Shape variation in the vertebrae of Anguilliform Clariidae (Ostariophysi: Siluriformes): a useful tool for taxonomy?

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ABSTRACT

De Schepper, N., Adriaens, D., Teugels, G.G., Devaere, S. & Verraes, W. 2007. Shape variation in the vertebrae of Anguilliform Clariidae (Ostariophysi: Siluriformes): a useful tool for taxonomy? *J. Afrotrop. Zool.* Special issue: 57-71.

This study focuses on the postcranial skeleton of *Channallabes apus*. This species is representative of a specialized family of African catfish, the Clariidae. This family is of special interest because of a trend towards an increasing anguilliformity. But owing to the lack of a reliable understanding of generic and specific characteristics, it is difficult to discern between the different eel-like species based on morphological characteristics, biometric data and vertebral counts. Vertebral shape has not been studied before and may provide a solution.

This study focused on the informative nature of vertebral shape variation for taxonomy and phylogeny, using anguilliform clariids as a case study. Shape variation was quantified and qualified within one species. This has been done for specimens from three different populations of *C. apus*, collected in northern, southern and eastern Gabon. Geometric morphometrics based on thin plate spline (TPS) were used to study the morphological variation of the vertebrae. Two vertebrae were isolated (the fifth precaudal and the fortieth caudal vertebrae) to compare aspects of shape in the vertebral column of all specimens. The results of the morphometric analysis reveal some geographic vertebral variation between the different populations of *C. apus*. Two hypotheses may explain the presence of two groups within one species: (1) interspecific variation and (2) intraspecific variation. It can be concluded that vertebral shape variation is a useful tool in systematics and phylogenetics. It is, however, still not clear in the case of *C. apus*, whose taxonomic-level (species or subspecies) vertebral shape variation is explicatory.

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INTRODUCTION

The Clariidae (Teleostei: Ostariophysi: Siluriformes), distributed throughout northern and central Africa and extending to western Asia, is a specialized family of African catfishes, comprising 12 African genera with 74 species and two endemic Asian genera (Teugels 1996). This family is of special interest because of a trend towards an increasing anguilliformity, as reported by Boulenger (1908) and Pellegrin (1927). Although this tendency is present in other families of teleosts, amphibians, reptiles and some mammals, the distinct transformation from fusiformity to complete anguilliformity has never been as extensive as in Clariidae (Lande 1978). The most notable feature of this transformation is the elongation of the postcranial skeleton. A whole set of morphological transformations have, however, been observed: disappearance of the adipose fin; continuous ventral, dorsal and caudal fins; reduction of the pectoral and pelvic fins (limblessness); reduction of the skull bones; reduction of the eyes; and hypertrophy of the adductor-mandibulae complex (Pellegrin 1927; Poll 1977; Cabuy *et al.* 1999; Devaere *et al.* 2001). The species *Channallabes apus* Günther, 1873 shows all of these traits. Currently, the taxonomic relationships of some species within the Clariidae, especially the anguilliform ones (as, for example, *C. apus*), are ambiguous (Teugels & Adriaens 2003). A reliable understanding of generic and specific characteristics, which is presently lacking, is consequently to study the functional implications of these adaptations. Based on the current determination key for the Clariidae (Poll 1977), it is difficult to discern between the different eel-like species because the range of variation for different features has not been adequately considered, largely owing to the limited numbers of specimens used to describe the species.

This paper focuses on the shape variation in vertebral elements to evaluate whether this proves to be useful as an additional set of characters for taxonomy. The vertebrae of most of the less-primitive teleosts are

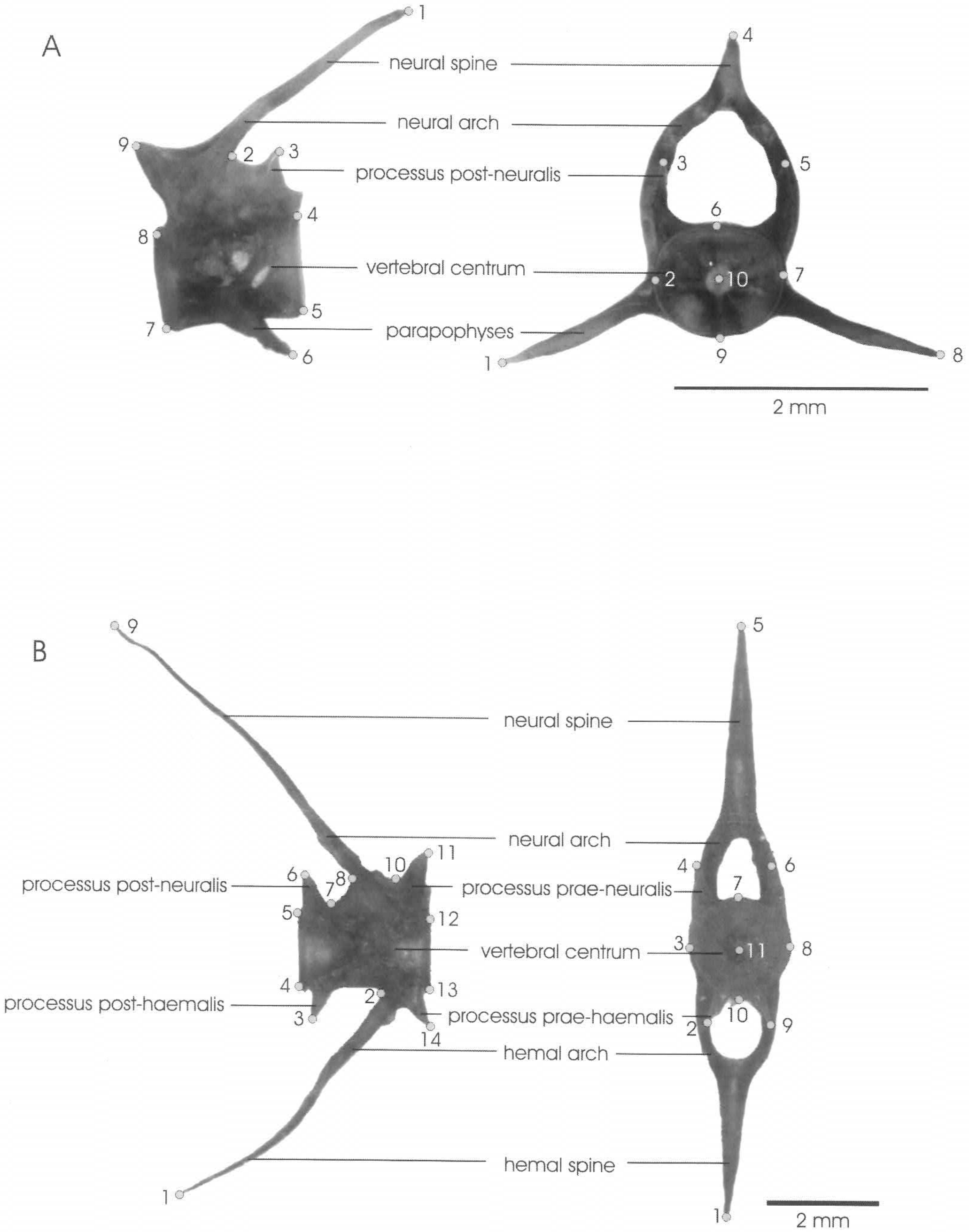

Fig. 1: Vertebral morphology and position of landmarks in *Channallabes apus*: A: 5th precaudal vertebra; B: 40th caudal vertebra (left is lateral view, right is caudal view).

composed of one centrum (monospondylous). In general, caudal vertebrae of teleosts are characterized by the presence of neural arches and spines and haemal arches and spines, while precaudal vertebrae have neural arches and spines and parapophyses, fused to their centra (Grassé 1958; Jarvik 1980; Arratia 2003). Vertical rotational movements between adjacent vertebrae are limited by the presence of bony extensions (zygapophyses) on each neural and haemal arch (Gosline 1971; Hildebrand 1995). These extensions are referred to as dorsal and ventral pre- and postzygapophyses by many authors, but these terms are used to describe the articulation processes of vertebral arches in Tetrapodes (Hildebrand 1995), while the extensions present in fish are considered non-homologous with those of Tetrapodes (Grassé 1958; Gosline 1971; Hildebrand 1995). Consequently, to avoid misinterpretations, the following terms are used in this paper: processus prae- and posthaemalis and processus prae- and postneuralis (Fig. 1).

The vertebral column can be divided into two zones: the precaudal and the caudal zones (Rockwell *et al.* 1938; Ramzu & Meunier 1999). The precaudal zone consists of vertebrae characterized by the presence of parapophyses, and sometimes supporting ribs, and the absence of haemal spines (Lakshmi & Srinivasa 1989). The vertebrae of the caudal zone have haemal arches, which enclose the large ventral blood vessels and are elongated by a haemal spine (Rockwell 1938; Ramzu & Meunier 1999). Both zones have neural arches and spines (Rockwell 1938).

At present the systematics and phylogeny of the Clariidae are still complex owing to the lack of unambiguous, diagnostic traits to distinguish the different anguilliform clariid species. The study of the postcranial skeleton may provide an solution to this problem. It is important to adequately quantify and qualify this variation in order to examine the informative nature of vertebral shape variation for taxonomy and phylogenetic research. To study the shape variation of the vertebrae, geometric morphometrics based on thin plate spline is used, as it completely uncouples variation in shape from variation in size (Rohlf 1990). Apparently, no geometric morphometric analyses based on landmark data have been used until now to describe intraspecific shape variation in the postcranial skeleton of teleosts.

MATERIAL AND METHODS

The sample of *C. apus* comprised 30 specimens, of which 14 were collected in northern Gabon, nine in eastern Gabon and 7 in southern Gabon by D. Adriaens, S. Devaere & A. Herrel in 1999 and 2000 (Fig. 2).

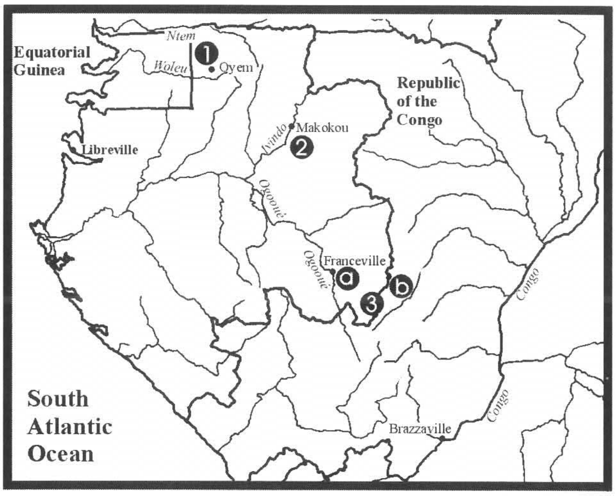

Fig. 2: Geographic location of Gabon showing sampling locations 1: northern population (Oyem-region), 2: eastern population (Makokou-region), 3: southern population 3a: Franceville region, 3b: Congo basin.

The specimens collected in northern Gabon, in Oyem (1), were found in the Woleu River, which is part of the Woleu-Ntem-system. The eastern population, collected in Makokou (2), occurred in the Liboumba River, lying within the Ivindo basin. The specimens collected in southern Gabon (3) were obtained from two different river systems. Two specimens were found in the Kahjaka Kanjaka River (Franceville 3a), which is part of the Ogooué basin, while the other specimens were collected in the Djou River (3b), part of the Congo basin. Institutional abbreviations are as listed in Levinton *et al.* (1985).

Two vertebrae were isolated: the fifth precaudal and the fortieth caudal vertebrae to compare aspects of shape in the vertebral column of all specimens. The counting of the vertebrae begins with the first vertebra not included in the weberian apparatus, which thus corresponds to the sixth vertebra. To allow a proper comparison of vertebral shapes, it is necessary to use homologue vertebrae. As the total number of vertebrae varies among specimens, it is difficult to identify which vertebrae are homologous for all specimens, and the homology of these two vertebrae among different specimens can be questioned. Four successive vertebrae (39, 40, 41, 42) from three different specimens collected in the same geographic region were isolated and compared. It was concluded that no significant differences between the four successive vertebrae and between the three specimens were present, based on a Discriminant Function Analysis (DFA) of the weight matrix of the partial warp scores (see below). An Anova was performed on the partial warp scores of three successive precaudal vertebrae. The analysis indicated no significant differences between the successive vertebrae. This indicates that these vertebrae may be considered homologous, or at least as shape homologues.

To investigate whether the total number of vertebrae is a discriminative characteristic, the total number of vertebrae was counted for most of the observed specimens as well. The average of the total number of vertebrae was calculated for each population.

For the precaudal as well as the caudal vertebrae, different lengths were defined and measured (Fig. 3, 4A). A total of nine and ten lengths are defined on the lateral and caudal view of the precaudal vertebrae, respectively (Fig. 3A, B). On the lateral view of the caudal vertebrae, 14 lengths were measured (Fig. 4A). A total of six measurements were defined on the lateral view of the pectoral spines (Fig. 4B). Each length was plotted to standard length to analyse which length can be used to distinguish different populations. All biometric data are expressed in relation to standard length to be able to compare lengths

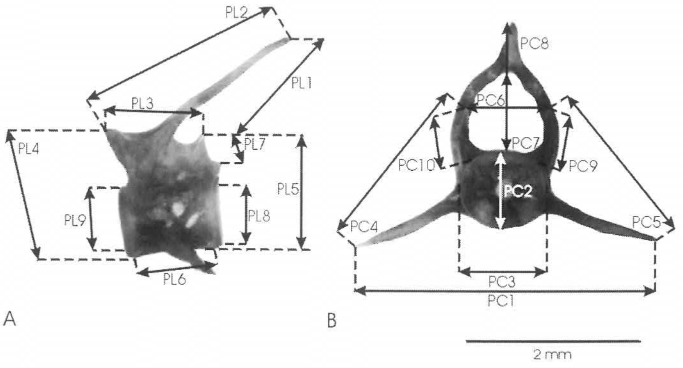

Fig. 3: Indication of measurements of the precaudal vertebra in (A) lateral and (B) caudal view of *Channallabes apus*.

between different specimens. Additionally, these data were analysed by a Principle Component Analysis (PCA).

For the geometric morphometric analyses, shape variation in both the precaudal and caudal vertebrae were studied based on landmark configurations, representing the shapes from a caudal and a lateral view (Bookstein 1991). Caudal and lateral vertebral images were captured using a digital camera (Colorview 8, Soft Imaging System) mounted on a stereomicroscope (WILD M5). Images were taken with Analysis Docu (Soft Imaging System GmbH, version 3.0). Ten landmarks were defined to describe the shape of the precaudal vertebrae in a caudal view and nine for the lateral view (Fig. 1A). For the caudal and lateral view of the caudal vertebrae, 11 and 14 landmarks were defined, respectively (Fig. 1B). In some specimens it was difficult to define the correct location of the landmarks because of the presence of soft tissue. To limit inaccuracies these vertebrae are consequently not included in the analysis. This explains the variable number of vertebrae in the geometric morphometric analyses (e.g. the eastern population counts eight specimens in the analysis of the lateral view of the caudal vertebrae, while the eastern populations counts five specimens in the analysis of the caudal view of the caudal vertebrae (Fig. 7, 8)). X and Y coordinates for each landmark were digitized using TPS-DIG (Rohlf 2001a). The Procrustes distances between the specimens were compared to the corresponding Euclidean distance using TPS-SMALL (Rohlf 1996, 1998) to evaluate the approximation of the distribution of the specimens in the Kendall shape space relative to that in the tangent space. The relationship between shape variation and an independent variable (degree of anguilliformity) was examined using TPS-REGR (Rohlf 2000). (Note: reports on material and methods should be cited in the past tense.)

The shape of both the precaudal and caudal vertebrae in caudal view is symmetrical. To avoid duplication of information and to avoid asymmetry noise, the landmark coordinates of both sides were averaged. To explore patterns in shape variation in the different populations, a relative warp analysis was conducted. Landmark configurations were aligned, translated, rotated and scaled to unit centroid size by Generalized Least-Squares (GLS) superimposition, using TPS-RELW (Rohlf & Slice 1990). Partial and relative

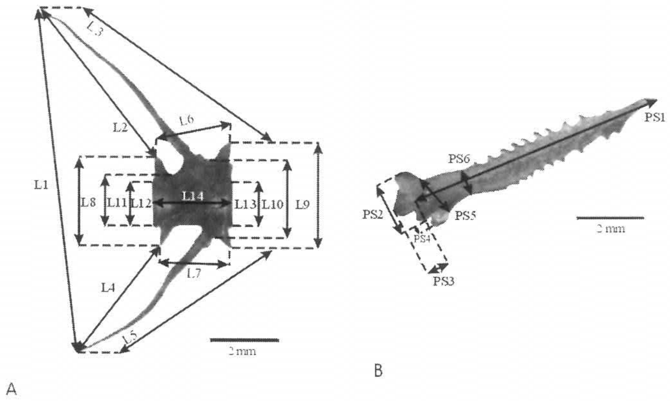

Fig. 4: Indication of measurements taken on caudal vertebrae in lateral view (A) and on pectoral spines (B) of *Channallabes apus*.

warp scores, calculated by TPS-RELW, were used as descriptors for the variation in shape at different scales of shape variation. Relative warp scores actually represent principle component scores of the partial warp scores, with the relative warps being analogous to principle components (Bookstein 1991; Rohlf 1993, 2001b). To investigate whether the different populations showed different patterns in shape variation, a backward stepwise Discriminant Function Analysis (DFA) (Statistica 5.5, Statosoft, Inc.) was done using the weight matrix of partial warp scores.

Visualization of the landmark plots and deformation grids are generated using TPS-RELW (Bookstein 1991; Rohlf 1993, 2001b). TPS-REGR was used to explore the relationship between shape variation and independent variables (degree of anguilliformity, size). A multivariate test, included in the TPS-REGR program, examines how well variation in shape can be predicted using one or more independent variables (Rohlf 2000).

RESULTS

For the analyses of the landmark configurations of the caudal and lateral views of the precaudal vertebrae and those of the caudal and lateral views of the caudal vertebrae, the distribution in tangent space is a sufficient approximation for that in the Kendall shape space, as indicated by a correlation coefficient between Procrustes distance (in Kendall shape space) and tangent distance (in tangent shape space) of 1.000.

The relative warp analysis based on the data set of the precaudal vertebrae in lateral view yielded 14 relative warps, of which Relative Warp 1 (RW1) explains most of the variation in shape (68.61 %). A plot of RW1 versus RW2 thus represents the pattern of the most important shape variation in the sample of landmark configurations. Despite the overall similarity in morphology of the vertebrae, this plot shows some marked shape variation. In the plot of RW1 versus RW2 of the lateral view of precaudal vertebrae, two geographical groups can be discerned (Fig. 5). Specimens collected in northern Gabon form the first group, whereas the second group originates from southern and eastern Gabon. RW1 explains most of the shape variation in the sample (44.35 %). This shape vector reflects the variation in length and inclination of the neural spines and the size of the postneural processes. With respect to the consensus, which is the calculated mean landmark configuration of the sample and situated at the origin of the biplot,

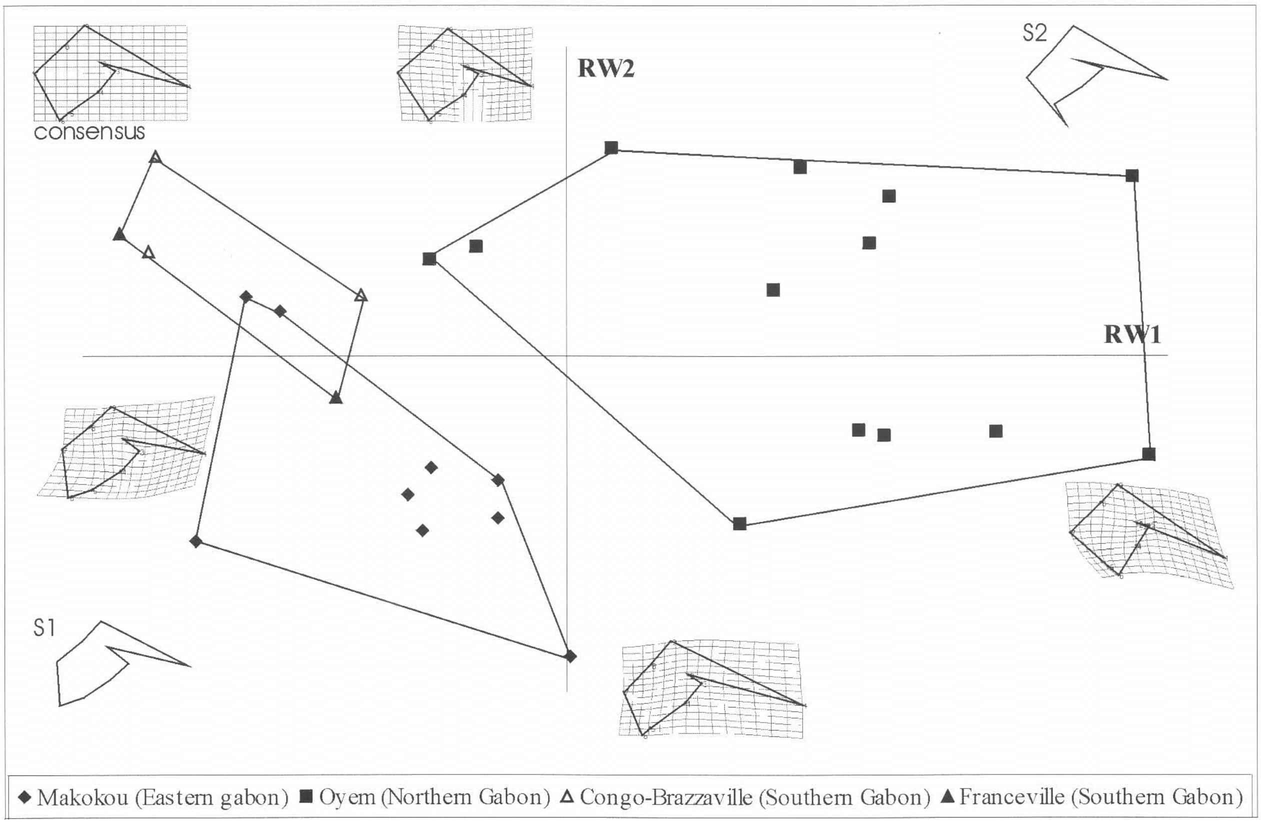

Fig. 5: Scatterplot of RW1 versus RW2 scores for the landmark configurations of the precaudal vertebrae (in a lateral view). Deformation grids visualising shape variation represented by RW1 (44.35 % of the total variance) and RW2 (23.81 % of the total variance) in relation to the consensus.

positive RW1-values represent longer, ventrally displaced neural spines and smaller processus postneurales. The opposite is true for the negative values. Variation expressed by RW2 (23.81 % of variation explained) involves the length of the neural spines and the vertebral centra (in rostro-caudal direction). Negative RW2 scores involve elongated neural spines and higher vertebral centra. The two geographic groups are separated along axis S. The landmark configurations of the extreme negative (S1) and positive values (S2) of this axis S is visualized in Fig. 5 and these represent the main shape differences between the two groups. A Discriminant Function Analysis reveals highly significant geographic variation and confirms the distinct shape differences in the two geographic groups (Table 1A).

Data based on the caudal view of the precaudal vertebrae are plotted in Fig. 6. The scatterplot of the scores of RW1 (explains 44.32 % of variation) versus those of RW2 (22.18 %) indicates a different ordination of three groups. The first group consists of the specimens collected in northern Gabon. The second group consists of the specimens collected in eastern and southern Gabon. The third group consists of specimens collected in Congo-Brazzaville. Positive RW1 scores represent vertebrae with shorter neural spines and with

Table 1A: Statistics of Discriminant Function Analysis of shape variation in precaudal vertebrae in lateral view: Squared Mahalanobis distances (D^2) in lower half and F-values in upper-right half (*$p < 0.01$, **$p < 0.001$).

		Makokou (Eastern Gabon)	Congo-brazzaville-Franceville (Southern Gabon)	Oyem (Northern Gabon)
Makokou (Eastern Gabon)	F-value		0,76	44,35**
Congo-brazzaville-Franceville (Southern Gabon)	F-value			38,30**
	D^2	0,28		
Oyem (Northern Gabon)				
	D^2	9,24**	12,77**	

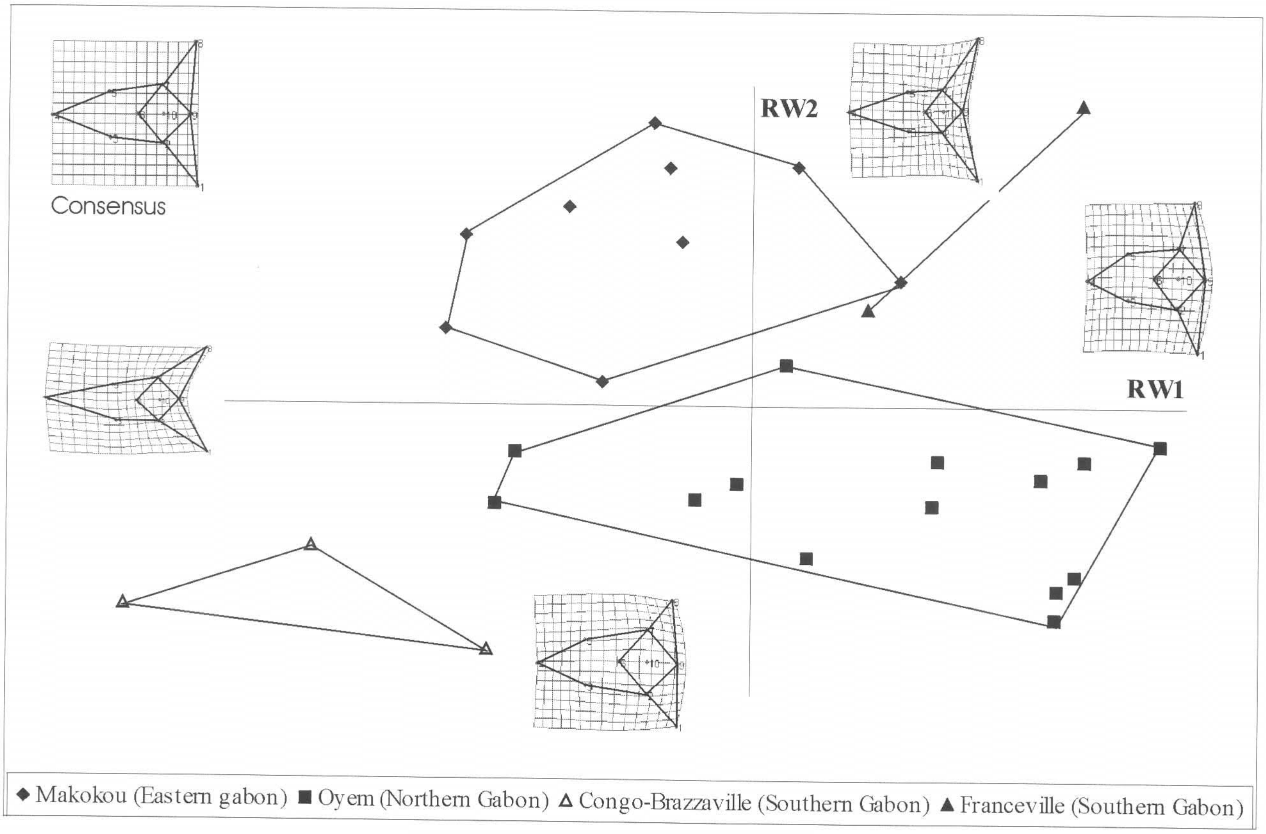

Fig. 6: Scatterplot of RW1 versus RW2 scores for the landmark configurations of the precaudal vertebrae (in a caudal view). Deformation grids visualising shape variation represented by RW1 (44.32 % of the total variance) and RW2 (22.18 % of the total variance) in relation to the consensus.

Table 1B: Statistics of Discriminant Function Analysis of shape variation in precaudal vertebrae in caudal view: Squared Mahalanobis distances (D2) in lower half and F-values in upper-right half (*p < 0.01, **p < 0.001).

		Makokou (Eastern Gabon) (Eastern Gabon)	Franceville	Oyem (Northern Gabon)	Congo-Brazaville
Makokou (Eastern Gabon)	F-value		0,01**	57,34**	1,55
Franceville	F-value			46,10**	10,44**
	D^2	23,47**			
Oyem (Northern Gabon)	F-value				13,32**
	D^2	29,28**	15,63**		
Congo-Brazaville					
	D^2	5,72	51,25**	46,95**	

parapophyses being more dorsally displaced. Along RW2, positive scores represent, with respect to the consensus, vertebrae with longer, ventrally displaced parapophyses and vertebral centra that have a smaller diameter, while negative scores involve dorsally displaced parapophyses and vertebral centra with broader diameter. The displacement of landmarks 1, 4 and 8 corresponds to the main shape variation observed on the caudal view of the precaudal vertebrae. A Discriminant Function Analysis of the data indicates significant differences between all groups except for the specimens collected in eastern Gabon and Congo-Brazzaville, which show no significant differences (Table 1B).

Based on the biometric results (see below) and the morphometric results of the lateral and caudal view of the precaudal vertebrae, the conclusion can be made that the northern population has neural spines, which are ventrally inclined and shorter. The biometric as well as the morphometric analyses indicate that the parapophyses of the northern population are dorsally inclined. The biometric results also confirm that the vertebral centrum of the northern population is larger. These shape differences of the precaudal vertebrae in lateral view between the northern population and the other populations are visualized by axis S in Fig. 5. Analyses of the data based on the lateral view of caudal vertebrae follow the pattern where a distinction between the specimens of northern Gabon and those of eastern and southern Gabon is observed even more clearly (Fig. 7). Both groups are separated along

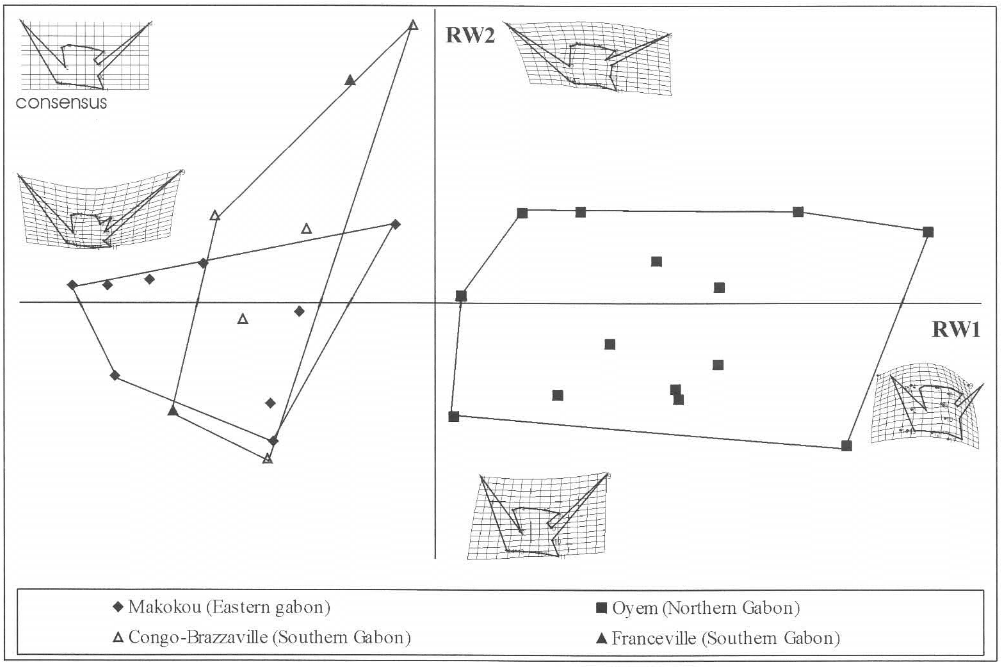

Fig. 7: Scatterplot of RW1 versus RW2 scores for the landmark configurations of the caudal vertebrae (in a lateral view). Deformation grids visualizing shape variation represented by RW1 (62.22 % of the total variance) and RW2 (16.35 % of the total variance) in relation to the consensus.

Table 1C: Statistics of Discriminant Function Analysis of shape variation in caudal vertebrae in lateral view: Squared Mahalanobis distances (D2) in lower half and F-values in upper-right half (*$p < 0.01$, **$p < 0.001$).

		Makokou (Eastern Gabon)	Congo-brazzaville-Franceville (Southern Gabon)	Oyem (Northern Gabon)
Makokou (Eastern Gabon)	F-value		2,87	61,76**
Congo-brazzaville-Franceville (Southern Gabon)	F-value			28,10**
	D^2	0,84		
Oyem (Northern Gabon)	D^2	12,47**	6,84**	

RW1, which explains 62.22 % of the shape variation. RW1 scores of the northern population all are positive, which involves (with respect to the consensus) shorter neural and haemal spines and larger vertebral centra. The second group, which has negative relative warp scores only, is formed by the eastern and southern population, and has caudal vertebrae with elongated neural and haemal spines and smaller vertebral centra. The RW2 explains 16.35 % of the variation. Positive scores of this axis include specimens that have, next to the elongated neural and haemal spines and smaller processus prae- and postneurales, neural and haemal spines that are respectively inclined in rostro-dorsal and rostro-ventral direction. These results are confirmed by the results of the biometric analysis of the data of the precaudal vertebrae in lateral view (see below). A Discriminant Function Analysis indicates highly significant differences between the Northern population and the eastern population and between the Northern population and the southern population (Table 1C).

The scatter plot of RW1 versus RW2 based on the data of the caudal view of the caudal vertebrae again shows a separation of two groups: the Northern population on the one hand and the eastern and southern population on the other hand (Fig. 8). The Northern

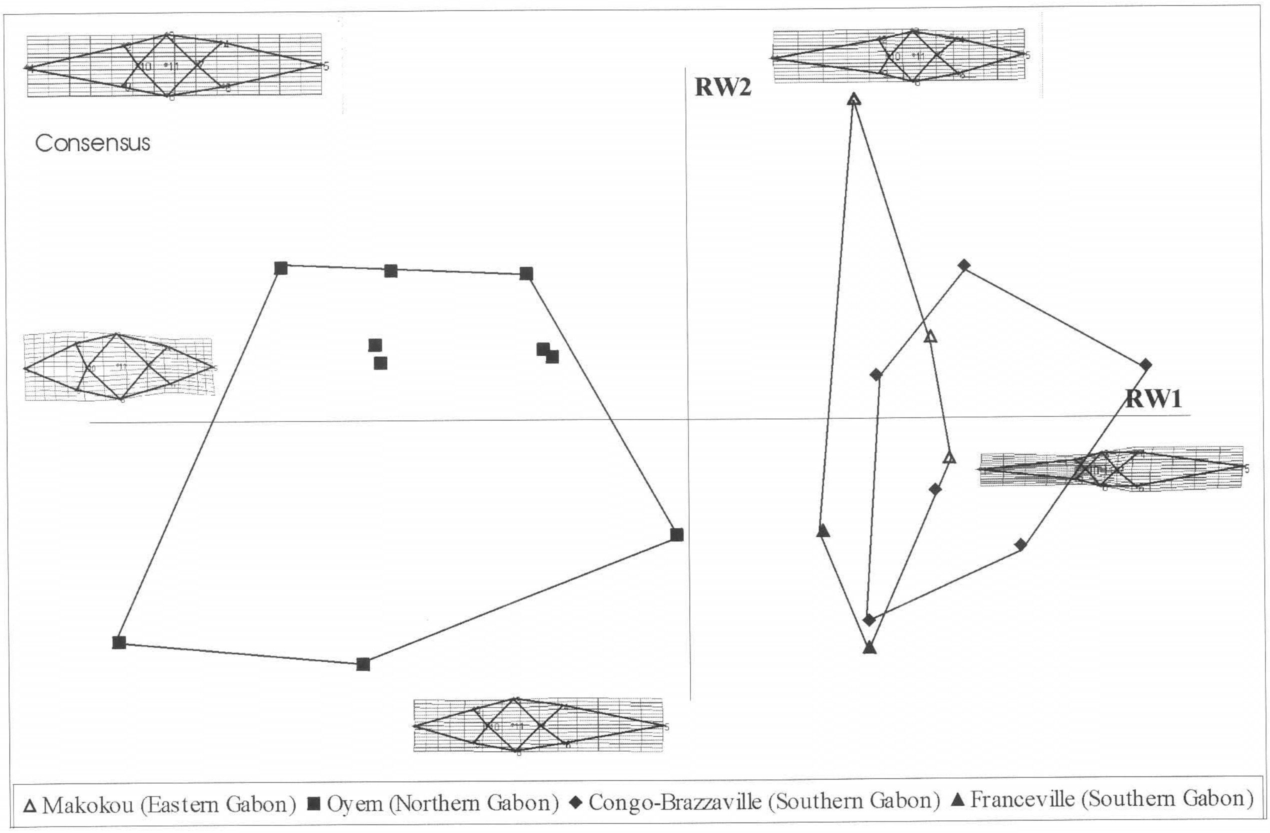

Fig. 8: Scatterplot of RW1 versus RW2 scores for the landmark configurations of the caudal vertebrae (in a caudal view). Deformation grids visualizing shape variation represented by RW1 (63.86 % of the total variance) and RW2 (22.49 % of the total variance) in relation to the consensus.

Table 1D: Statistics of Discriminant Function Analysis of shape variation in caudal vertebrae in caudal view: Squared Mahalanobis distances (D2) in lower half and F-values in upper-right half (*$p < 0.01$, **$p < 0.001$).

		Makokou (Eastern Gabon)	Congo-brazzaville-Franceville (Southern Gabon)	Oyem (Northern Gabon)
Makokou (Eastern Gabon)	F-value		0,79	101,40**
Congo-brazzaville-Franceville (Southern Gabon)	F-value			106,93**
	D^2	0,36		
Oyem (Northern Gabon)	D^2	31,55**	38,61**	

population has negative RW1 scores only, with RW1 explaining 63.86 % of the variation. Combined with the information obtained from the lateral view of the caudal vertebrae, it can be concluded that the vertebrae in the specimens of the Northern population have shorter neural and haemal spines, vertebral centra with a larger diameter and shorter processus postneuralis in contrast to the vertebrae of the other populations. Specimens of the group composed by the eastern and southern populations, which all have positive RW1 scores, have caudal vertebrae with elongated neural and haemal spines, vertebral centra with smaller diameter and longer processus postneuralis. The second relative warp axis explains 22.49 % of the shape variation. As could be suspected from the results of the relative warp analyses, the differences are highly significant (Table 1D).

The degree of anguilliformity can be defined as the ratio of the abdominal body depth versus the standard length. A Discriminant Function Analysis (Table 2) indicates that the differences, based on the degree of anguilliformity, between the northern population and the eastern and southern populations are significant. The specimens of northern Gabon show a higher degree of anguilliformity.

A biometrical analysis of the measurements of the precaudal and caudal vertebrae also led to the separation of the Northern population. The relative values of two measurements with respect to the standard length, the distance between the processus praehaemalis and the processus praeneuralis (PL4) (Fig. 3A) and the distance between the processus posthaemalis and the processus postneuralis (PL5) of the precaudal vertebrae in lateral view, are lower compared to those of the other populations. These lower relative values correspond with smaller processus prae- and postneurales because the relative values of the vertebral centra are similar in the different populations. These results are confirmed by the geometric morphometric analysis.

The relative values (with respect to the standard length) of the distance between the parapophyses (PC1) (Fig. 3B), the length of the neural canal (PC7), the distance between the vertebral centrum and the tip of the neural spine (PC8) and the distance between the tip of the processus postneurales and the tip of the parapophyses (PC4, 5) are lower for the Northern population. The geometric morphometric analysis showed that the neural spines of the Northern population are ventrally inclined and that their parapophyses are dorsally inclined, which explains the relatively lower values of PC7, PC8 and PC4, PC5.

A total of 14 measurements were defined on the caudal vertebrae in lateral view (Fig. 4A). Based on the values of six measurements (with respect to the standard length), again the specimens collected in northern Gabon are separated from those collected in the

Table 2: Statistics of Discriminant Function Analysis of the degree of anguilliformity and abdominal depth: Squared Mahalanobis distances (D2) in lower half and F-values in upper-right half (*$p < 0.01$, **$p < 0.001$).

		Makokou (Eastern Gabon)	Congo-brazzaville-Franceville (Southern Gabon)	Oyem (Northern Gabon)
Makokou (Eastern Gabon)	F-value		0,01	57,34**
Congo-brazzaville-Franceville (Southern Gabon)	F-value			46,10**
	D^2	0,01		
Oyem (Northern Gabon)	D^2	11,95**	11,53**	

other parts of Gabon. The relative values of (L1) the distance between the tip of the neural spine and the tip of the haemal spine, (L2) distance between the tip of the neural spine and the processus postneuralis, (L3) distance between the tip of the neural spine and the processus praeneuralis, (L4) distance between the tip of the haemal spine and the processus posthaemalis, and (L5) distance between the tip of the haemal spine and the processus praehaemalis are lower for the northern Gabon population. The lengths of the pterygiophores, which support the fin rays of the anal and the dorsal fins, also have lower values compared to the other populations. All abovementioned measurements explain the lower relative values of the abdominal body depth of the specimens of the Northern population.

Geometric morphometric analysis revealed the presence of shorter neural and haemal spines in the Northern population. This is confirmed by the biometric analysis, which shows that the L2, L3, L4 and L5 have lower relative values. The neural and haemal spines are, respectively, dorsally and ventrally inclined, which corresponds with the biometric results (L1).

With respect to skull length, the measurements (Fig. 4B), defined on the pectoral spines also distinguish the Northern population from the eastern and southern populations. The Northern population has lower relative values for the total length of the pectoral spine (PS1), height of the ridge on the articulation head (PS2), distance between the ridge and the caudal processus (PS3), distance between lateral processus and ridge (PS4), width of the base of the ridge (PS5) and width of the spine (PS6). It is remarkable that based on higher relative values for the spine length the specimens collected in Franceville are completely separated from the other specimens (Fig. 9C).

The total dataset of measurements of the precaudal and caudal vertebrae in both views and of the pectoral spine is analysed using a Principle Component Analysis. The measurements with the highest factor loadings are (1) on the precaudal vertebrae in lateral view: PL 4, 5, 8, 9; (2) on the precaudal vertebrae in caudal view: PC 2, 3, 5, 7, 8; (3) on the caudal vertebrae in lateral view: L 1-12; and (4) on the pectoral spine: PS 1-6. The combination of these measurements has the capacity to separate the different populations. The measurements with the highest loading factors are shown in Fig. 9A. Based on measurements of the pectoral spine, it is possible to separate the specimens originating from Franceville (Fig. 9B). The biometric data reflect and confirm the shape variation observed using the geometric morphometric analysis.

The plot in Fig. 9C shows that the Northern population, in contrast to the other populations, has low values (with respect to standard length) for L 2 and L 4. Plotting measurements with high loading factors (e.g. L1, PL 8), the same distribution of specimens is present. This means that the Northern population has precaudal vertebrae with larger vertebral centra and caudal vertebrae with shorter and, respectively, ventral and dorsal inclined neural and haemal spines. These results confirm the morphometric data.

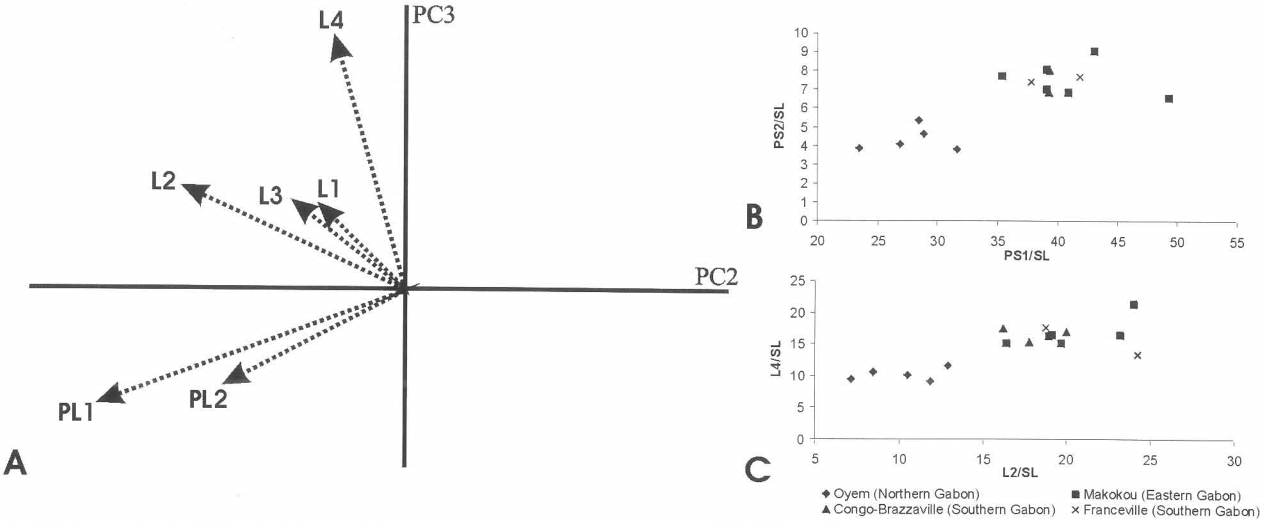

Fig. 9A: PCA-plot of biometric data based on measurements of the precaudal and caudal vertebrae and the pectoral spine, with indication of those measurements with highest loading factors, separating the northern population from the other populations; 9B: plot of (PS1) the total length of the pectoral spine (with respect to standard length) in function of (PS2) the height of the ridge on the articulation head (with respect to standard length); 9C: Plot of (L2) the distance between the tip of the neural spine and the processus postneuralis (with respect to standard length) in function of (L4) distance between the tip of the haemal spine and the processus posthaemalis (with respect to standard length).

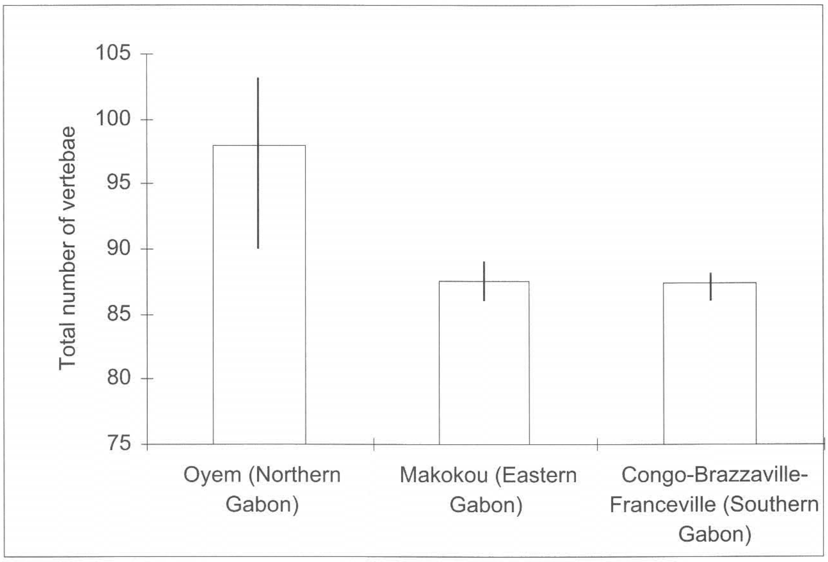

Fig. 10: Total number of vertebrae of the studied specimens. The bars reflect the average number of vertebrae while the vertical bar indicates the minimum and maximum number of vertebrae.

Based on the number of vertebrae, it is possible to distinguish the Northern population, which has more vertebral elements (90-103), from the eastern (86-89) and the southern populations (86-88) (Fig. 10). Based on the data of the lateral view of the caudal vertebrae, it is not possible to conclude whether a relationship exists between shape and degree of anguilliformity, because the sample size is too small and too many landmarks are defined. This implies that too few (?) degrees of freedom are defined, so a multivariate regression cannot be done. Regressing the shape variation based on the data of the precaudal vertebrae in lateral and caudal view, the relation found between shape and the degree of anguilliformity is in both cases low. Only 21 % of the variance is explained by the regression of shape to degree of anguilliformity, which is significant ($F = 5.986$, $p < 0.00001$), based on the data of the caudal view of the precaudal vertebrae. The program also performs a permutation test, where all of the shape variables for each specimen are randomly assigned to any specimen and where the regression is performed 1000 times. With the permutation procedure, it calculates how many times it found a better regression than the one the program indicates. Performing a Goodall F test, only one alternative regression is found, which yielded a better or equal result than the one observed. The amount of variance explained by the regression based on the data of the lateral view of precaudal vertebrae is 29 % and is also significant ($F = 8.7855$, $p < 0.00001$). In this case, no better or equal alternative regression was found. The relationship between the shape of caudal vertebrae in caudal view and the degree of anguilliformity is rather low. However, in this case, 41 % of the variance (significance: $F = 13.413$, $p < 0.00001$) is explained by the regression. The Goodall F test found no better than equal alternative regression. The relationship between shape variation and standard length has also been examined. The variance explained by the regression based on the precaudal and caudal vertebrae in lateral and caudal view is, respectively, 22.01 %, 31.89 %, 49.95 and 45.27 %. In all cases, no better or equal alternative regressions were found by the Goodall F tests. Despite the fact that the northern specimens have higher standard lengths than the other populations, no relationship is present between shape variation and standard length.

We may consequently conclude that shape variation observed in these vertebrae is independent of size and the degree of anguilliformity, and presumably thus reflects geographic differences. This implies that the degree of anguilliformity could thus be considered a taxonomic trait.

DISCUSSION

Vertebral counts are often used in systematics and phylogenetic research. The aim of this study was to investigate whether the shape of vertebrae is also a useful tool for taxonomic purposes.

The results indicate that vertebral shape differences are present between the different populations of anguilliform clariids. The analysis of the data based on the lateral and caudal view of the precaudal as well as the caudal vertebrae revealed a pattern of at least two geographical groups. The specimens originating from northern Gabon are always completely separated from those collected in eastern and southern Gabon, forming a geographical group. The specimens from eastern and southern Gabon form the second geographical group. However, based on the caudal view of the precaudal vertebrae, the specimens of Congo-Brazzaville can be separated from those collected in Franceville & Makokou.

The northern population is characterized by the following features: (1) the precaudal vertebrae have smaller processus postneurales, ventrally inclined neural spines, dorsally displaced parapophyses and larger vertebral centra; (2) the caudal vertebrae show shorter neural and haemal spines, respectively, dorsally and ventrally inclined, vertebral centra with a larger diameter and shorter processus postneurales. These shape differences are observed based on the geometric morphometric analysis and are confirmed by biometric data.

Owing to the lack of a reliable understanding of generic and specific characteristics, it is difficult to discern between the different eel-like species. The current determination key of Poll (1977) did not consider the range of variation of the different features because few specimens were used to describe the species.

For example, one specimen, found in Makokou, was cataloged in the collection of the Royal Museum for Central Africa (RMCA) as *Gymnallabes alvarezi* Roman, 1970, based on the currently available determination key of Poll (1977). According to Poll (1977) the presence of the pelvic fins is considered to be a diagnostic character for *G. alvarezi*. However, the presence or absence of these fins has been demonstrated to be a highly variable trait in some anguilliform clariids, including *G. alvarezi* (Adriaens *et al.* 2002). Specimens of the northern population, which show the highest degree of anguilliformity, lack pelvic fins, while in specimens collected in eastern Gabon pelvic fins are present. This leads to the conclusion that proposed diagnostic characters to distinguish different species are not unambiguous and thus cannot be used to distinguish species or subspecies (at least as they are described today).

A Discriminant Function Analysis on several metric characters, performed by Adriaens *et al.* (2002), shows that differences between *G. alvarezi* and *C. apus* are significant. This analysis is based on only few specimens of *G. alvarezi* and thus could give a wrong representation of the group differences. A more recent PCA analysis of metric and meristic characters of *C. apus* (collected in northern, southern and eastern Gabon), C. apus (present in RMCA) and *G. alvarezi* (present in RMCA), based on more specimens of each species, shows a substantial degree of overlap, and distinct groups can no longer be recognized (Fig. 11). However, since DFA reflects

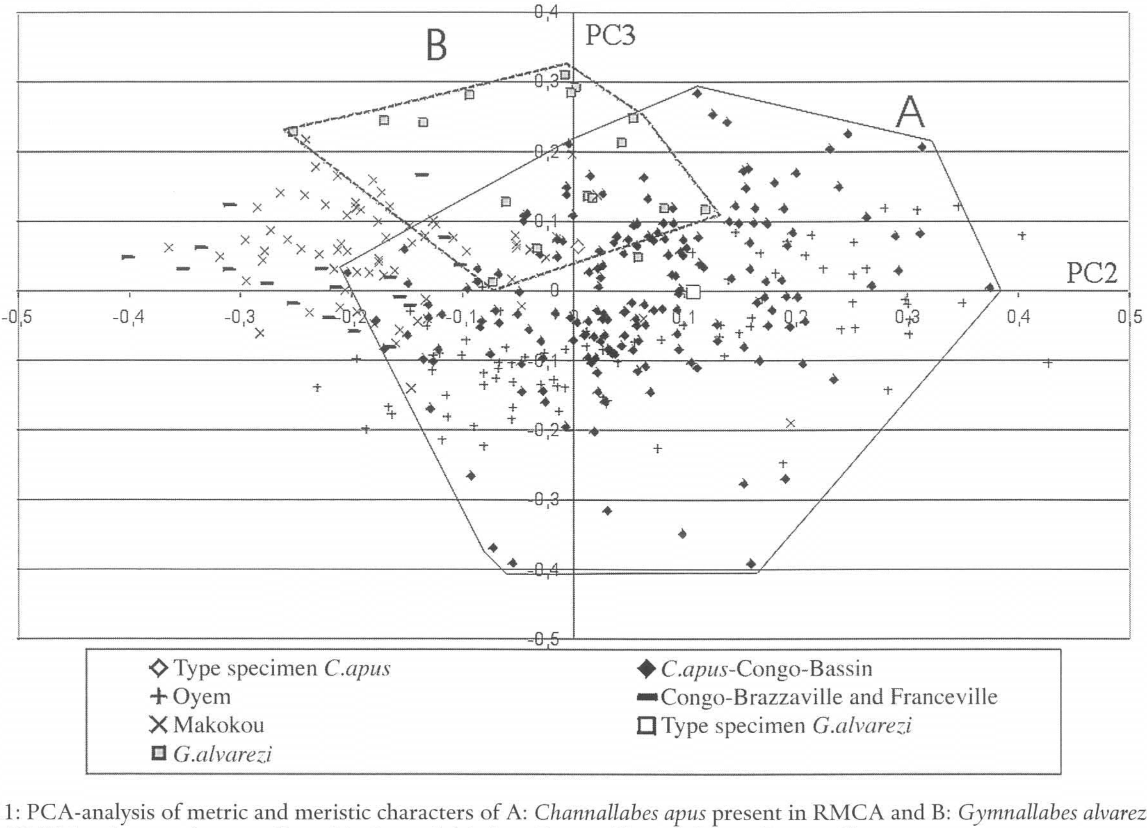

Fig. 11: PCA-analysis of metric and meristic characters of A: *Channallabes apus* present in RMCA and B: *Gymnallabes alvarezi* present in RMCA; other specimens collected in Oyem, Makokou, Franceville and Congo-Brazzaville.

the maximal variation between groups, which is not the case for PCA, a DFA should be done on this data set as well. Currently, a Discriminant Function Analysis of this data is not possible because the different groups of species or subspecies are not clearly understood. Additionally, the type specimen of *G. alvarezi*, is located in the plot near specimens belonging to *C. apus*, as on-going research is showing.

This indicates the difficulty of assigning a species name to the specimens collected in the different regions of Gabon and the need of diagnostic tools to distinguish the different species.

The morphometric analyses of both precaudal and caudal vertebrae thus reveal some kind of geographic variation in the postcranial skeleton of '*C. apus*'. Two hypotheses may explain the presence of two groups within the currently examined specimens: (1) interspecific variation and (2) intraspecific, geographic variation.

(1) Morphometric analyses of the precaudal as well as the caudal vertebrae reveal shape differences between the northern population on the one hand and the eastern and southern population on the other hand. Next to the vertebral differences between the different populations, other postcranial differences can be observed as well within the northern population. These differences are the absence of serrations on the anterior side of the pectoral spine, shorter and more slender pectoral spines, the absence of pelvic fins, a higher total number of vertebrae, a higher degree of anguilliformity and shorter pterygiophores. Features such as serrations of the pectoral spine, abdominal height, the presence of the pelvic fin and the number of vertebrae are characteristics that are often used to describe species (Poll 1942; 1957; Robert & Stewart 1976). The differences in these characters may be additional arguments for the hypothesis that the observed morphological differences result from interspecific variation rather than intraspecific variation. But, as mentioned before, some of these features (e.g. pelvic fins) are highly variable within the Clariidae (Adriaens *et al.* 2002) and thus may not be useful in systematic research. If the morphological vertebral differences and other postcranial differences between the northern population on the one hand and the eastern and southern population on the other hand result from interspecific variation, the differences may indicate that the former belongs to *C. apus* and the latter belongs to *G. alvarezi.* Further studies are required and are in progress to confirm these statements. If the on-going research reveals the presence of two species, the morphology and shape of precaudal and caudal vertebrae can be used to distinguish both species.

(2) The morphological vertebral differences may be caused by intraspecific variation. In this case, all specimens should be considered as *C. apus*, where the observed vertebral shape differences occur at a subspecies level. Intraspecific variation can be the result of different natural processes. Owing to the isolation of a population, genetic drift may occur (Jockush 1997). Additionally, variation of environmental factors will select for different adaptations, which supports (sub)speciation (Holcik & Jedlicka 1994). Within a geographic population, differences in the vertebral column have been observed which can be related to fluctuating abiotic factors, such as temperature (Gabriel 1944), climatologic or geographic circumstances (Holcik & Jedlicka 1994). Each year the number of vertebral elements of specimens of a population can vary, which depends on the temperature to which the embryo has been exposed during development (Gabriel 1944). Since the studied populations were collected in different regions of Gabon, different factors control the development of the embryos and may be responsible for the observed geographic variation. The habitats of the northern and eastern populations are similar. These two populations live in the moist soil of the rainforest, while the southern population lives in a dryer region, closer to the Gabonese savannah. But the results suggest that the morphology of the eastern and southern populations is more similar. This may indicate that shape variation is not a consequence of different developmental controlling factors.

As mentioned above, a recent PCA analysis of metric and meristic characters of *C. apus* specimens of different geographic regions and of *G. alvarezi* shows no distinct groups. The hypothesis of intraspecific variation is furthermore supported by the high degree of phenotypic plasticity in the fusion of hypurals in the caudal skeleton, the absence or presence of the pectoral and pelvic fins, and the presence of shorter neural and haemal spines (De Schepper *et al.* 2004; Adriaens *et al.* 2002).

The length of the pterygiophores of the specimens of the northern population is short compared to that of the other populations. These observations are reflected in the higher degree of anguilliformity. However, the statistical analysis (TPS-REGR) reveals no relation between shape of precaudal and caudal vertebrae and the degree of anguilliformity. Furthermore, no relations between vertebral shape and the presence or absence of paired fins and hypural fusions of the caudal fin are observed. Thus these characteristics may be examples of features, that are highly variable and can not be used as diagnostic traits.

The geometric morphometric analysis of vertebral shape variation in three different populations of *C. apus* collected in Gabon leads us to the conclusion that vertebral shape is a useful diagnostic tool in taxonomy. But at this moment it is not clear at which species level (species or subspecies) vertebral shape variation can be considered in the case of these anguilliform clariids.

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