPI$^{3)}$ (ms)	gIPI$_{(3\text{-}13)}$$^{4)}$ (ms)	gPRR$^{3)}$ (Hz)	gPAF1$^{3)}$ (Hz)	gPAF$^{3)}$ (Hz)
P.	Grand Mean$^{2)}$ / Median$^{1)}$	20.6	4.5	674.1	21.5$^{1)}$	3.4	33.1	31.4	30.7	197.0	1035.0
castelnaui	SD/SIQ$^{1)}$	12.37	1.86	184.40	5.93$^{1)}$	0.19	3.75	3.87	3.16	18.11	118.60
	SE	4.12	0.62	61.47	1.98$^{1)}$	0.06	1.25	1.29	1.05	6.03	39.53
	Range	8.6-43	2.8-8.8	457-1110	15.6-34.1$^{1)}$	3.1-3.8	29.0-41.2	26.9-39.2	24.3-34.5	176-235	868-1206
	number of individuals	9	9	9	9	9	9	9	9	9	9
	ANOVA $F_{8,78}$			**7.330**	**8.455**	**9.683**	**59.651**		**47.992**	**15.702**	**23.981**
	ANOVA p			**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		**<0.0001**	**<0.0001**	**<0.0001**
P.	Grand Mean$^{2)}$ / Median$^{1)}$	21.3	3.2	546.2	16.2$^{1)}$	3.6	35.2	35.0	28.6	186.5	1003.0
marianne	SD/SIQ$^{1)}$	7.12	0.24	49.40	1.95$^{1)}$	0.35	2.88	3.59	2.33	13.50	81.03
	SE	3.56	0.12	24.70	0.97$^{1)}$	0.17	1.44	1.79	1.16	6.75	40.51
	Range	15.1-31.1	2.9-3.5	488-608	13.6-18.1$^{1)}$	3.3-4.1	32.6-38.7	31.5-39.4	25.8-30.7	168-200	925-1117
	number of individuals	4	4	4	4	4	4	4	4	4	4

Water temperature, 25.0 ± SD 0.28°C (*P. castelnaui*) and 24.9 ± SD 0.34°C (*P. marianne*). All sounds were passband-filtered at 40 Hz-10 kHz.

1) Median and SIQ (= semi-interquartiles) for count data.

2) At least eight sounds were averaged to determine an individual's mean. The grand means were calculated by averaging the individual means of nine *P. castelnaui* specimens and of four *P. marianne* specimens.

3) Sound characteristics used in MANOVA and univariate ANOVAS.

4) gIPI$_{(3\text{-}13)}$ was not used in MANOVA and univariate ANOVA because this parameter's distribution was not Normal.

The Kwando male was not observed to vocalise grunts.

Table 4. Long-term stability of moan and grunt characteristics in *P. marianne* male 01.

		Moan		Grunt		
		Duration (s)	PAF1 (Hz)	Duration (ms)	PAF (Hz)	PRR(Hz)
June 2002	M	2.58	154	573	922	31.5
	SD	1.39	13.3	195.7	103.4	2.3
	N	106	104	57	57	57
June 2004	M	2.81	166	563	888	32.5
	SD	1.03	9.3	144	85	1.69
	N	23	23	46	45	46
July 2005	M	1.90	148	539	925	30.3
	SD	0.83	15.7	67.6	31.5	0.57
	N	10	10	10	10	10

N, number of vocalisations.

than in *P. castelnaui* because of one *P. marianne* whose mean of 4.1 ms exceeded that of all other fish. Between grunt pulses, sinusoidal oscillations of the superimposed moan were visible in both species. In moans with long mPGPs, mPGP receded to longer terminal values after a grunt had finished, similar to those that had been generated preceding the grunts (Figure 3A′). The grunt amplitude spectrum peaked at 1051 Hz in this particular example (gPAF). Lower-frequency spectral peaks were caused by the superimposed moan, such as the one at 215 Hz (Figure 3E). *P. marianne* males vocalised grunts with similar PAF of 925-1117 Hz that were within the range of *P. castelnaui* individuals (868-1206 Hz). Grunt pulses were separated by mean gIPIs of similar duration for both species (33 and 35 ms, Table 3). A few initial gIPIs of long duration were followed by a sequence of gIPIs that were nearly constant or only slowly increasing ($\text{gIPI}_{3\text{-}13}$), with the very last gIPI of much longer duration (see Figure 3F, G). The mean $\text{gIPI}_{3\text{-}13}$ varied considerably between males, but only little within males. Table 4 illustrates the long-term variability of some sound characteristics of *P. marianne* male 01 over three years (June 2002-July 2005). This male's mean gPRR varied between 30.3 Hz in 2005 and 32.5 Hz in 2004 at similar temperature. Regarding the sound characteristics of the grunt we were unable to recognize clear species differentiation between *P. castelnaui* and *P. marianne*.

In many animals, sound characteristics depend on body size. As a rough indicator for body size we used SL × BD. Our *P. castelnaui* ranged from 5.4

to 6.3 cm in SL, and from 1.45 to 1.91 cm in BD. None of the *P. castelnaui* sound characteristics depended on body size, as revealed by least-squares regression analysis ($F_{1,8} \leqslant 2.791$, $p \geqslant 0.1333$ for mD, mPAFl, mPAF1$_{\mathrm{min}}$, mPAF1$_{\mathrm{max}}$, mPGP, mBW$_{-10\ \mathrm{dB}}$, IGI, gD, number of grunt pulses, gPD, gPAF, gPRR, gIPI, gIPI$_{3\text{-}13}$; one mean value per individual entered in the analysis). The number of *P. marianne* individuals that were available to this study was too small for regression analysis.

Intraspecific variability of sound characteristics in P. castelnaui and P. marianne

We examined the variability of moan and grunt characteristics between *P. castelnaui* males vs the within-male variability.The hypothesis of no between-male differences among any one of 6 moan characteristics (Table 2) was rejected by MANOVA ($F_{\geqslant 9,86} \geqslant 4.808$, $p < 0.0001$; test variables, Wilks' Lambda, Roy's Greatest Root, Hotelling-Lawley Trace, and Pillai Trace). A similar MANOVA result was obtained for the seven grunt characteristics marked on Table 3 ($F_{\geqslant 8,78} \geqslant 9.453$, $p < 0.0001$). Subsequent univaritate ANOVAs showed significant between-male differences for all six moan characteristics (Table 2), and all seven grunt characteristics (Table 3) that were included in the MANOVAs. Therefore, sound characteristics may function as individual markers, perhaps of male quality, in intra- or intersexual selection.

Figure 4 illustrates the considerable between-male variability, using mPGP, gPAF and gIPI as examples for both *P. castelnaui* and *P. marianne*. Table 5 contrasts the two species' within- (CV$_{\mathrm{w}}$) and between-male (CV$_{\mathrm{b}}$) variabilities of sound characteristics. In *P. castelnaui*, the mean within-male variability of mD, gD, IGI, and grunt pulse number was very high (29.4-52.7%), and these sound characteristics therefore appear unsuitable as individual markers ($\mathrm{CV_b/CV_w} \leqslant 0.93$). However, in *P. castelnaui* mPAF1$_{\mathrm{max}}$, mPGP and mBW$_{-10\ \mathrm{dB}}$ might serve as individual-specific traits ($\mathrm{CV_b/CV_w} \geqslant 1.13$); a similar conclusion is suggested for the characteristics gPRR, gIPI, gIPI$_{3\text{-}13}$, and gPAF ($\mathrm{CV_b/CV_w} \geqslant 1.7$).

Four *P. marianne* males showed higher between- than within-male variability in the characteristics mPAF1, mPAF1$_{\mathrm{max}}$, and mPGP, and also in gPAF, gPD, gPRR, gIPI and gIPI$_{3\text{-}13}$. All are potentially suitable as individual markers (Table 5). However, in mBW$_{-10\ \mathrm{dB}}$, gD, IGI and number of pulses to a grunt *P. marianne* males showed ratios of $\mathrm{CV_b/CV_w} < 1.0$.

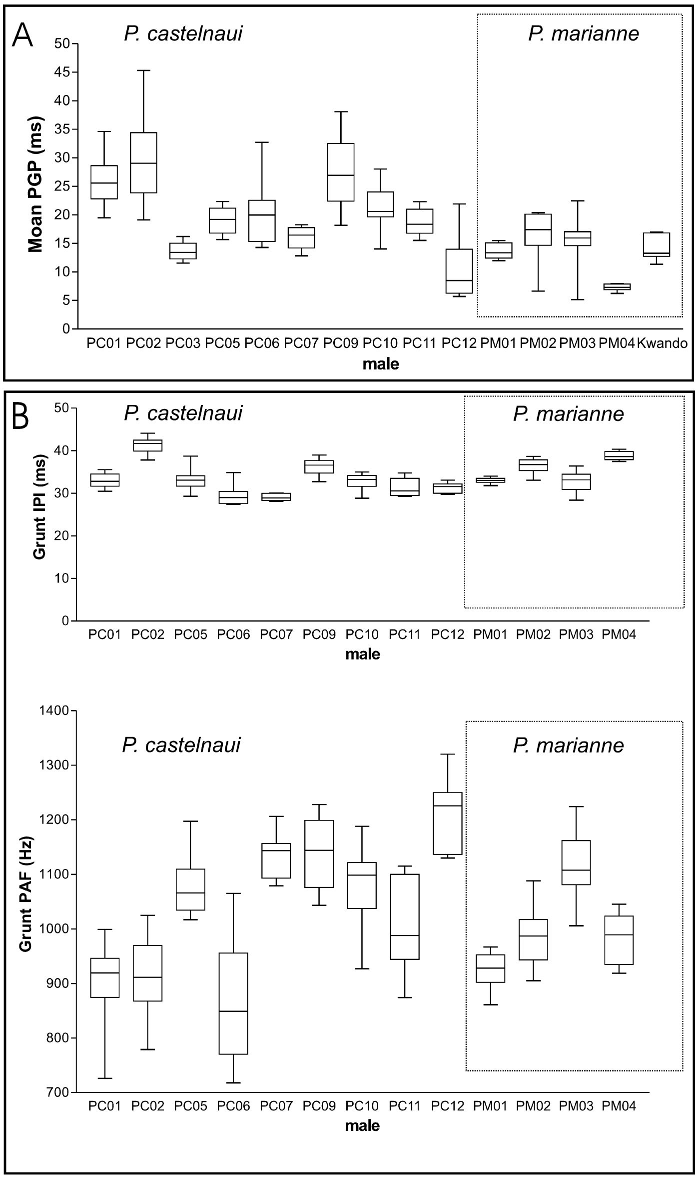

Figure 4. Between-male variability for (A) mPGP, (B) gIPI and gPAF. For gIPI and gPAF, all *P. marianne* males were within the broad range of variation of *P. castelnaui* males. For mPGP, seven of ten *P. castelnaui* males produced longer mean mPGPs than the *P. marianne* male with the longest mPGP of 16 ms. The horizontal bar within a box indicates the median. The whiskers give the minimum and maximum value for each individual. The lower and upper box boundaries indicate the 25th and 75th percentiles.

Table 5. Within- and between-male variability of moan (10 individuals) and grunt sounds (nine individuals) for *P. castelnaui*, and *P. marianne* (four individuals), as measured by the coefficient of variation. Sound characteristics that are potentially individual markers are shown in bold print ($CV_b/CV_w > 1.1$). Additionally, the within-male CV of moan characteristics for one *P. marianne* male from the Kwando River is given.

Sound characteristic	*P. castelnaui*				*P. marianne*				Kwando male
	Mean within-male CV (%)	Range of within-male CV (%)	Between-male CV (%)	Ratio CV_b/CV_w	Mean within-male CV (%)	Range of within-male CV (%)	Between-male CV (%)	Ratio CV_b/CV_w	Within-male CV (%)
Moan Duration	52.71	31.13-98.28	39.81	0.75	37.84	14.94-52.05	39.64	1.04	64.39
MoanPAFl	8.14	3.06-12.98	8.26	1.01	7.71	4.82-10.61	11.77	**1.52**	2.09
MoanPAFl$_{min}$	8.52	5.85-14.02	6.04	0.70	9.94	8.23-12.53	10.46	1.05	7.14
Moan PAFl$_{max}$	6.66	3.01-9.90	8.08	**1.21**	9.41	3.51-12.72	12.96	**1.37**	8.13
Moan PGP	20.55	11.19-50.91	30.08	**1.46**	17.07	7.38-26.44	31.08	**1.82**	15.16
Moan BW$_{-10\ dB}$	43.25	14.48-68.61	49.27	**1.13**	39.72	20.50-59.22	22.73	0.57	39.82
Grunt Duration	30.86	18.64-40.06	27.35	0.88	18.71	12.52-26.26	9.04	0.48	
Inter-Grunt Interval	50.30	32.42-58.70	41.28	0.82	43.74	34.20-56.52	7.60	0.17	
Number of Grunt Pulses	29.40	21.12-39.07	27.55	0.93	18.54	10.48-26.34	12.07	0.65	
Grunt PRR	5.03	2.61-6.80	10.33	**2.05**	4.28	1.88-7.77	8.14	**1.90**	
Grunt PAF	6.73	3.39-12.30	11.45	**1.70**	4.82	3.38-5.98	8.07	**1.67**	
Grunt IPI	5.11	2.63-7.51	11.35	**2.22**	4.15	1.87-7.43	8.21	**1.97**	
Grunt IPI$_{3\text{-}13}$	4.08	1.97-6.50	12.32	**3.04**	6.90	4.40-8.32	10.23	**1.48**	
Grunt Pulse Duration	5.71	3.33-7.37	5.755	1.00	4.75	3.43-6.53	9.91	**2.08**	

The single *P. marianne* male from the Kwando River resembled *P. castelnaui* in its high within-male variability of mD (64.4%) and low within-male variability of $\text{mPAF1}_{\text{min}}$ (7.1%; Table 5). In mPAFl (2.1%) and mPGP (15.2%) this male's within-male variability was lower than those of both *P. castelnaui* and *P. marianne* from the Upper Zambezi River. The Kwando male resembled *P. marianne* from the Upper Zambezi River in its higher within-male variability of $\text{mPAF1}_{\text{max}}$ (8.1%) and in its lower within-male variability of $\text{mBW}_{-10\ \text{dB}}$ (39.8%) compared to *P. castelnaui.*

Discussion

Courtship songs within the genus Pollimyrus

Like males of the sibling species *P. marianne* (Lamml & Kramer, 2005), territorial *P. castelnaui* males built nests at the beginning of a reproductive cycle and courted females with two different sound types which they combined into courtship songs: relatively long-lasting moans and short, pulsatile grunts. The vocalisations of both southern African species differed from those of the two West African species, *P. adspersus* and *P. isidori* (Crawford et al., 1997b), in the typical sequence of sound patterns, in quantitative parameters of certain sound characteristics, and by the lack of growls and hoots. *Pollimyrus castelnaui* and *P. marianne* generated moans and grunts of much longer duration, and grunts of lower gPRR, than the two West African species (Figure 5). In *P. adspersus*, grunts and moans are generated by sonic muscles drumming against the swimbladder (as demonstrated by Crawford & Huang, 1999), and the same mechanism is assumed for the two southern African species. Differences between southern African and West African species in body size and shape are likely to affect the size of the resonant structure (presumably the swimbladder). However, morphology does not explain differences in display duration, such as in mD and gD. It is unexplained why the southern African species, called the dwarf stonebashers because they are so small, show lower mPAF1 and gPRR values than the two West African species. This seems to indicate that mPAFl is not only determined by the morphology (size) of the swimbladder but also by the sonic muscle twitch rate.

Nest construction, parental care and complex courtship songs may be shared derived behavioural traits for the genus *Pollimyrus*. Members of other

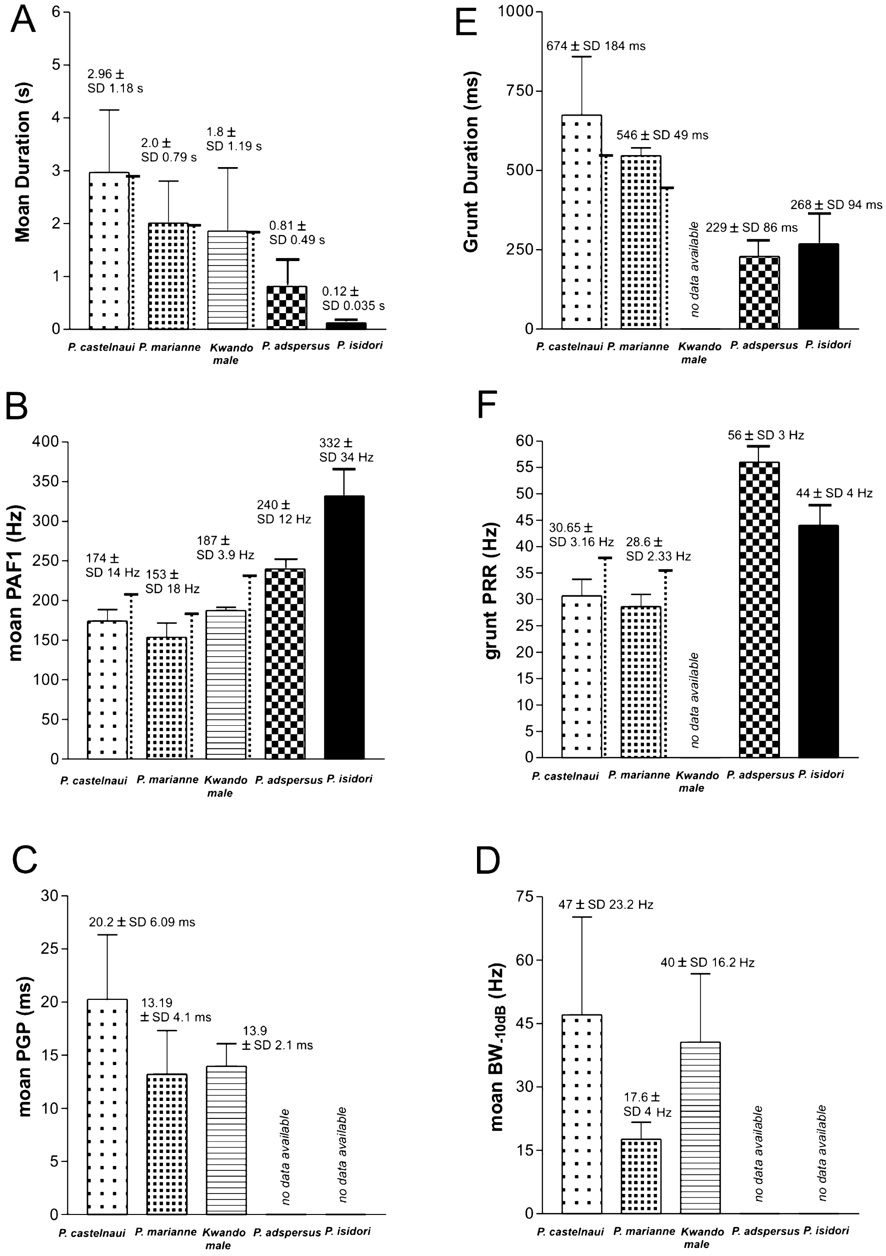
A
Moan Duration (s)
0
1
2
3
4
5
6
2.96 ± SD 1.18 s
2.0 ± SD 0.79 s
1.8 ± SD 1.19 s
0.81 ± SD 0.49 s
0.12 ± SD 0.035 s
P. castelnaui
P. marianne
Kwando male
P. adspersus
P. isidori
E
Grunt Duration (ms)
0
250
500
750
1000
674 ± SD 184 ms
546 ± SD 49 ms
no data available
229 ± SD 86 ms
268 ± SD 94 ms
P. castelnaui
P. marianne
Kwando male
P. adspersus
P. isidori
B
moan PAF1 (Hz)
0
50
100
150
200
250
300
350
400
174 ± SD 14 Hz
153 ± SD 18 Hz
187 ± SD 3.9 Hz
240 ± SD 12 Hz
332 ± SD 34 Hz
P. castelnaui
P. marianne
Kwando male
P. adspersus
P. isidori
F
grunt PRR (Hz)
0
5
10
15
20
25
30
35
40
45
50
55
60
30.65 ± SD 3.16 Hz
28.6 ± SD 2.33 Hz
no data available
56 ± SD 3 Hz
44 ± SD 4 Hz
P. castelnaui
P. marianne
Kwando male
P. adspersus
P. isidori
C
moan PGP (ms)
0
5
10
15
20
25
30
20.2 ± SD 6.09 ms
13.19 ± SD 4.1 ms
13.9 ± SD 2.1 ms
no data available
no data available
P. castelnaui
P. marianne
Kwando male
P. adspersus
P. isidori
D
moan BW-10dB (Hz)
0
15
30
45
60
75
47 ± SD 23.2 Hz
17.6 ± SD 4 Hz
40 ± SD 16.2 Hz
no data available
no data available
P. castelnaui
P. marianne
Kwando male
P. adspersus
P. isidori

Figure 5.

mormyrid genera which have reproduced in captivity, such as *Marcusenius macrolepidotus* from South Africa (Werneyer & Kramer, 2005), and *Mormyrus rume proboscirostris* and *Campylomormyrus cassaicus* (Schugardt, 1997), are unknown to build nests. Whether or not *Mormyrus* and *Campylomormyrus* species vocalise is unstudied at present. Growl sounds are also generated by male *M. macrolepidotus angolensis* from the Upper Zambezi during courtship (Lamml & Kramer, subm.), which are rather simple calls compared to the complex courtship songs within the genus *Pollimyrus*.

Acoustic differentiation between P. castelnaui and P. marianne

Few are the data in support of species-specific vocalisations among closely related fish species (Myrberg et al., 1978; Lugli et al., 1995; Crawford et al., 1997b; Lobel, 1998; Amorim et al., 2004). The parapatric sibling species *P. marianne* and *P. castelnaui* are differentiated morphologically, genetically, and in their EOD waveforms (Kramer et al., 2003). The Upper Zambezi is sporadically connected with the Okavango via the Linyanti-Chobe system during high floods (at least seven brief periods since 1942). Kwando *P. marianne* are distinct from Upper Zambezi *P. marianne* in morphology and EOD waveform, following a geographical cline (increasing differentiation with distance). This is evidence in support of migration and possibly hybridisation between the Kwando and Upper Zambezi Rivers (Kramer et al., 2003).

Although courtship behaviour, the sound elements (moan and grunt), and their combination into songs was similar for both sibling species of the present study, a practised human listener distinguishes between the ‘husky’ moans of *P. castelnaui*, and *P. marianne*’s more tonal moans. Because mPGP in *P. castelnaui* was more variable than in *P. marianne*, *P. castelnaui* moans

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Figure 5. Comparison of acoustic characteristics for all members of the genus *Pollimyrus* known to vocalise courtship songs (data for *P. adspersus* and *P. isidori* taken from Crawford et al., 1997b). A-D, moans; E, F, grunts. Shown are means +1 SD. The vocalisations of *P. castelnaui* and *P. marianne* were recorded at a mean water temperature of 25°C, whereas those of *P. adspersus* and *P. isidori* were recorded at about 28°C. Note that with temperature compensation to 28°C applied, means change to values indicated by vertical broken lines. Notwithstanding, note clearly higher or lower means for the two tropical species compared with the two southern African species. Note the differences between the two southern African species in mPGP and $\mathrm{mBW}_{-10\ \mathrm{dB}}$, indicating differentiation. The Kwando male fell in with *P. castelnaui* for $\mathrm{mBW}_{-10\ \mathrm{dB}}$, but with *P. marianne* for mPGP.

showed a broad-band spectral structure compared to the more narrow-band, harmonic structure of *P. marianne* moans ($\mathrm{mBW}_{-10\ \mathrm{dB}}$, Figure 5). Grunts were quite similar for both species, but *P. castelnaui* generated more pulses per grunt than *P. marianne* (15-34 in *P. castelnaui*; 13-18 in *P. marianne*) which resulted in a longer mean gD in *P. castelnaui*. However, the *P. marianne* range was within the limits of the broad range of *P. castelnaui*. As far as can be ascertained from the small number of *P. marianne* males available to this study, the differences in moan characteristics between both species indicate acoustic differentiation between *P. castelnaui* and *P. marianne*, congruent with morphological, genetic, and EOD waveform differentiation as documented by Kramer et al. (2003).

Intraspecific variability and sexual selection

The cues required for 'specific mate recognition', as proposed by H.E.H. Paterson (1985), may, in certain mormyrids, be provided by the EOD waveform (e.g., the spectacular sexual dimorphism in EOD pulse duration found in *Marcusenius macrolepidotus angolensis* from the Upper Zambezi; Kramer, 1997). Individual EOD waveform discrimination of μs-resolution has been demonstrated in *P. adspersus* (Graff & Kramer, 1992; Paintner & Kramer, 2003), and is apparently present also in *M. macrolepidotus* (South African form; Hanika & Kramer, 2005). Untrained, unrewarded *P. marianne* preferred conspecific playback EODs over those of *P. castelnaui*, whereas the reverse experiment was less clear (Markowski et al., submitted). Is species- or individual recognition in *P. castelnaui* and *P. marianne* not entirely restricted to the electric modality? Given the presumably very high costs associated with vocalisations that may attract predators, many of which have keen hearing, such as catfish (*Clarias gariepinus* and *C. ngamensis* were reported to prey on *P. castelnaui* in the Okavango delta; Merron, 1993), and characids (such as the tigerfish and the African pike), there must be an important benefit associated with courtship songs.

In *P. adspersus*, auditory sensitivity is best between 200-900 Hz, and matches the spectral range of moans and grunts with peak amplitudes at about 400 Hz (Marvit & Crawford, 2000a, 2000b; Fletcher & Crawford, 2001; Large & Crawford, 2002). Behavioural studies revealed a discrimination limen of ±8.5 Hz at a base frequency of around 500 Hz (Marvit & Crawford, 2000b), and best temporal click resolution with just noticeable differences (jnds) of approximately 0.3 ms in the 10-15 ms inter-click interval range (Marvit & Crawford, 2000b). For another mormyrid generating acoustic clicks, *Gnathonemus petersii* (Rigley & Marshall, 1973), auditory evoked brainstem responses showed similar jnds of about 0.5 ms (Wysocki & Ladich, 2002). Assuming a similarly acute hearing for *P. castelnaui*, females should discriminate the tonal moans of *P. marianne* males with a mean mPGP of below 16 ms from the more 'husky' moans of *P. castelnaui* with longer and more variable mPGPs of about 20 ms. Therefore, mate recognition based on specific courtship songs should be possible between *P. marianne* and *P. castelnaui* individuals, during an encounter which is clearly possible in nature.

Additionally, some sound characteristics might have the potential for individual signatures. Grunts were vocalised during courtship almost exclusively. Mean gIPI was surprisingly stable within, but quite variable between males of both species (29.0-41.2 ms in all *P. castelnaui* males; 32.6-38.7 ms in all *P. marianne* males; Figure 4, Table 3). A high between- but low within-male variability in sound characteristics is the prerequisite for inter- and intrasexual selection, and also individual recognition (Table 5). In *P. castelnaui* we did not recognize any dependency of sound characteristics on body size (SL × BD), indicating that none of the characteristics we studied may be under sexual selection. By contrast, specific information on sex, size, and perhaps condition is available from *P. castelnaui*'s (much less so from *P. marianne*'s) EOD waveform (Markowski et al., subm.). Compared to *P. castelnaui* that produces pentaphasic EODs, *P. marianne* generates triphasic EODs encoding less information, in line with its simpler EOD waveform. A female may identify her partner by individual-specific vocalisations at a greater distance than would be possible by its EOD alone, the reach of which is smaller than that of the vocalisations (Crawford et al., 1986, 1997a).

Pollimyrus marianne may have evolved more precisely harmonic moans than *P. castelnaui* that compensate for being softer by contrasting better from a noisy background. The maximum within-male variability for mPGP was 50% in *P. castelnaui* males, but only 26% in *P. marianne* males (Table 5). In contrast to *P. castelnaui*, size dependency for mPAF1 seems to be present in *P. marianne*, because a small male (5.5 cm SL) produced moans of high mPAF1 at about 180 Hz, and the still smaller Kwando male (SL, 5.2 cm) even at 187 Hz, whereas in three larger males this value was around 140 Hz. Therefore, in contrast to *P. castelnaui*, *P. marianne* females may assess mate size or quality by acoustic characteristics, indicating a difference in the relative importance of the electric and the acoustic modality in both species that, regarding the EOD, became already apparent from EOD playback experiments (Markowski et al., subm.).

Mormyrids produce electric signals day and night from the time they are small juveniles to the end of their adult lives. In contrast, males of the genus *Pollimyrus*, including the two southern African species studied here, only sing courtship songs when they are ready to reproduce. This suggests that courtship songs can be at least as important as EODs for intra- and intersexual selection in this sonic mormyrid genus. We suggest species-specific courtship songs as an additional field of study providing useful information for the taxonomy, ethology, and behavioural ecology of mormyrid fish.

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