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Adaptive radiation in African weakly electric fish (Teleostei: Mormyridae: *Campylomormyrus*): a combined molecular and morphological approach

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Abstract

We combined multiple molecular markers and geometric morphometrics to revise the current taxonomy and to build a phylogenetic hypothesis for the African weakly electric fish genus *Campylomormyrus*. Genetic data (2039 bp DNA sequence of mitochondrial cytochrome *b* and nuclear S7 genes) on 106 specimens support the existence of at least six species occurring in sympatry. We were able to further confirm these species by microsatellite analysis at 16 unlinked nuclear loci and landmark-based morphometrics. We assigned them to nominal taxa by comparisons to type specimens of all *Campylomormyrus* species recognized so far. Additionally, we showed that the shape of the elongated trunk-like snout is the major source of morphological differentiation among them. This finding suggests that the radiation of this speciose genus might have been driven by adaptation to different food sources.

Introduction

Africa hosts a great number of so-called fish species flocks (i.e. speciose monophyletic groups with restricted distributions). Among these, lacustrine cichlid species flocks are probably the most renowned and well studied. However, such explosive speciation phenomena are not limited to this taxonomic group. Specifically, Sullivan *et al.* (2002, 2004) proposed weakly electric fish of the family Mormyridae as potential model organisms to study species flock evolution in rivers. Mormyrids comprise one of the most diverse clades of freshwater fish from Africa and the single largest known group of electric fish (Alves-Gomes & Hopkins, 1997). Being nocturnal, these fishes use electric cues to actively locate objects in darkness (Lissman & Machin, 1958; Bastian, 1994; von der Emde, 1999). More importantly from an evolutionary point of view, the electric organ discharge (EOD) plays a key role in pair formation, mating and social attraction (Bratton & Kramer, 1989; Crawford, 1991; Kramer & Kuhn, 1994). This is particularly evident in *Campylomormyrus*, the main object of the study presented here. This genus exhibits a wide diversity of EOD waveform types, which are almost invariably species specific. Therefore, EOD can be used as an important character to discriminate species that are otherwise cryptic (Hopkins, 1999).

By monitoring ontogenetic changes in EOD and by combining these observations with the analysis of multiple molecular markers, we recently demonstrated the existence of cryptic species hidden under the formal name *Campylomormyrus numenius* (Feulner *et al.*, 2006). Our findings suggest that *Campylomormyrus* could potentially be another compelling system to investigate speciation in the light of competing models of diversification in ecological, morphological and behavioural traits. While morphological and genetic analyses strongly support the monophyly of mormyroids as well as the sister taxa relationship between gymnarchids and mormyrids (Lauder & Liem, 1983), the existing molecular data sets

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suggest conflicting phylogenies at and near the species level. In addition, very few studies considered the genus *Campylomormyrus*, whose systematics are extremely puzzling. Based on the analysis of morphological characters, the number of described species fluctuated through the years from 16 (Taverne, 1972) to three (Roberts & Stewart, 1976) and again to 14 (Poll *et al.*, 1982) (Table 1). Most of the species considered valid nowadays are endemic to a single river system, the Congo and its tributary streams (Table 1 and Fig. 1). Some of them can be found throughout the entire Congo Basin, others are restricted to certain areas (Luapula River/Lake Moero or Kasai River). *Campylomormyrus phantasticus* is the only *Campylomormyrus* species not present in the Congo Basin, being limited to the Sanaga River (Cameroon). *Campylomormyrus tamandua* is the most widely distributed species and the only one whose range extends across different river systems, including Congo, Volta, Niger and Tchad/Shari (Gosse, 1984). So far, only two species (*C. numenius and C. tamandua*) have been included in molecular phylogenies (Sullivan *et al.*, 2000; Lavoué *et al.*, 2003).

While this genus may be a unique system to study general evolutionary phenomena of speciation and adaptation, neither a robust phylogenetic hypothesis nor a thoroughly tested taxonomic arrangement have been proposed so far. Our study aims at addressing both these essential prerequisites towards a proper understanding of the relationship between speciation and ecological/phenotypic diversification in this still enigmatic group. To this end, we rely on two lines of evidence: (i) we evaluate morphology by quantitative morphometric assessment based on 11 landmarks. We analysed type specimens representing all the 17 *Campylomormyrus* species recognized to date, deposited at the Royal Museum for Central Africa (MRAC) Tervuren (Belgium), also including three type specimens not available there

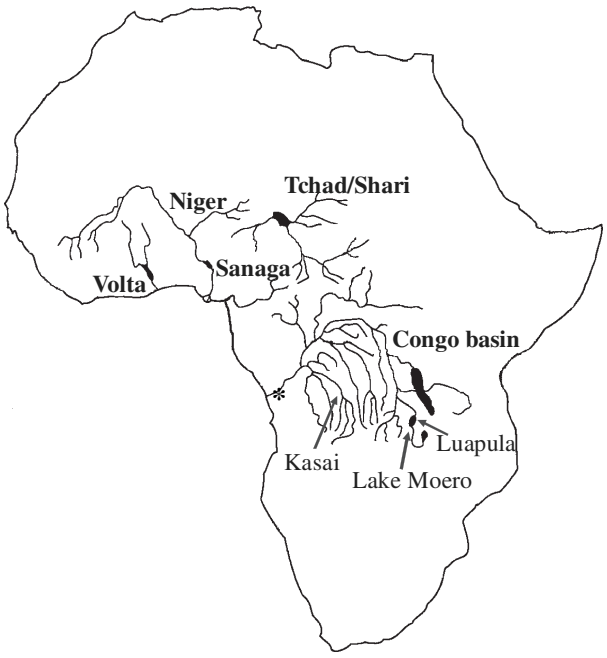

Fig. 1 Geographic location of African river systems in which *Campylomormyrus* occurs. Most species are endemic to the Congo Basin. * indicates the sampling location at Brazzaville/Kinshasa.

Table 1 Described species according to three different authors, their distribution and type museum material used for morphometric comparison.

Taverne (1972)	Roberts & Stewart (1976)	Poll *et al.* (1982)	Distribution (Gosse, 1984)	Type
C. alces	*C. mirus*	*C. alces*	Congo Basin	S
C. bredoi	*C. rhynchophorus*	*C. bredoi*	Lake Moero and Luapula River	H
C. cassaicus	*C. mirus*	*C. cassaicus*	Afflux Kasai River	P
C. christyi	*C. mirus*	*C. christyi*	Congo Basin	S
C. curvirostris	*C. rhynchophorus*	*C. curvirostris*	Congo Basin	H
C. elephas	*C. mirus*	*C. elephas*	Congo Basin	S
C. luapulaensis	*C. rhynchophorus*	*C. luapulaensis*	Upper Luapula	H
C. mirus	*C. mirus*	*C. mirus*	Congo Basin	H
C. numenius	*C. rhynchophorus*	*C. numenius*	Congo Basin	S
C. ibis	*C. rhynchophorus*	*C. numenius*	Congo Basin	S
		C. orycteropus	Lake Moero	H*
C. phantasticus	*C. rhynchophorus*	*C. phantasticus*	Sanaga River	H*
C. rhynchophorus	*C. rhynchophorus*	*C. rhynchophorus*	Congo Basin	S
C. compressirostris	*C. rhynchophorus*	*C. rhynchophorus*	Congo Basin	H
C. lualabaensis	*C. rhynchophorus*	*C. rhynchophorus*	Congo Basin	H
C. tamandua	*C. tamandua*	*C. tamandua*	Volta, Niger, Tchad/Shari and Congo Basin	H*
C. tshokwe	*C. rhynchophorus*	*C. tshokwe*	Kasai River and afflux	H

H, S, P refer to inclusion of holotypus, syntypus or paratypus in the analysis. Type museum specimens were analysed at the Royal Museum for Central Africa (MRAC), Tervuren (Belgium), except for *, the type specimen information of which was taken from Harder (2000). *C.*, *Campylomormyrus*

(Harder, 2000). We compared these museum specimens to 106 individuals caught in the wild in the course of our project, to match our samples to the typed representatives of the genus *Campylomormyrus*. Such an approach should ensure a cross-check of correct identification of the wild samples as well as reveal patterns of morphological variation among them. (ii) We produced for the first time a comprehensive molecular phylogeny for the genus *Campylomormyrus* by using sequence polymorphisms of one mitochondrial (cyt *b*: the complete cytochrome *b* gene, 1142 bp) and one nuclear gene (S7: the first and second introns as well as the second exon of the gene coding for the S7 ribosomal protein, 897 bp). These genes proved useful in studies on mormyrids at a comparable level of taxonomic separation (Sullivan *et al.*, 2002; Lavoué *et al.*, 2003; Feulner *et al.*, 2006). Milinkovitch *et al.* (2002) suggested that to genetically distinguish among different species in sympatry, it is essential to independently confirm any phylogenetic hypothesis based on molecular data (here, sequences of cyt *b* and S7 genes) by either morphological or unlinked molecular characters. Therefore, we also screened all individuals included in the study for length polymorphisms at 16 microsatellite loci. We previously demonstrated for *C. numenius* that combining such different and unlinked molecular markers is a powerful method to correctly identify distinct evolutionary lineages and cryptic species (Feulner *et al.*, 2006). Ultimately, by relating genetic distinctness to morphological divergence, we aim at identifying those morphological traits which might relate to adaptation in the radiation of the genus *Campylomormyrus*.

Materials and methods

Field sampling

We sampled 66 *Campylomormyrus* specimens during an expedition to Brazzaville (Republic of the Congo) in August 2004 (Fig. 1). All but seven specimens were sampled at the same location (rapids south of Brazzaville). Four specimens (K01, K66, K67 and K68) were sampled a few kilometres southwards at the inflow of the Foulakari River, while three specimens (K69, K70 and K71) are from Kintele, just north of Brazzaville. Fin clips of the right pectoral fin were taken and stored in 1 ml of tissue buffer (Seutin *et al.*, 1991).To expand the sample size, we included the data on 40 *Campylomormyrus* specimens from Brazzaville/Kinshasa from our previous study (Feulner *et al.*, 2006) in the statistical analyses. As outgroup species, we used one *Hippopotamyrus wilverthi* and one *Marcusenius* sp. specimen.

Phylogenetic analysis

DNA extraction, polymerase chain reaction (PCR) amplifications and sequencing was performed as described in Feulner *et al.* (2006). All obtained sequences for cyt *b* and S7 were submitted to GenBank (accession no. DQ630551–DQ630652). We aligned sequences using BIOEDIT version 7.0.0 (Hall, 1999) and reconstructed a phylogeny for the two genes (cyt *b* and S7) separately, for the combined data set and for the third codon positions of cyt *b* alone. We used PAUP 4.0B10 (Sinauer, Inc., Sunderland, MA, USA) to calculate variability estimates, the number of transitions (Ti) and transversions (Tv) and to perform chi-square test for homogeneity of base frequencies. Saturation of sequences was investigated by plotting the absolute number of Ti and Tv against the percentage of sequence divergence. This was done for the cyt *b* gene at third codon position only. Aligned sequences were analysed by neighbour-joining (NJ) (Saitou & Nei, 1987) and Bayesian methods (Rannala & Yang, 1996; Mau & Newton, 1997; Larget & Simon, 1999; Mau *et al.*, 1999; Huelsenbeck *et al.*, 2000). We used MODELTEST version 3.06 (Posada & Crandall, 1998) to identify the best model of sequence evolution for each data set. These models were then applied to calculate genetic distances and to construct trees via NJ in PAUP version 4.0B10. To gain statistical support, we performed 1000 bootstrap replicates. Complex models of nucleotide substitution for estimating evolutionary distances and the application of NJ methods are recommended for large data sets, like in the case of our combined data set (Tamura *et al.*, 2004). For the Bayesian approach, we employed the same models of sequence evolution used in the NJ analyses, allowing for site-specific rate variation partitioned by gene and for cyt *b* by codon position. We ran one cold and three heated Markov chains for 2 million generations using MRBAYES version 3.0B4 (Ronquist & Huelsenbeck, 2003). We saved trees every 100 generations for a total sample size of 20 000. We discharged the first 2000 sampled trees as burn-in and used the remaining to calculate a 50% majority rule consensus tree. Phylogenetic tree topologies generated with different phylogenetic methods and competing phylogenetic hypotheses were statistically evaluated with the approximately unbiased tree selection test (AU; Shimodaira, 2002), as implemented in the software package CONSEL (Shimodaira & Hasegawa, 2001). For comparison, we also performed the more conservative Shimodaira and Hasegawa test (SH; Shimodaira & Hasegawa, 1999) as implemented in PAUP version 4.0B10 with the resampling estimate log-likelihood (RELL) technique. We always compared tree topologies simultaneously (Shimodaira & Hasegawa, 1999).

Microsatellite analysis

For genotyping we used microsatellites specifically developed for *Campylomormyrus* (Feulner *et al.*, 2005). Specifically, we used the same 16 microsatellite loci and the same experimental conditions as in Feulner *et al.* (2006). We calculated observed and expected heterozyg-

osity using ARLEQUIN version 2.000 (Schneider *et al*., 2000) and tested for linkage disequilibrium and deviation from Hardy–Weinberg equilibrium using GENEPOP on the web (Raymond & Rousset, 1995). Significance was tested after correction for multiple comparisons via sequential Bonferroni correction at an experiment-wise error rate of $\alpha = 0.05$. In addition, we used microsatellites to assess the accuracy of classifications based on morphology and sequence data. To summarize the degree of genetic differentiation, we calculated pairwise F_{ST}-values using *F*-statistics (Weir & Cockerham, 1984). The significance of F_{ST} was tested by permutation analyses and an analysis of molecular variance (AMOVA; Excoffier *et al*., 1992) was conducted as implemented in ARLEQUIN version 2.000 (Schneider *et al*., 2000). By means of pairwise *t*-tests, we compared mean pairwise differences between expected heterozygosities to contrast genetic variability within groups. Assignment tests were performed with GENECLASS version 2.0 (Piry *et al*., 2004). Two Bayesian-based tests (Rannala & Mountain, 1997; Baudouin & Lebrun, 2001) and one frequency-based test (Paetkau *et al*., 1995) were used to calculate the probability of each individual's assignment to a particular clade. To evaluate genetic subdivision independently from classifications based on morphology and/or sequence data, we used the STRUCTURE software, estimating the likelihood and sample composition of different numbers of subgroups ($k = 4$,5 and 6; Pritchard *et al*., 2000).

Morphometric analysis

In addition to the specimens we sampled in the field, we performed morphometric analysis on the type material of all the 17 recognized taxa in *Campylomormyrus* (Table 1). Digital images of the types were taken at MRAC Tervuren (Belgium). Images of the three types not available at MRAC were taken from Harder (2000). Digital photographs were taken with a scale bare forthright beside the animal. Landmark-based geometric morphometric methods were used to record *x*, *y* coordinates of 11 homologous landmarks and capture information of body shape using TpsDig (Rohlf, 2003). Landmark configuration is shown in Fig. 4a. All the following morphometric analyses were conducted using the IMP package (Sheets, 2002). First, differences due to size, orientation and position were removed by generalized procrustes analysis (Rohlf, 1999; Slice, 2001). After superimposition, the data were converted into principal warps using the thin-plate spline model (Bookstein, 1989). These variables can then be used in conventional multivariate analyses because they posses the same number of variables as degrees of freedom (Zeldith *et al*., 2004). We used canonical variates analysis (CVA) of morphological variables to demonstrate the discrimination among groups identified by the screening for genetic polymorphisms. In addition, we also performed a principal component analysis for subsets of samples whose placement was not resolved in the phylogenetic tree. Shape-based assignments were performed with CVAgen6N (part of IMP) following the method outlined by Nolte & Sheets (2005) which includes a jackknifing procedure as a test of performance of the assignment. In 500 replicates, 10% of the data were left out and assigned to groups in the remaining data set. In this way, we were able to test the distinctiveness of the different groups. We used the same approach to assign the samples screened to the type specimens for genetic variation. To this purpose, we calculated mean Mahalanobis distances between each type specimen and each clade recovered in the phylogenetic analyses. The rationale behind this analysis was to assess whether our genetically and morphologically identified groups matched the already described species.

Results

Phylogenetic analysis of sequence data

We sequenced a total of 2039 bp for each individual included in the study. The alignment of the S7 gene included 10 indels, while no indels were found in the alignment of cytochrome *b* (cyt *b*) gene. As typical for mitochondrial genomes, there was a low frequency of *G*'s in cyt *b*, especially in third codon position ($G = 0.033$). Most of the variation in the combined data set is in the third codon positions of the cyt *b* gene. Inspection of the saturation plots (not shown) revealed that saturation is not apparent in our data set. The chi-square test for base homogeneity indicates that base frequency distribution is always homogenous among taxa, both when genes are analysed separately (tests were also performed on each codon position on cyt *b*) and when genes are combined in a single data set. Figure 2 shows the Bayesian tree obtained on the complete data set using the unequal-frequency Kimura 3-parameter plus Gamma (K81uf + I + *G*) model of evolution (Rodriguez *et al*., 1990; model chosen with MODELTEST) and summarizes the results of the NJ analysis. Trees obtained with different methods (Bayesian or NJ) and based on different data sets (cyt *b*, S7 or both combined) were statistically indistinguishable with the AU and the SH tests ($0.137 \leq P \leq 0.991$). We consistently recovered five strongly supported monophyletic clades (A, B, D, E and F). Clade F is clearly separated from the rest, while clade D forms a monophylum with A, B and C. The node grouping A, B, C vs. D is strongly supported. Within C there are two weakly supported clades (C_a and C_b) plus three specimens (K13, K16 and Cn29) whose placement could not be resolved by the data. In the case of Cn34, the two homologous alleles we detected at the S7 gene (Cn34a and Cn34b; differing by a single indel) do not cluster together. Rather, Cn34a is placed within the C_a clade while Cn34b is embedded within the C_b clade. To further evaluate resolution among clades A, B and C as well as within clade C, we used the AU and SH test to

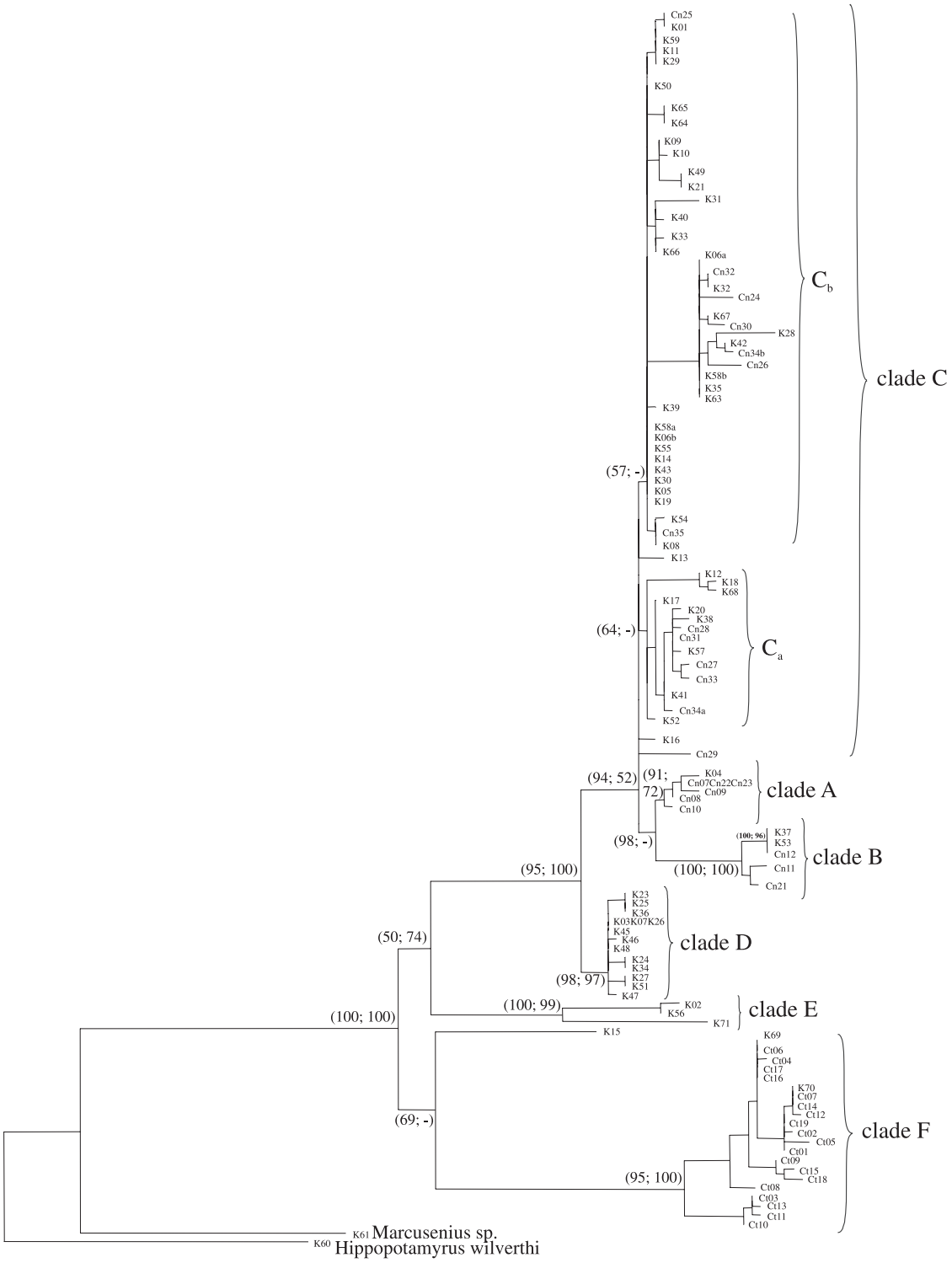

Fig. 2 Bayesian phylogeny based on the combined data set of mitochondrial cytochrome *b* and nuclear S7 gene (2039 bp). Branch length is proportional to the amount of character changes. Numbers in brackets give statistical support (Bayesian and neighbour-joining analysis) for the respective clade.

evaluate two alternative hypotheses. First, we forced clade C to be monophyletic with A and B basal to it. Second, we took advantage of the morphometric results to constrain four individuals (K13, K14, K16 and K52, see below) to form a monophyletic clade within C. Both the AU and SH tests rejected these two alternative hypotheses ($0.003 \leq P \leq 0.027$).

Microsatellite analysis

Summary statistics for microsatellite variation are shown in Table 2. We found between seven and 33 alleles per locus. The loci are inherited independently, as just one significant linkage was detected between locus GAII26 and locus GAII42 in a single clade (clade C). The mean observed heterozygosity within morphs ranged from 0.51 to 0.70, the mean expected heterozyosity ranged from 0.61 to 0.78. Only five significant deviations from Hardy–Weinberg expectations were detected, but they are scattered among loci and populations. We performed microsatellite analysis as an independent line of molecular evidence to further evaluate the degree of genetic divergence among clades identified by analyses of sequence and morphometric data. Indeed, microsatellite analysis further corroborates our phylogenetic analysis: all the five identified clades (A, B, C, D and F) were also significantly differentiated from one another at microsatellite loci, as pairwise F_{ST} values were between 0.08 and 0.33 and the overall fixation index was 0.19, all values being highly significant (Table 3). We did not consider clade E in the microsatellite analysis due to its small sample size. The F_{ST} value comparing the two poorly supported subclades of C (C_a and C_b) was lower but still significant ($F_{ST} = 0.01$, $P = 0.007$). However, within C the four samples identified morphologically (K13, K14, K16 and K52) were clearly differentiated

Table 2 Genetic diversity at 16 microsatellite loci in clades of *Campylomormyrus*.

Locus	Number of alleles	Range of allele size	Test	Clade A	Clade B	Clade C	Clade D	Clade F	Mean
CampGAI14	23	189–243	H_O	1.00	0.60	0.78	0.71	0.43	0.71
			H_E	0.97	0.67	0.82	0.92	0.37	0.90
CampGAI28	22	165–233	H_O	0.57	0.60	0.69	0.36	0.62	0.62
			H_E	0.57	0.73	0.86	0.71	0.88[a]	0.84
CampGAI8	19	173–215	H_O	1.00	0.80	0.85	0.86	0.71	0.83
			H_E	0.90	0.64	0.92	0.84	0.66	0.92
CampGAII17	15	216–246	H_O	0.71	0.40	0.89	0.21	0.57	0.70
			H_E	0.76	0.87	0.89	0.27	0.68	0.88
CampGAII26	24	204–284	H_O	0.43	0.60	0.78	0.71	0.86	0.75
			H_E	0.60	0.87	0.80	0.71	0.84	0.88
CampGAII42	13	346–376	H_O	1.00	0.60	0.89	0.42	0.05	0.65
			H_E	0.86	0.80	0.91	0.61	0.09	0.88
CampGAIII8	27	377–451	H_O	0.86	0.40	0.33	0.29	0.95	0.48
			H_E	0.88	0.67	0.93*	0.74*	0.92	0.94
CampGTI18a	18	136–192	H_O	0.71	0.00	0.43	0.64	0.45	0.47
			H_E	0.66	0.00	0.52	0.69	0.91*	0.69
CampGTI19	8	188–204	H_O	0.29	0.60	0.53	0.79	0.62	0.57
			H_E	0.40	0.67	0.50	0.69	0.64	0.69
CampGTI39	31	159–227	H_O	0.71	0.80	0.93	0.93	1.00	0.92
			H_E	0.74	0.80	0.91	0.80	0.96	0.94
CampGTII27	7	257–281	H_O	0.00	0.00	0.16	0.07	0.33	0.17
			H_E	0.00	0.00	0.20	0.37	0.48	0.47
CampGTII2a	28	193–273	H_O	0.57	0.60	0.82	0.71	0.95	0.80
			H_E	0.69	0.53	0.93	0.80	0.90	0.93
CampGTII6a	18	184–228	H_O	0.86	0.60	0.84	0.64	0.05	0.65
			H_E	0.75	0.53	0.92	0.69	0.15	0.89
CampGTII6b	33	136–204	H_O	0.86	0.60	0.87	0.86	0.90	0.86
			H_E	0.90	0.98	0.94*	0.90	0.95	0.96
CampGTIII41	12	177–217	H_O	0.00	0.00	0.40	0.00	0.62	0.34
			H_E	0.00	0.00	0.44	0.00	0.87	0.58
CampGTIII4b	30	188–248	H_O	1.00	1.00	0.93	1.00	0.76	0.91
			H_E	0.95	0.93	0.95	0.94	0.88	0.96
All loci			H_O	0.66	0.51	0.70	0.58	0.62	0.65
			H_E	0.66	0.61	0.78	0.68	0.70	0.83

For each clade and each microsatellite locus, expected (H_E) and observed (H_O) heterozygosity is given. The range of allele sizes is given for the entire data set. *Significant heterozygote deficiency after Bonferroni correction at an experiment-wise error rate of $\alpha = 0.05$.

Table 3 Genetic variation and pairwise divergence at 16 microsatellite loci in clades of *Campylomormyrus*.

Clade	A	B	C	D	F
A	0.66	0.06 (0.345)	0.11 (0.008)	0.00 (0.924)	0.04 (0.741)
B	0.20 (<0.001)	0.61	0.17 (0.003)	0.06 (0.385)	0.09 (0.391)
C	0.08 (<0.001)	0.13 (<0.001)	0.78	0.11 (0.070)	0.08 (0.391)
D	0.17 (<0.001)	0.23 (<0.001)	0.12 (<0.001)	0.68	0.03 (0.739)
F	0.29 (<0.001)	0.33 (<0.001)	0.23 (<0.001)	0.29 (<0.001)	0.70

Diagonal, mean expected heterozygosity (H_E); above, pairwise difference in H_E; below, pairwise F_{ST}. *P*-values are given in parentheses.

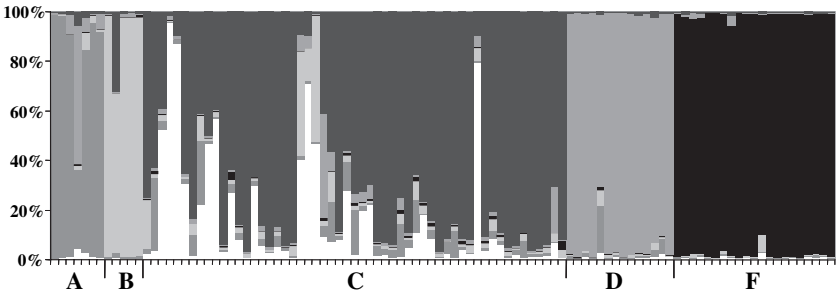

Fig. 3 Sample composition (clades A, B, C, D and F) by the STRUCTURE analysis yielding the highest likelihood ($k = 6$). Clear structuring into five clades is visible as well as heterogeneity within clade C.

from the remaining individuals ($F_{ST} = 0.08$, $P < 0.001$). The assignment test based on three different methods assigned all individuals correctly to the five phylogenetic clades, with very few exceptions: all methods were unable to assign Cn10 to clade A, and one method failed in assigning K16 and K17 to clade C. The sample composition by the STRUCTURE analysis yielding the highest likelihood ($k = 6$) showed similar results (Fig. 3). All five clades were clearly distinct. Again, Cn10 was either placed in A or D. Clade C was separated from the other clades and a large amount of genetic heterogeneity was evident within this clade.

Morphometric analysis

All the six distinct clades (A, B, C, D, E and F) identified by our phylogenetic analysis are clearly differentiated also on morphometric grounds. All of them formed discrete clusters in a CVA along the first two axes, which displayed the greatest separation between groups relative to within-group variance (Fig. 5a). The differentiation in shape as captured by CV axes could be visualized as displacement vectors for each landmark on a deformation grid relative to a reference (Fig. 4). The first axis (Lambda = 0.0007; $\chi^2 = 619.1097$; d.f. = 108; $P < 0.001$) described variation in trunk length, whereas the second axis (Lambda = 0.0076; $\chi^2 = 417.0081$; d.f. = 85; $P < 0.001$) was mainly related to body height. There were four more significant but less differentiating CV axes (Lambda > 0.04). By means of a distance-based assignment test, all the individuals could be assigned correctly to their source cluster. This proved the utility of the derived axes to discriminate among groups and to determine a given specimen's group affinity. The robustness of the CV axes and the assignment test were evaluated by a jackknife test. In this way, 89.8% of the specimens were assigned significantly and correctly into their source groups. PCA analysis identified four specimens within C (K13, K14, K16 and K52), which differed clearly from the other individuals in the clade. These individuals formed a distinct cluster in CVA with one significant CV axis (Lambda = 0.1068; $\chi^2 = 87.2181$; d.f. = 18; $P < 0.001$) and could be assigned to an additional morphological group (Fig. 5b). Jackknifing-based tests (500 replicates, 10% unknowns) resulted in 90.6% of correct and significant assignments. Table 4 reports the shortest Mahalonobis distances between each cluster of the morphometric analyses (clades recovered by phylogenetic analyses of sequence data) and the assigned type specimen. Clade A was assigned to *Campylomormyrus rhynchophorus*, clade B to *C. numenius*, clade C-I to *Campylomormyrus compressirostris*, clade C-II to *Campylomormyrus curvirostris*, clade D to *Campylomormyrus tshokwe*, clade E to *Campylomormyrus elephas* and clade F to *C. tamandua*. Specimen K15 achieved the shortest distance to *Campylomormyrus bredoi*.

Discussion

Phylogeny and taxonomy within the genus *Campylomormyrus*

To our knowledge, we were the first to prove that there are genetically distinct clades within *Campylomormyrus* which correspond to characteristic waveform types of the EOD (Feulner *et al.*, 2006). In the study presented here, we were able to substantially expand our sample set to 106 *Campylomormyrus* specimens. In addition, we associ-

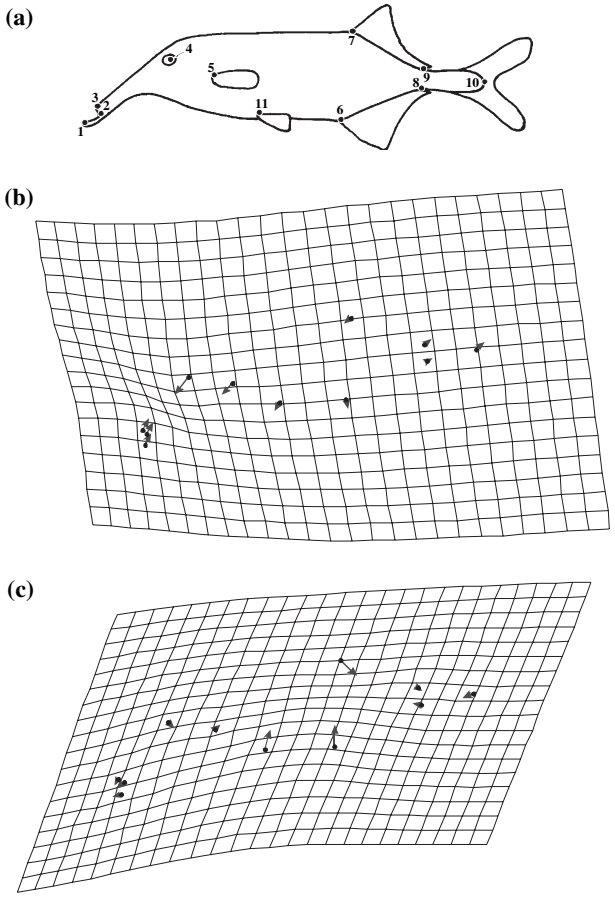

Fig. 4 Landmark configuration and displacement vectors that distinguish clades of *Campylomormyrus*. (a) The 11 landmarks chosen to analyse variability in *Campylomormyrus* body shape. (b, c) Deformation grid with relative displacement vectors visualizing the shape changes for each landmark captured by CVA axes, due to which different groups can be discriminated. The first two of the six significant axes are shown. The two shown axes possess by far the smallest Lambda values (Wilks' Lambda 0.0007 and 0.0076 respectively), indicating the greatest differentiation between the groups along these axes.

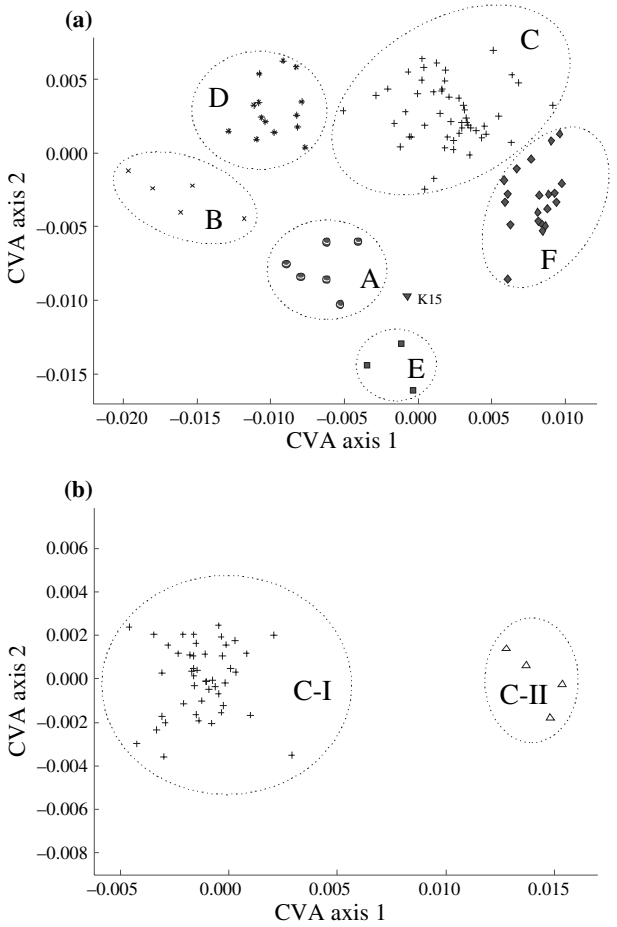

Fig. 5 (a) Results of the canonical variates analysis (CVA) conducted on morphological variables for all *Campylomormyrus* samples analysed genetically. Letters (A–F) match coding of clades in the tree of Fig. 2. The main six different clades identified on genetic grounds are clearly separated due to the first two CVA axes. (b) CVA restricted to individuals of clade C in the tree of Fig. 2. Four individuals (K13–14–16–52) are clearly differentiated.

ated phylogenetic clades with taxonomic units by morphometric means. Morphometric analysis has already proven able to resolve complex taxonomic problems at the species level (Fink & Zelditch, 1997; Fadda & Corti, 2001; Dobigny *et al*., 2002; Baylac *et al*., 2003). Here we used a distance-based method already successfully used in teleosts for identifying hybrids and assigning species into their source population (Nolte & Sheets, 2005). By this approach, distinct phylogenetic clades could be associated with type specimens to obtain a classification. This classification is generally in line with the currently accepted taxonomy proposed by Poll *et al.* (1982), with a few exceptions. The genetically supported clade C-I was assigned to *C. compressirostris*. *Campylomormyrus compressirostris* was first described by Pellegrin (1928) and later also denominated in the classification of Taverne (1972). Conversely, it was considered synonymous to *C. rhynchophorus* by Poll *et al.* (1982). Both genetically and morphologically, our analysis significantly supported the distinct clades A and C-I, assigned to *C. rhynchophorus* and *C. compressirostris* types respectively. Genetically, this distinctiveness is not only proven by our phylogenetic results based on two unlinked genes, but further independently confirmed by our microsatellite data. Hence, we consider *C. compressirostris* a valid species.

Generally, the resolution within clade C was complicated by its high variation, both in morphology and genetics. The genetic heterogeneity is particularly well reflected in the results of our microsatellite analysis: clade C had the highest mean expected heterozygosity

Table 4 Assignment of type specimens to phylogenetic clades based on morphometric analysis of 11 landmarks.

		Least distance	
	n	Species	Value
Clade A	6	*Campylomormyrus rhynchophorus*	4.672
Clade B	5	*Campylomormyrus numenius*	5.122
Clade C-I	46	*Campylomormyrus compressirostris**	4.789
Clade C-II	4	*Campylomormyrus curvirostris*	7.272
Clade D	14	*Campylomormyrus tshokwe*	6.080
Clade E	3	*Campylomormyrus elephas*	5.396
Specimen K15	1	*Campylomormyrus bredoi*	6.984
Clade F	20	*Campylomormyrus tamandua*	3.663

Clade C-II consists of K13, K14, K16 and K52; clade C-I of the remaining specimens of clade C. Types with the nearest Mahalanobis distances were assigned to each clade. *Clade C-I yielded a lower distance (3.595) to *C. tamandua* in this analysis. However, based on a unique coloration pattern not captured in the landmarks, *C. tamandua* can unambiguously be assigned to clade F.

(Table 3) and STRUCTURE revealed specimens with diverse genotype composition (Fig. 3). By means of our morphometric analysis we could identify four specimens within C (C-II) which were differentiated from the rest of C (C-I) (see Fig. 5b) and could be assigned to *C. curvirostris*. While this separation was not visible in the phylogenetic tree, it was supported by the multilocus microsatellite data, which yield a highly significant pairwise F_{ST}. Our molecular data obviously reject the assumption that *Campylomormyrus* includes only three species, as suggested by Roberts & Stewart (1976). Nevertheless, all taxa called 'species' by these authors form well-supported clades in our molecular phylogeny, although some of them consist of several valid species (Fig. 2, Table 4): *C. tamandua*, considered a separate species by all authors (Table 1), is confirmed as such (clade F; Fig. 2). *Campylomormyrus mirus* (sensu Roberts & Stewart, 1976) is reflected by clade E which we assigned to *C. elephas*, a taxon incorporated in the '*C. mirus*' summation (Table 1). In fact, the number of species within clade E could not be resolved here, because of small sample size and the large differentiation among these specimens within this robust clade. At least, the three specimens analysed here show slight but pronounced morphological differences. Finally, *C. rhynchophorus* (sensu Roberts & Stewart, 1976) formed a large and strongly supported monophyletic group in our phylogenetic analyses, consisting of clades A–D (Fig. 2). By combining genetics and morphology, we could, however, unambiguously demonstrate that these clades (A, B, C-I, C-II and D) represent five sympatrically occurring reproductively isolated groups (i.e. biological species) hidden under the name '*rhynchophorus*'.

Basal in the phylogenetic tree we found a single specimen (K15) not significantly associated with any other clade. In our morphological assignment to type material, K15 appeared most similar to *C. bredoi* (Table 4). We are, however, in doubt about this assignment, for two reasons: (i) K15 showed special morphological features, e.g. a relatively narrow elongated caudal peduncle, which were not shared by the holotype of *C. bredoi*. (ii) *C. bredoi* is so far only known from the headwater region of Congo and has never been detected in our sampling region, the Lower Congo. Unfortunately, genetic comparison to the type material is precluded by the fact that all type specimens have been stored in concentrated formalin for prolonged periods of time. Based on its distinct position in our phylogenetic tree, the peculiar morphology and the lack of a morphologically similar specimen among the *Campylomormyrus* types, it appears possible that K15 might constitute a so far undescribed new species. This has to be verified by further extensive examination of this specimen, which was beyond the scope of the study presented here.

Adaptive radiation within the genus *Campylomormyrus*

Beside the taxonomic clarification, the major aim of this study was to better understand the adaptive radiation of this group of weakly electric fish. According to Schluter (2000), an adaptive radiation is the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage. Adaptive radiations can be identified by certain criteria, i.e. common ancestry, rapid speciation, phenotype/environment correlation and trait utility: common ancestry has been proven multiple times for *Campylomormyrus*, a single genus clearly characterized by its morphology. *Campylomormyrus* formed a well-supported monophyletic taxon in our phylogeny with two different outgroup genera, as well as in various other phylogenies which included a higher number of mormyrid genera (Alves-Gomes & Hopkins, 1997; Lavoué *et al.*, 2000, 2003; Sullivan *et al.*, 2000). Rapid speciation is apparent both generally in the entire group of mormyrids and specifically in the genus *Campylomormyrus*. This can be shown by a comparison of numbers of species among contemporary clades: while the mormyrids comprise almost 200 described species, their sister taxon, the gymnarchids, are monotypic (Nelson, 1994). Moreover, the species-rich mormyrids belong to the Osteoglossomorpha, an otherwise species-poor group (Lavoué & Sullivan, 2004). In our study on *Campylomormyrus*, particularly rapid speciation has apparently occurred in the monophyletic group consisting of clades A–D (see Fig. 2; '*C. rhynchophorus*' sensu Roberts & Stewart, 1976), as we could detect five species, some of which genetically similar, yet significantly different. This is in particular true for the clades C-I and C-II, which were significantly separated in the morphological and multilocus microsatellite analysis, but had not acquired reciprocal monophyly at the cytochrome *b* and S7 loci.

Finally, our morphometric analysis allows us to discuss this speciation in the light of phenotype–environment

correlation and trait utility. We were able to show that our distinct phylogenetic clades are associated with significant morphological differences (Fig. 5a); therefore, the variation at neutral genetic markers is consistent with phenotypic traits. Visualization of the morphological changes captured by the CV axis reveals that shape changes are mainly caused by differences in the trunk-like snout (Fig. 4b). *Campylomormyrus* feed on insect larvae that burrow into, or hide within, interstitial spaces and holes in clay sediment or river channels (Marrero & Winemiller, 1993). It is therefore reasonable to hypothesize that different trunk shapes are associated with different diets, as the accessibility of certain food items might depend on the morphology of the trophic apparatus. At present, we cannot verify this hypothesis by direct evidence, as neither feeding behaviour nor stomach content have been analysed so far in these nocturnal tropical fishes. Nevertheless, all species identified on genetic grounds significantly differed in an important morphological trait, i.e. their trophic apparatus. We therefore consider this a first indication for a correlation between the shape of the snout and the substrate structure (criterion of 'phenotype–environment correlation'); similarly, the variation of this important morphological trait presumably impacts the accessibility of specific food resources (criterion of 'trait utility').

We previously showed that reproductive isolation in *Campylomormyrus* is strongly correlated with divergence in waveform types of the EOD (Feulner *et al*., 2006). Here we could demonstrate that these reproductively isolated groups, i.e. biological species, have significantly diverged in their feeding apparatus. Therefore, we are arguing for a diversification of *Campylomormyrus* caused by an adaptation to different food sources and triggered by EOD differences as prezygotic isolation mechanism. Proving sympatric speciation in a huge open habitat like the Congo Basin is virtually impossible. Nevertheless, our findings of clear-cut differences in both an ecological trait (feeding apparatus) and a prezygotic isolation mechanism (EOD) strongly raise the possibility that the vast genus' diversification we demonstrated might have occurred in sympatry. A few other studies have also postulated sympatric speciation due to disruptive natural selection as adaptation to different food sources and sexual selection via assortative mating as the isolation mechanism (Schliewen *et al*., 2001; Salzburger *et al*., 2005; Barluenga *et al*., 2006). While theoretical models show that speciation by sexual selection alone is unlikely, because of the lack of ecological differentiation to stabilize coexistence of incipient species (Arnegard & Kondrashov, 2004; van Doorn *et al*., 2004; Kirkpatrick & Nuismer, 2004), sexual selection can promote speciation during an adaptive radiation. While our findings on *Campylomormyrus* are fully consistent with such a hypothesis of sexual selection as a trigger for speciation, we have so far no observational data confirming mate choice based on EOD. We nevertheless argue that, if the species-specific EODs are subject to sexual selection, they should be particularly diverse in adult males. This is indeed confirmed by experimental data, as morphologically indistinguishable juveniles with a common EOD develop into morphologically (slightly) distinct adults with very diverse male EOD (Feulner *et al*., 2006). In summary, we conclude that *Campylomormyrus* has undergone a rapid, possibly sympatric speciation with disruptive selection for diverse feeding apparatus and promoted by sexual selection based on strikingly different adult male electric signals (EODs), serving as an effective prezygotic isolation mechanism.

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