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Habitudes alimentaires de *Brycinus longipinnis* dans le complexe fluvio-lacustre de la Bia, Côte d'Ivoire

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RÉSUMÉ. Le régime alimentaire de 171 spécimens de *Brycinus longipinnis* a été étudié en fonction de la taille, des stations et des saisons hydrologiques. L'échantillonnage a porté sur 24 prélèvements mensuels. Le plan d'eau concerné est la rivière Bia sur laquelle a été construit en 1959 un barrage hydroélectrique. L'indice d'importance relative combinant les pourcentages d'occurrence, numérique et pondéral a été utilisé. Il apparaît que l'espèce indiquée est omnivore. Elle consomme des macrophytes ainsi que des invertébrés terrestres et aquatiques avec comme aliments principaux les Chironomidae et les Formicidae. En amont du barrage de retenue, le régime alimentaire de *B. longipinnis* varie en fonction de la taille des individus. Il est également fonction des saisons hydrologiques dans toutes les stations (amont, aval, lac).

MOTS CLÉS : Alestidae, *Brycinus longipinnis,* habitudes alimentaires, complexe fluvio-lacustre, bassin Bia, Afrique occidentale.

Feeding Habits of *Brycinus Longipinnis* in Bia River, Côte d'Ivoire

The diet of 171 specimens of *Brycinus longipinnis* was examined in relation to the study site, to the specimen size and hydrological seasons during 24 months in the Bia River (Côte d'Ivoire) on which has been constructed a hydroelectric dam in 1959. The relative importance index combining the occurrence, numerical and weight of percentage of the items identified in the stomach contents was computed. This examination revealed for this species an omnivorous diet mainly composed of invertebrates and higher plant materials. The dominant food items were Chironomidae and Formicidae. This study showed differences in diet in relation to the hydrological seasons in all site of study and the size of the specimens upstream of the man-made lake.

Alestidae, *Brycinus longipinnis*, feeding habits, lake, Bia River, West Africa.

INTRODUCTION

Brycinus longipinnis (Günther, 1864) est un Alestidae largement répandu sur toute la frange atlantique depuis la Gambie jusqu'en République Démocratique du Congo (PAUGY, 1986). Ainsi, le rencontre-t-on dans les bassins côtiers de Côte d'Ivoire où il est très abondant dans les cours supérieurs des rivières (MERONA, 1981).

Par ailleurs, les *Brycinus* constituent avec les Cichlidae l'essentiel de la biomasse piscicole débarquée du lac de barrage d'Ayamé et l'espèce *B. longipinnis* est un poisson très apprécié et largement consommé par les populations autochtones malgré sa petite taille (taille maximale observée = 101 mm LS d'après PAUGY, 2003). Cet Alestidae constitue, en outre, une proie préférentielle dans l'alimentation de poissons prédateurs comme l'espèce *Mormyrops anguilloides* (KOUAMELAN, 1999). Cependant, très peu d'informations sont d'une manière générale disponibles sur l'écologie de *B. longipinnis,* notamment les travaux sur les habitudes alimentaires. En effet, ceux-ci demeurent assez rares et fragmentaires.

En Côte d'Ivoire, l'étude du régime alimentaire de *B. longipinnis* a été réalisée sur quelques spécimens examinés dans le Bandama blanc par PLANQUETTE & LEMASSON (1975). Quant aux travaux effectués dans les autres régions, ils se résument le plus souvent en un simple inventaire des proies ingérées (LAUZANNE, 1988 ; PAUGY & BENECH, 1989 ; VICTOR & BROWN, 1990).

Aussi, la présente étude apporte-t-elle des informations sur l'alimentation et les variations du régime alimentaire de *B. longipinnis* en fonction du milieu, de la taille du poisson et des saisons hydrologiques aussi bien en milieu fluviatile que lacustre.

MATERIEL ET METHODES

Milieu d'étude

La Bia est une rivière côtière qui développe son cours dans le sud-est de la Côte d'Ivoire. Elle prend sa source au Ghana et après 120 km en territoire ivoirien, se jette dans la lagune Abi au sud-est (Fig. 1). Son bassin versant couvre une superficie de 9300 km^2 pour une lon-

gueur totale d'environ 300 km (GIRARD et al., 1971; DURAND & GUIRAL, 1994). Un barrage (Ayamé I), a été construit près du site de l'ancien village d'Ayamé en 1959. Le lac d'Ayamé qui en est résulté est situé entre 5°30' et 5°50' de latitude Nord et entre 3° et 3°15' de longitude ouest. Il couvre une superficie de 180 km^2 pour une côte maximale de 91m et une profondeur pouvant atteindre 30m.

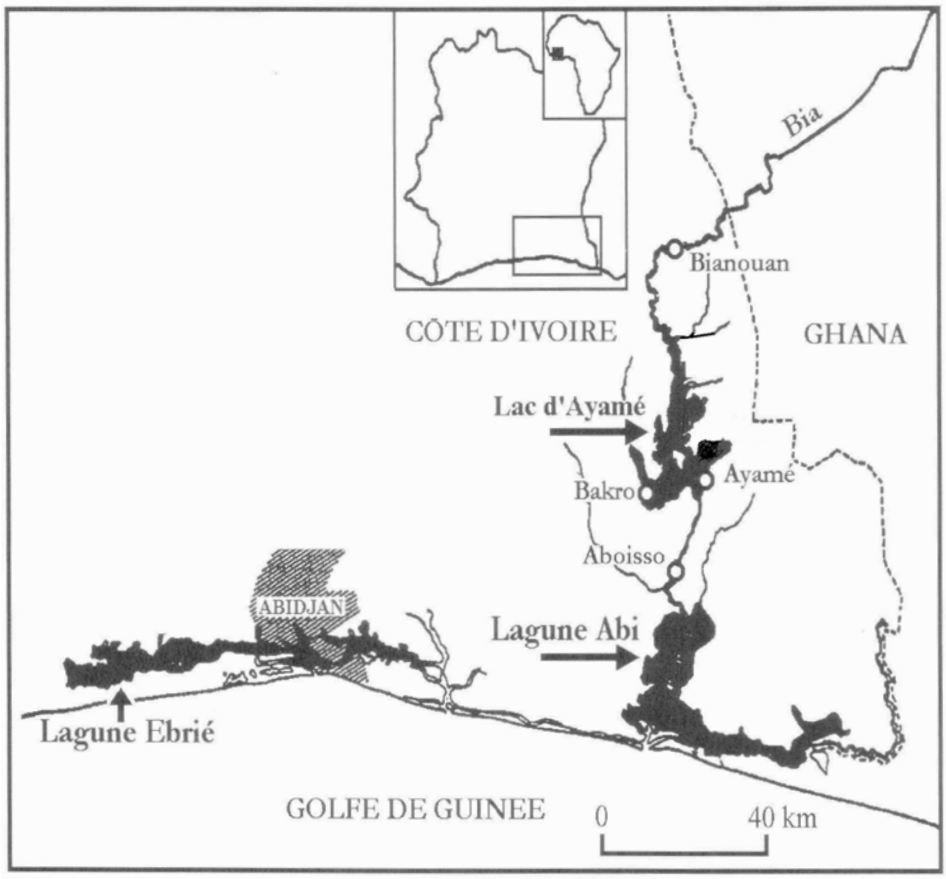

Fig. 1. – Localisation des sites d'échantillonnage sur la rivière Bia (d'après GOURÈNE et al., 1999) O = site d'échantillonnage.

Echantillonnage et analyses

Quatre stations ont été retenues sur la Bia : deux dans le lac (Bakro et Ayamé) et deux en milieu fluviatile dont un en amont (Bianouan) et un en aval (Aboisso). Les prélèvements d'échantillons de *B. longipinnis* ont été effectués chaque mois pendant deux ans à l'aide de deux batteries de filets maillants (de 10, 12, 15, 20, 25 et 30 mm de vide mailles) de 30m de longueur pour une hauteur de chute de 1.5 m. Dans chaque station, les filets sont posés à 17 heures et visités à 7 heures le lendemain pour la pêche de nuit, puis visités à nouveau à 13 heures pour la pêche de jour.

Après identification (selon PAUGY, 2003), chaque spécimen est pesé au gramme près et mesuré au millimètre près (longueur standard). L'estomac est prélevé, puis conservé dans du formol à 5%. Au laboratoire, l'estomac est pesé puis ouvert. Le contenu stomacal est ensuite délayé dans de l'eau et examiné à la loupe binoculaire. Les différents taxons-aliments sont triés, dénombrés, essorés puis pesés et identifiés, quand leur état de digestion le permettait, d'après les travaux de NEEDHAM (1962), DEJOUX et al. (1981), SCHOLTZ & HOLM (1985) et DIERL & RING (1992).

Les formules utilisées pour les analyses prennent en compte :

- le nombre d'estomacs vides : Ev
- le nombre total d'estomacs examinés : N
- le nombre d'estomacs contenant l'item i : Nie
- le nombre total d'estomacs pleins examinés : Net
- le nombre total de l'item i : Ni
- le nombre total de tous les items : Nt
- le poids total de l'item i : Wi
- le poids total de tous les items : Wt.

Ces formules sont :

- le coefficient de vacuité V = Ev/N x 100
- le pourcentage d'occurrence F = Nie/Net x 100
- le pourcentage numérique N = Ni/Nt x 100 (dans le cas des fruits, débris animaux et débris végétaux, le chiffre 1 a été attribué à leur présence dans un estomac d'après ROSECCHI & NOUAZE, 1987).
- le pourcentage pondéral W = Wi/Wt x 100
- l'indice d'importance relative IRI = F * (N+W) de PINKAS et al. (1971), qui est un indice mixte ayant l'avantage d'intégrer les trois pourcentages précédents et permettant une interprétation beaucoup plus réelle du régime alimentaire en minimisant les biais occasionnés

par chacun de ces pourcentages a été utilisé. Chaque pourcentage employé seul entraînerait éventuellement des biais au niveau de l'appréciation du régime alimentaire. En effet, selon LAUZANNE (1977), le pourcentage d'occurrence n'apporte pas d'indication sur l'importance quantitative des différents aliments tandis que le pourcentage numérique sous-estime l'importance des aliments peu nombreux mais de poids élevé. Quant au pourcentage pondéral, il n'apporte pas d'indication sur les préférences alimentaires.

Afin de pouvoir déterminer les préférences alimentaires des poissons, les différentes catégories d'aliments (préférentiels, secondaires et accidentels) ont été regroupées selon la classification proposée par SIMENSTAD (1979).

Une analyse de classification hiérarchique ascendante ("cluster analysis, single linkage") a été employée pour mettre en évidence les similitudes trophiques entre les stations d'étude ou les classes de taille.

Les classes de tailles ont été déterminées selon la règle de Sturge (SCHERRER, 1984) :

Nombre de classes (NC) = 1 + $(10\log_{10}N)/3$, ou N = nombre total de spécimens examinés. Avec : I = (LSmax – LSmin) / NC

Où I = intervalle de classe, NC = nombre total de classes, LSmax = longueur standard maximale, LSmin = longueur standard minimale.

Cette analyse a été réalisée à partir des coefficients de similarité de Jaccard calculés entre stations d'échantillonnage. Le coefficient de Jaccard a été calculé selon la formule : J = c x 100 / (a + b – c)

où a = nombre d'items à la station 1, b = nombre d'items à la station 2 et c = nombre d'items communs aux deux stations.

Le test statistique du coefficient de rang de Spearman (programme XLSTAT 2006), réalisé sur les pourcentages indiciaires des aliments a été utilisé pour comparer les compositions trophiques afin de tester si les aliments sont exploités dans les mêmes proportions aux différentes stations. Ce coefficient a également permis de comparer les régimes selon les classes de tailles et les saisons hydrologiques. Afin d'illustrer les différences et les ressemblances de régime alimentaire entre les populations de *B. longipinnis* provenant des différentes stations, une analyse en composantes principales a été réalisée à l'aide du programme XLSTAT 2006.

RESULTATS

Profil général du régime alimentaire

Au total, cent soixante onze (171) estomacs de *B. longipinnis* provenant de la Bia ont été examinés dont 125 contenaient des aliments et 46 vides. Vingt catégories d'aliments repartis entre 6 groupes principaux ont été identifiées (Tableau 1). Il s'agit des insectes, des arachnides, des crustacés, des myriapodes, des annélides, des macrophytes et d'autres aliments (fibres, écailles). La fraction végétale est essentiellement composée de fruits (pulpes, graines) et de débris végétaux (feuilles fraîches, tiges, morceaux de racines).

La classification des aliments à partir des pourcentages indiciaires (IRI) indique que les aliments préférentiels de *B. longipinnis* dans la Bia sont les Chironomidae avec 67.3% d'indice d'importance relative. Les Formicidae (19.6%) constituent les aliments secondaires.

TABLE 1

Régime alimentaire et pourcentages d'indice d'importance relative (IRI) correspondants de *Brycinus longipinnis* au cours des deux saisons hydrologiques dans la rivière Bia (n = nombre de spécimens examinés; SP = saison des pluies; SS = saison sèche).

ALIMENTS	Bianouan		Lac		Aboisso	
	SP	SS	SP	SS	SP	SS
	(n = 13)	(n = 10)	(n = 44)	(n = 16)	(n = 31)	(n = 11)
INSECTES						
DIPTERES						
Chironomidae	21,0	37,0	55,6	28,1	0,8	26
EPHEMEROPTERES						
Povilla adusta	0,0	0,0	2,2	0,0	0,0	0,0
Centroptilum sp.	0,0	0,0	4,4	21,1	0,0	0,0
COLEOPTERES						
Dytiscidae	31,3	0,0	0,0	0,0	5,1	0,0
TRICHOPTERES						
Ecnomus sp.	0,0	0,0	3,3	20,6	1	0,0
Amphipsyche sp.	0,0	0,0	0,0	29,4	0	13,1
Leptoceridae	0,0	0,0	0,0	0,0	0,8	0,0
PLECOPTERES						
Neoprla spio	14,4	0,0	0,0	0,0	0,0	0,0
HYMENOPTERES						
Formicidae	21,4	63,0	10,7	0,8	38,2	14,0
LEPIDOPTERES						
Nymphula sp.	0,0	0,0	0,0	0,0	1,1	0,0
HEMIPTERES						
Plea sp.	0,0	0,0	0,3	0,0	0,0	0,0

TABLE 1 (SUITE)

Régime alimentaire et pourcentages d'indice d'importance relative (IRI) correspondants de *Brycinus longipinnis* au cours des deux saisons hydrologiques dans la rivière Bia (n = nombre de spécimens examinés; SP = saison des pluies; SS = saison sèche).

ALIMENTS	Bianouan		Lac		Aboisso	
	SP	SS	SP	SS	SP	SS
	(n = 13)	(n = 10)	(n = 44)	(n = 16)	(n = 31)	(n = 11)
ISOPTERES						
Termitidae	0,0	0,0	0,0	0,0	3,6	0,0
CRUSTACES						
Cladocères	0,0	0,0	18,1	0,0	0,0	0,0
Copépodes	0,0	0,0	0,1	0,0	31,2	0,0
ARACHNIDES						
Hydracarina sp.	0,0	0,0	0,0	0,0	1,7	0,0
MYRIAPODES	12,3	0,0	0,0	0,0	0,0	0,0
MACROPHYTES						
Fruits	0,0	0,0	1,6	0,0	8,2	18,6
Débris végétaux	0,0	0,0	3,9	0,0	5,3	28,3
AUTRES ALIMENTS						
Fibres	0,0	0,0	0,0	0,0	3,0	0,0
Ecailles	0,0	0,0	0,0	2,0	0,0	0,0

Variation du régime alimentaire en fonction des stations

La Fig. 2 représente le plan (1-2) de l'analyse en composantes principales effectuée à partir de la matrice "stations d'échantillonnage/indice d'importance relative des différents aliments". Le pourcentage d'information restituée par l'analyse est de 70%. Le plan factoriel (1-2) montre un regroupement des stations fluviatiles (Bianouan, Aboisso) par rapport aux stations lacustres (Bakro, Ayamé). Les populations de *B. longipinnis* dans les stations du milieu fluviatile sont caractérisées principalement par un régime alimentaire dominé par les Formicidae tandis que celles du milieu lacustre consomment majoritairement des Chironomidae et des Cladocères.

Les corrélations des rangs de Spearman calculés entre les pourcentages indiciaires des différentes compositions trophiques ne présentent pas de valeurs significatives entre les différentes stations.

Variation du régime en fonction de la saison hydrologique

Les stations de Bakro et d'Ayamé, n'étant pas statistiquement différentes du point de vue composition trophique (Rs = 0.078, p = 0.735), elles ont été regroupées pour constituer l'échantillon du lac. Le Tableau 1 donne la composition du régime alimentaire de *B. longipinnis* en fonction des saisons hydrologiques à Bianouan, dans le lac et à Aboisso.

A Bianouan, les spécimens pêchés en saison des pluies se nourrissent préférentiellement de Dytiscidae (31.3%) et de Formicidae (21.4%). Les larves de Chironomidae (21%) et *Neoperla spio* (14.4%) constituent l'aliment secondaire. En saison sèche, les aliments préférentiels sont les Formicidae (63%); les larves de Chironomidae (37%) représentent les aliments secondaires.

Dans le lac, les aliments préférentiels des spécimens de *B. longipinnis* pêchés en saison des pluies sont les larves de Chironomidae (55.6%). Les Cladocères et les Formici-

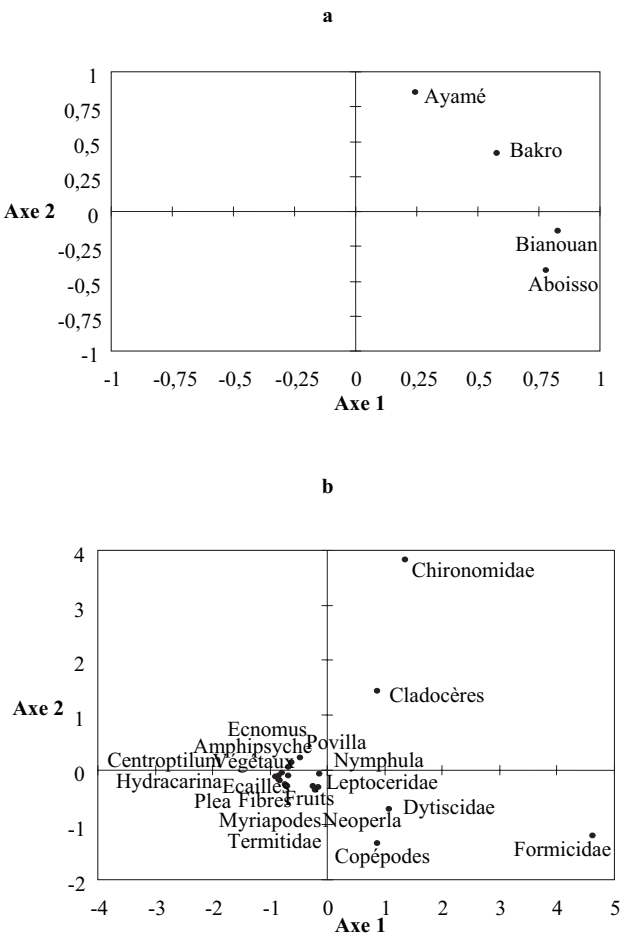

Fig. 2. – Variation du régime alimentaire de *B. longipinnis* en fonction des stations définies sur la rivière Bia (Côte d'Ivoire). Analyse en composantes principales effectuées à partir de la matrice de 4 stations et 20 types d'aliments. **a** : représentation des stations; **b** : représentation des aliments.

dae ne représentent respectivement que 18.1% et 10.7%. En saison sèche, les larves de *Amphipsyche* sp. (29.4%) et de Chironomidae (28.1%) dominent dans le régime alimentaire de l'espèce. A ces deux aliments s'ajoutent les Ephéméroptères du genre *Centroptilum* (21.1%).

A Aboisso, le régime alimentaire en saison des pluies est à base de Formicidae (38.2%) et de Copépodes (31.2%). Les fruits (8.2%) sont consommés secondairement. En saison sèche, les débris végétaux (28.3%) et les larves de Chironomidae (26%) constituent l'essentiel du bol alimentaire de *B. longipinnis*. Ils sont suivis dans l'ordre d'importance relative par les fruits (18.6%) et les Formicidae (14%).

Le spectre alimentaire comprend à Bianouan 5 types d'aliments en saison des pluies contre 2 en saison sèche. Dans le lac et à Aboisso, on enregistre respectivement 8 et 12 aliments en saison pluvieuse contre 6 et 5 en saison sèche.

La corrélation des rangs de Spearman calculée entre les pourcentages indiciaires des aliments consommés au cours des deux saisons hydrologiques est très significative à Bianouan (Rs = 0.709, p = 0.0003), et juste significative dans le lac (Rs = 0.452, p = 0.039) et à Aboisso (Rs = 0.476, p = 0.028).

Variation du régime en fonction de la taille des individus

Les spécimens examinés mesurent entre 38 et 95mm de longueur standard. Sept classes de tailles ont été déterminées selon la règle de Sturge (Tableau 2). En raison du très faible effectif des classes 1 (3 spécimens) et 7 (1 spécimen), les classes 1 et 2 ont été fusionnées, de même que les classes 6 et 7.

L'analyse de classification hiérarchique ascendante réalisée sur la base des pourcentages d'occurrence des différents aliments calculés dans chaque classe de taille (Fig. 3) permet de considérer deux groupes de classes de taille. Le groupe 1 se compose de tous les spécimens de classe de taille inférieure à 70mm. Le groupe 2 comprend tous les individus de longueur standard supérieure ou égale à 70mm. Ce sont ces deux groupes de classes de taille, constitués dans l'ensemble des stations de la Bia, qui ont été utilisés pour les analyses quantitatives du régime alimentaire. La composition du régime alimentaire des spécimens appartenant à ces deux groupes de classes de taille est détaillée dans le Tableau 3.

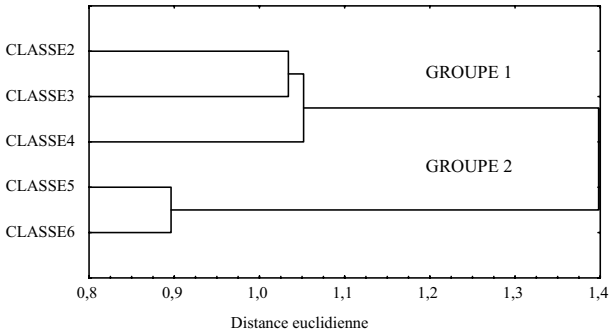

Fig. 3. – Dendrogramme montrant les similarités alimentaires entres les classes de taille de *Brycinus longipinnis* provenant de la rivière Bia (classe 2 : LS<54mm; classe 3 : 54mm ≤LS<62mm; classe 4 : 62mm ≤LS<70mm classe 5 : 70mm ≤LS<78mm; classe 6 : LS≥78mm).

TABLE 2

Différentes classes de tailles de *Brycinus longipinnis* obtenues par la règle de Sturge et leurs effectifs dans la rivière Bia.

Classes	Etendue	Effectif	Groupe
1	[38; 46 [	3	
2	[46; 54[	8	1
3	[54; 62[	23	
4	[62; 70[	32	
5	[70; 78[	41	
6	[78; 86 [	17	2
7	[86; 95[	1	

TABLE 3

Composition du régime alimentaire et pourcentages d'indice d'importance relative (IRI) correspondants des deux groupes de classes de taille de *Brycinus longipinnis* dans la rivière Bia (n = nombre de spécimens examinés; groupe 1 = LS < 70mm et groupe 2 = LS ≥ 70mm).

ALIMENTS	**Bianouan**		**Lac**		**Aboisso**	
	Groupe1	**Groupe2**	**Groupe1**	**Groupe2**	**Groupe1**	**Groupe2**
	(n = 14)	**(n = 9)**	**(n = 38)**	**(n = 22)**	**(n = 19)**	**(n = 23)**
INSECTES						
Chironomidae	12,5	1,9	54,1	0,0	5,4	2
Povilla adusta	0,0	0,0	2,2	0,0	0,0	0,0
Centroptilum sp.	0,0	0,0	4,4	0,0	0,0	0,0
Dytiscidae	17,5	40,0	0,0	0,0	32,6	0,0
Ecnomus sp.	0,0	0,0	2,7	77,6	6,3	0,0
Amphipsyche sp.	0,0	0,0	1,1	0,0	1,1	1
Leptoceridae	0,0	0,0	0,0	0,0	0,0	1,1
Neoprla spio	14,2	0,0	0,0	0,0	0,0	0,0
Formicidae	43,3	58,1	10,6	0,0	0,0	49,9
Nymphula sp.	0,0	0,0	0,0	0,0	0,0	1,6
Plea sp.	0,0	0,0	0,0	21,1	0,0	0,0
Termitidae	0,0	0,0	0,0	0,0	0,0	4,6
CRUSTACES						
Cladocères	0,0	0,0	18,1	0,0	0,0	0,0
Copépodes	0,0	0,0	0,0	0,0	0,0	39,8

TABLE 3 (SUITE)

Composition du régime alimentaire et pourcentages d'indice d'importance relative (IRI) correspondants des deux groupes de classes de taille de *Brycinus longipinnis* dans la rivière Bia (n = nombre de spécimens examinés; groupe 1 = LS < 70mm et groupe 2 = LS ≥ 70mm).

ALIMENTS	Bianouan		Lac		Aboisso	
	Groupe1	Groupe2	Groupe1	Groupe2	Groupe1	Groupe2
	(n = 14)	(n = 9)	(n = 38)	(n = 22)	(n = 19)	(n = 23)
ARACHNIDES						
Hydracarina sp.	0,0	0,0	1,3	0,0	11,3	0,0
MYRIAPODES	12,5	0,0	0,0	0,0	0,0	0,0
MACROPHYTES						
Fruits	0,0	0,0	1,6	0,0	20,7	0,0
Débris végétaux	0,0	0,0	3,9	0,0	4,0	0,0
AUTRES ALIMENTS						
Fibres	0,0	0,0	0,0	0,0	18,6	0,0
Ecailles	0,0	0,0	0,0	1,3	0,0	0,0

A Bianouan, les aliments préférentiels des spécimens du groupe 1 sont les Formicidae (43.3%) et les Dytiscidae (17.5%). L'aliment secondaire est *Neoperla spio* (14.2%) et les Chironomidae (12.5%). Les représentants du second groupe consomment préférentiellement les Formicidae (58.1%) et secondairement les Dytiscidae (40%).

Dans le lac, les larves de Chironomidae en réunissant 54.1% de l'indice d'importance relative (IRI) des aliments, dominent dans la nourriture des individus du groupe 1. Avec respectivement 18.1% et 10.6%, les Cladocères et les Formicidae représentent les aliments secondaires. Chez les individus de grande taille (groupe 2), le régime alimentaire est essentiellement à base de larves de Trichoptères du genre *Ecnomus* (77.6% de IRI).

A la station d'Aboisso, les représentants du groupe l consomment préférentiellement les Dytiscidae (32.6%) et les fruits (20.7%) tandis que les fibres (18.6% de IRI) représentent les aliments secondaires. En revanche, ceux du groupe 2 ont pour aliments préférentiels les Formicidae (49.9%) et les copépodes (39.8%).

Le coefficient de corrélation des rangs de Spearman calculés entre les pourcentages indiciaires des aliments consommés par ces deux groupes de classes de tailles n'est significatif qu'à Bianouan (Rs = 0.774, p = 0.00004).

DISCUSSION

Le profil général du régime alimentaire de *B. longipinnis* dans la Bia montre que cette espèce est majoritairement entomophage. L'examen qualitatif de ce régime révèle en effet que 12 des 20 aliments inventoriés dans les contenus stomacaux sont des insectes qui globalement constituent en terme quantitatif 60% du régime. Un tel régime avait déjà été mis en évidence par d'autres auteurs dans plusieurs cours d'eau chez cette espèce. En effet, PLANQUETTE & LEMASSON (1975) notent que *B. longipinnis* consomme essentiellement des insectes aquatiques dans le Bandama (Côte d'Ivoire). Dans la rivière Mono au Togo, PAUGY & BENECH (1989) révèlent également une consommation prépondérante d'insectes terrestres et aquatiques.

Du point de vue des ressources exploitées par cette espèce, aucune corrélation statistiquement significative n'a été observée entre les indices d'importance relative des aliments consommés. Cela indique que les aliments ne sont pas quantitativement exploités de la même manière par *B. longipinnis* dans les différentes stations. Ce qui s'expliquerait par le fait que les stations sont écologiquement différentes. En effet, dans le lac, les larves de Chironomidae sont les aliments préférentiels. Ces larves constituent l'une des composantes les plus abondantes parmi les insectes aquatiques du lac (DIOMANDÉ & GOURÈNE, 2005). On note également que des microcrustacés planctoniques comme les cladocères, généralement mieux représentés en milieux lentiques, sont les aliments secondaires. En milieu fluviatile (Bianouan et Aboisso), la majorité des aliments est constituée de végétaux et d'insectes terrestres. Ces derniers vivent dans la forêt environnante et se retrouvent accidentellement dans le cours d'eau où ils sont consommés par les poissons. C'est le cas des Formicidae qui selon DIÉTOA (2002), constituent un groupe abondant et diversifié dans les échantillons de dérive en amont et en aval de la rivière Bia. Dans ces stations, les insectes aquatiques sont très peu représentés dans l'alimentation de *B. longipinnis*. Par ailleurs, on constate qu'en aval du lac (Aboisso), *B. longipinnis* consomme en plus des insectes terrestres, des microcrustacés en particulier les copépodes. Ces invertébrés planctoniques proviendraient des eaux lacustres. Ceci semble indiquer que cette espèce se nourrit des ressources alimentaires importantes dans le milieu. Ce résultat confirme l'opportunisme alimentaire noté par VICTOR & BROWN (1990) dans la rivière Ikpoba au Nigéria.

Pour ce qui est de la variation du régime alimentaire en fonction des saisons hydrologiques, *B. longipinnis* consomme un plus grand nombre de catégories d'aliments en saison des pluies par rapport à la saison sèche. Par ailleurs, les Formicidae font partie des aliments préférentiels en toute saison à l'exception du lac où les larves de Chironomidae sont les plus consommées aussi bien en saison des pluies qu'en saison sèche. Le nombre élevé de catégories d'aliments enregistré en saison pluvieuse pourrait être attribué à la remontée de l'eau. En effet, *B. longipinnis* fait partie des nageurs de surface (LAUZANNE, 1988). Il a donc la facilité de capturer les insectes terrestres de taille réduite qui tombent dans l'eau. En effet, la présence le long de cette rivière d'une abondante végétation où vivent de nombreuses espèces de Formicidae favoriserait la disponibilité de ces organismes en toute

saison. La montée des eaux met à la disposition de ces poissons un grand nombre d'aliments notamment les invertébrés vivant sur la végétation riveraine. De plus, les fortes pluies provoquent également la chute de nombreuses branches hébergeant des invertébrés qui deviennent des proies accessibles aux poissons. L'abondance des Formicidae dans la dérive de la partie fluviatile expliquerait leur prépondérance dans le régime alimentaire de l'espèce considérée.

On ne note aucun changement du régime alimentaire de *B. longipinnis* en fonction de la taille à Bianouan. En revanche, dans le lac et en aval de la rivière, une variation de compositions trophiques est observée entre les spécimens étudiés. En effet, les plus grands individus consomment majoritairement *Ecnomus* dans le lac alors que leur alimentation est à base de Formicidae et de copépodes en aval. Quant aux individus de petite taille, leur régime est principalement composé de Chironomidae et de cladocères dans le milieu lacustre, de Dytiscidae et de végétaux dans le cours inférieur.

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A contribution to the knowledge of the behaviour of *Anodontites trapesialis* (Bivalvia: Mycetopodidae). The effect of sediment type on burrowing

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ABSTRACT. Large specimens of *Anodontites trapesialis* (n = 17) were observed in the laboratory and three locomotory patterns are described : burrowing, horizontal locomotion and rotation. Burrowing which consists of successive digging cycles (events that occur in association with each downward movement) was observed and recorded in large specimens added on two types of substrate : mixed sand (0.28%< 62µm; n = 6) and native sediment (40.65%<65µm; n = 6) at 25ºC. The burrowing process was similar in both sediments and the rate of digging cycles, expressed as number of digging cycles per 10min interval, decreased gradually as the shell intruded into the substrate, probably due to a decrease in penetrability with depth. In both types of sediment, time and number of digging cycles associated with the deepening into the substrate (burrowing) were significantly greater than those associated with penetration of the foot into the substrate and lifting of the shell which occurred on the surface of the substrate. Compared to other bivalves, *A. trapesialis* is a very slow burrower. Although the Burrowing Rate Index (BRI = [specimen mass $(g)^{0.33}$/burrowing time(s)]x10^4) in mixed sand (6.0±1.5)(mean±SE) did not differ significantly from that in native sediment (8.3±1.1), the total number of digging cycles performed during the burrowing was significantly greater in mixed sand (69.7±7.1) than in native sediment (43.7±6.8) which, while penetrable, is probably more cohesive than mixed sand, affording a better purchase of the foot and thus increasing its power to drag the shell into the sediment on each digging cycle. Thus, each digging cycle in native sediment probably produces a greater deepening than in mixed sand and so a smaller number of digging cycles is needed to finish burrowing.

KEY WORDS : *Anodontites trapesialis*, bivalve, burrowing behaviour, sediment, Mollusca.

INTRODUCTION

Bivalves show a limited repertoire of locomotory movements, including surface leaping behaviour, locomotion over the surface of the substrate, swimming, vertical migration, and burrowing behaviour, the most ubiquitous of all, which has been studied in a large range of species (see ANSELL, 1967; TRUEMAN, 1968; THOMPSON et al., 1980; MAURER et al., 1981; ALEXANDER et al., 1993).

Burrowing can play a critical role in the life history of soft-sediment invertebrates since it enables individuals to escape unfavourable conditions such as dislodgment and predation (HULL et al., 1998; TALLQVIST, 2001). Burrowing consists of successive cycles of activity during penetration of the animal into the substrate. The events that occur in association with each downward movement are known as the digging cycle which involves opening the valves that press against the substrate (penetration anchor), protraction of the foot and dilatation of its distal tip that acts as a second anchor (terminal anchor), closure of the siphons, adduction of the valves and retraction of the foot which drag the shell into the sediment (see TRUEMAN, 1983). While the basic events occurring during burrowing are essentially similar in most bivalves, the movements involved may differ in detail (ANSELL & TRUEMAN, 1967; CHECA & CADÉE, 1997). In a number of species, the time and number of digging cycles necessary for complete burrowing may also vary significantly with sediment grain size (ALEXANDER et al., 1993; DE LA HUZ et al., 2002).

Although many studies on burrowing of different species of bivalves are available, except for a superficial mention of the burrowing time of a single specimen by HEBLING (1976), to our knowledge, there are no studies concerning the burrowing behaviour of *Anodontites trapesialis* (Lamarck, 1819), a bivalve which is found almost totally buried in a variety of sediments from sandy to muddy or compact clay, in rivers with slow currents or in ponds subjected to periodical floods during the rainy season (HEBLING, 1976; SIMONE, 1994). All the studies consulted on this animal are anatomical (HEBLING, 1976), systematic (SIMONE, 1994) and ecotoxicological (e.g., AVELAR et al., 1991) in nature. Despite its importance as a biomonitoring organism which can concentrate pesticides in its soft parts (LOPES, et al. 1992), many essential aspects of its biology remain poorly understood.

To contribute to the overall understanding of the biology of *A. trapesialis*, the goal of this study was to observe and describe some locomotory patterns, with special emphasis on burrowing behaviour. With the aim to find a suitable and practical substrate to be used in laboratory experiments, the importance of sediment type for burrowing behaviour was also studied.

MATERIALS AND METHODS

Animals

Large specimens of *Anodontites trapesialis,* collected in Jardinópolis, State of São Paulo, at a selected site along the Pardo River (21º01'54"S, 47º56'10"W) were kept in outdoor tanks (7.0 x 4.0 x 0.7 m), with running water from a natural spring and aquatic plants. Temperatures fluctuated daily with the ambient air (23 to 27ºC). The native sediment, removed from the area where the specimens were collected, was sorted with sieves into seven grades : silt and clay, <65µm; very fine sand, 65-125µm; fine sand, 125-250µm; medium sand, 250-500µm; coarse sand, 500-1000µm; very coarse sand, 1000-2000µm; and gravel, >2000µm.

Locomotory patterns

Seventeen animals (length = 11 to 14cm) were observed systematically in the laboratory in an experimental aquarium (40 x 30 x 30cm) provided with 17cm of substrate (mixed sand, n = 8; native sediment, n = 9) and 11cm of water aerated with an air pump.

Influence of sediment type on burrowing ability

Two types of sediment were used : mixed sand (0.28%< 62µm; 59.32% from 62 to 500µm; 40.39% from 500 to 2000µm; 0.01>2000µm) and native sediment (40.65%<65µm; 58.10% from 65 to 500µm; 1.11% from 500 to 2000µm; 0.14%>2000µm). Burrowing was observed and recorded in two different groups (G) of animals (length = 11 to 14cm) by placing them horizontally left side uppermost on the substrate surface (G1 : mixed sand, n = 6; G2 : native sediment, n = 6) of the experimental aquarium and allowing them to burrow freely at 25ºC. Symbols corresponding to the different components of the digging cycles were recorded on the paper of a Nihon Kohden polygraph model RM-6100 (v = 1mm/s). A continuous recording was performed during the period in which the digging cycles occurred regularly until the instant that the animal remained quiet for 30min without executing any shell valve movement. At this point burrowing was considered to have finished. This occurs often when about two thirds of the shell is buried (Fig. 1A). Bivalves that did not start burrowing within 2h were excluded from the experiments. The recordings were analyzed by computing the time and number of digging cycles associated with each of the three phases of burrowing behaviour : phase I (penetration of the foot into the substrate), phase II (lifting of the shell) and phase III (deepening of the shell into the substrate). The time associated with phase III was termed burrowing time and was expressed as a Burrowing Rate Index (BRI) using a modification of the equation of Stanley proposed by ALEXANDER et al. (1993) : BRI = [specimen mass $(g)^{0.33}$/burrowing time(s)]x10^4. The mass of each specimen was measured to the nearest 0.1g. A multiplication factor of 10 000 was used so that the slowest individuals would have BRI values of 1.0 or greater.

Statistical Analysis

All data were tested for normality and homogeneity of variances prior to analysis. Data from repeated measurements made on the same animals were analyzed by one-way analysis of variance for repeated measures followed by Newman-Keuls test. Data from measurements made on two different groups of animals were compared by Student *t*-test. All comparisons having a probability $P<0.05$ were considered to be significant. Data are expressed as the mean ± S.E.M.

RESULTS

Locomotory patterns

Three locomotory patterns were observed to occur by a similar basic process in both mixed sand and native sediment :

1. Burrowing – The animal, lying horizontally on the substrate surface, opens the valves and the foot is dilated and gradually emitted through the pedal gap. After several extension-retraction movements the foot is turned sideward toward the sediment, touching it. The subsequent burrowing process, observed in 14 of 17 animals, was divided into three phases :

I. Penetration of the foot into the substrate : After touching the substrate the foot starts a series of wavy movements around an axis parallel to the antero-posterior axis of the shell, excavating the substrate (Fig. 1B). Sporadic adductions may be observed. When the foot has penetrated far enough to obtain sufficient anchorage, we may observe sequences of protraction/dilatation of the foot, adduction of the valves with water ejection and pedal retraction which pulls the shell down toward the foot, followed by opening of the valves. The digging cycles are repeated many times, with the animal completely burrowing its foot.

II. Lifting of the shell : The animal then starts to lift the postero-dorsal portion of the shell while the anterior-ventral half, from where the foot protrudes, is pulled inside the substrate by the movement of retraction of the foot at the end of each digging cycle. Most of the time, the siphons are kept closed during the adduction of the valves. As a consequence, the water of the mantle cavity is ejected trough the pedal gap loosening the substrate, a procedure that facilitates the deepening of the foot and the erection of the shell. After several digging cycles the shell is finally drawn erect with the long axis about 40-50º inclined from the vertical (Fig. 1C-E).

III. Deepening of the shell : The digging cycles, similar to those described earlier, follow in regular succession, each one producing a little downward displacement. The adduction of the valves is always preceded by siphon closure, preventing water from leaking out through them which, as we have seen, facilitates the deepening. Immediately after each adduction, a deepening of the anterior part of the shell occurs, followed by a similar movement of the posterior part resulting in a rocking motion. Siphons reopen and the gape of the valves increases. In some cases in which the animal dug close to the aquarium glass wall, we observed successive protrusions of the foot

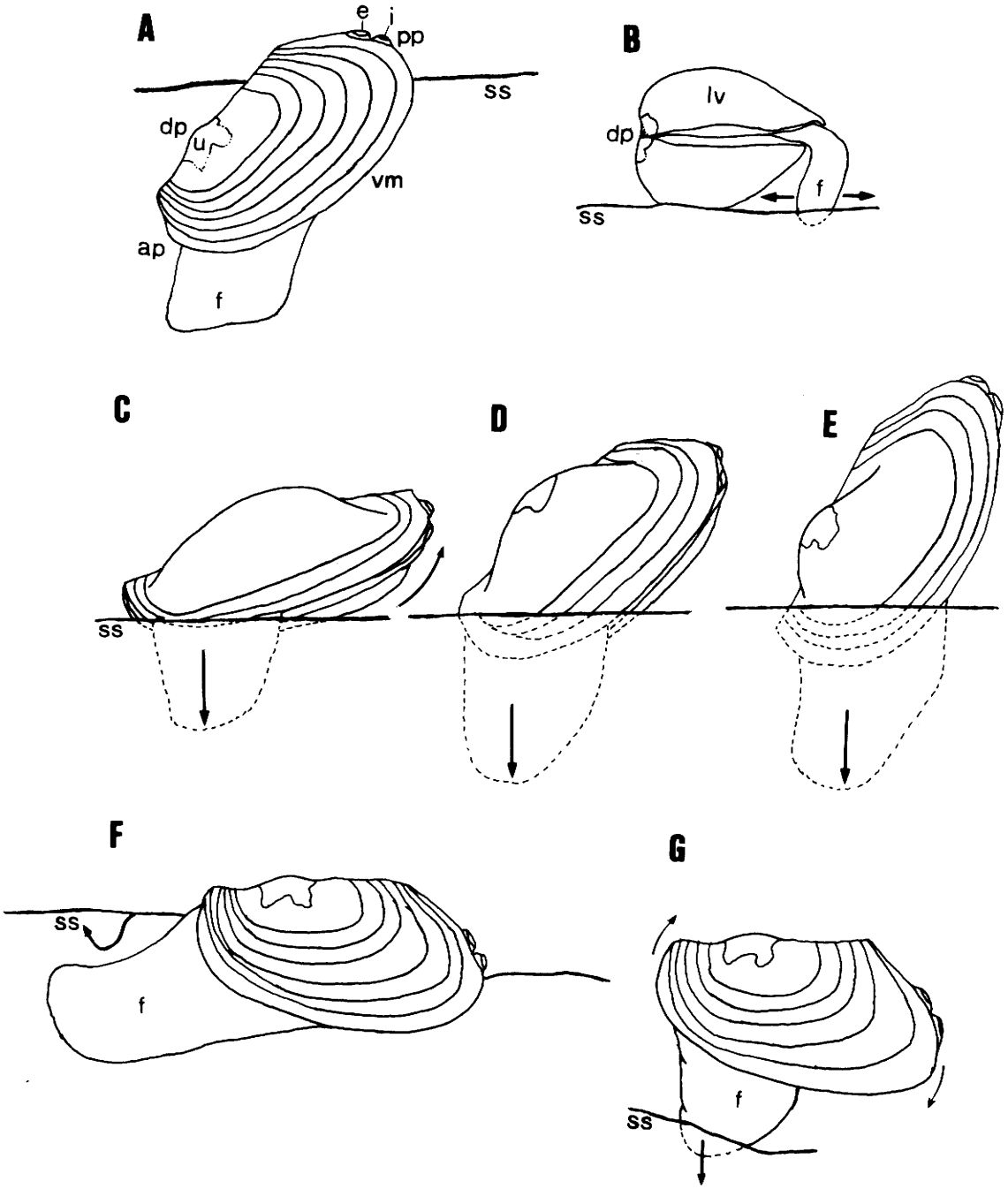

Fig. 1. – Diagrams of *Anodontites trapesialis* in different behavioural patterns. (A) Buried into the substrate; (B-E) Phases of Burrowing – (B) Penetration of the foot into the substrate; (C-D) Lifting of the shell; (E) beginning of Deepening into the substrate; the buried portion of the animal is traced in dashed lines; (F) Horizontal locomotion; (G) Rotation. Abbreviations : ap, anterior portion; dp, dorsal portion; e, exhalant siphon; f, foot; i, inhalant siphon; lv, left valve; pp, posterior portion; ss, surface of the substrate; u, umbo; vm, ventral margin; In all figures movements of the shell and foot are indicated by arrows. Further explanation in the text.

into the substrate (pedal probing) until a new adduction was produced, starting a new cycle. The digging cycles become more and more sparse until activity ceases.

Once burrowed, the bivalve lays immobile, with the anterior-ventral part from where the wide foot protrudes deepened into the substrate with the long axis about 40-50º inclined from the vertical, leaving the posterior part (about 1/3 of the shell) containing the inhalant and exhalant siphonal apertures exposed above the substrate (Fig. 1A).

2. Horizontal locomotion – This behaviour may occur after the animals have concluded phases I and II of burrowing. Instead of performing the deepening of the shell, the animal starts a displacement on the surface of the substrate due to the more anterior location of the foot, followed by a succession of digging cycles (Fig. 1F). This behaviour, observed in 8 (5 in mixed sand; 3 in native sediment) of 17 animals, leaves furrows in the surface of the substrate and ceases when the animal touches the aquarium wall which precludes its progress. Deepening into the substrate then starts.

3. Rotation – This behaviour, observed in 5 (1 in mixed sand; 4 in native sediment) of 17 animals, may occur when the animal is lying on one of its valves and only the distal part of the foot is burrowed forming an angle of about 90º with the antero-posterior axis of the shell. The bivalve then extends its foot abruptly pushing the anterior region of the shell, which results in a clockwise displacement of the posterior part of the shell (Fig. 1G). The valves then adduce and the foot retracts, resulting in unburying. This movement occurs several times, also leading to a displacement of the support point of the shell on the substrate, resulting in a spiral course. Then the animal restarts the burrowing from phase I.

Burrowing ability and type of substrate

The basic burrowing process was similar in mixed sand and native sediment. In both types of sediment the burrowing time (mixed sand, 187.0 ± 29.6min; native sediment, 128.5 ± 18.6min) was significantly greater than the time associated with phases I (mixed sand, 19.8 ± 3.3min; native sediment, 12.8 ± 3.1min) and II (mixed sand, 20.8 ± 6.6min; native sediment, 22.5 ± 6.9min), which did not differ significantly from one another (ANOVA for repeated measures followed by the Newman-Keuls test, $p<0.05$) (Fig. 2A). Also, the number of digging cycles associated with phase III (mixed sand, 69.7 ± 7.1, native sediment, 43.7 ± 6.8) was significantly greater than the number of those associated with phase I (mixed sand, 18.7 ± 2.7, native sediment, 8.2 ± 1.7) and phase II (mixed sand, 15.8 ± 3.4, native sediment, 19.7 ± 6.8) (Fig. 2B). As shown in Fig. 3, in both types of sediment the rate of digging cycles, expressed as number of digging cycles per 10min interval, decreased gradually as the shell intruded into the substrate in all experimental animals. Although the BRI in mixed sand (6.0 ± 1.5) did not differ significantly (Student's *t*-test, p>00.5) from that in native sediment (8.3 ± 1.1), the total number of digging cycles associated with phase III was significantly greater in mixed sand than in native sediment (Student's *t*-test, p = 0.0246). It is noteworthy that the values of the standard error of the mean associated with burrowing time were high due to large differences among specimens.

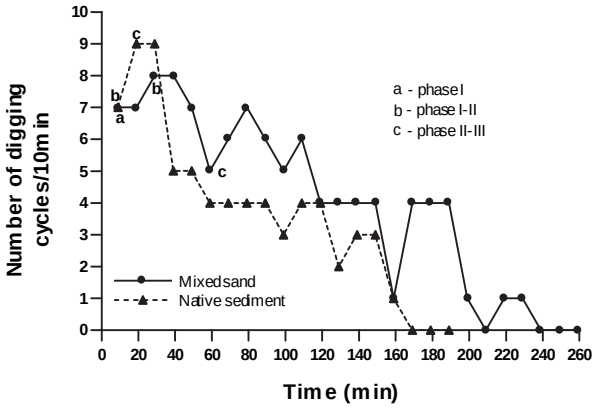

Fig. 3. – Rate of digging cycles, expressed as number of digging cycles per 10min interval, at 25ºC, during the course of burrowing behaviour in two specimens of *Anodontites trapesialis*. The letters above the points indicate the burrowing phase or the transition between phases.

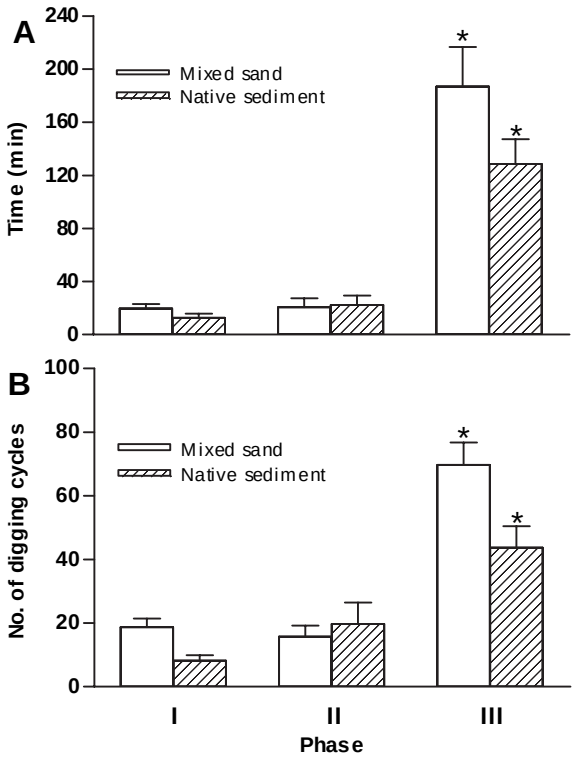

Fig. 2. – Mean time (A) and number of digging cycles (B) in each phase of the burrowing behaviour of *Anodontites trapesialis* in mixed sand and native sediment (n = 6 per group), at 25ºC. The vertical lines above the bars represent the standard error of the mean and the asterisks, the significance. $p<0.05$ compared to the respective values in phase I and II (ANOVA for repeated measures followed by the Newman-Keuls test).

DISCUSSION

Anodontites trapesialis, once dislodged from the substrate, may perform in the laboratory horizontal locomotion or rotation preceding the deepening into the substrate, which ends with the animal lying immobile/burrowed, the state in which it is generally found in nature and that was early reported by HEBLING (1976). Although we did not find in the literature reports on the occurrence of rotation in other bivalve species, horizontal locomotion was described in *Margaritifera margaritifera* (Linnaeus, 1758) (TRUEMAN, 1968), *Corbula gibba* (Olivi, 1792) (PISAROVIC et al., 2000) and reported in *Elliptio complanata* (Lightfoot,1786)(AMYOT & DOWNING, 1997). This surface locomotion, previously observed by HEBLING (1976), may be the means of finding a more suitable condition when the animal is dislodged from its buried position (TRUEMAN, 1968). Also, rotation probably enables the animal to find a better position to start burrowing. The basic events occurring during burrowing of *A. trapesialis* are essentially similar to those described in other bivalve species. The rocking motion observed during the deepening of the shell is characteristic of bulging shells and must greatly assist penetration by providing movements oblique to the plane of greatest resistance (TRUEMAN et al., 1966; ANSELL & TRUEMAN, 1967; TRUEMAN, 1968).

The basic burrowing mechanism was also similar in mixed sand and native sediment. As expected, in both types of sediment the time and number of digging cycles associated with phase III, which corresponds to the deepening of the animal into the substrate, were significantly greater than those associated with phases I and II, performed on the surface of the substrate. In native sediment as well as in mixed sand, the frequency of digging cycles decreased gradually as the animal penetrated into the substrate. This decrease was observed in several bivalves such as *Tellina tenuis* Da Costa, 1778 and *Macoma balthica* (Linnaeus, 1758) (TRUEMAN et al., 1966), in *Mercenaria mercenaria* (Linnaeus, 1758) (ANSELL & TRUEMAN, 1967), *Margaritifera margaritifera* (TRUEMAN, 1968), and *Donax denticulatus* Linnaeus, 1758 (TRUEMAN, 1971) and is caused by an increase in time per cycle due to the increasing difficulty that the foot has in penetrating the substrate whose compactness and resistance increase with depth (TRUEMAN et al., 1966, BROWN & TRUEMAN, 1991, CHECA & CADÉE, 1997).

Our results also showed that BRI in silty sand native sediment did not differ significantly from that measured in mixed sand. Similar results were obtained by CHECA & CADÉE (1997) for *Mya arenaria* Linnaeus, 1758 in its silty sand native sediment and in medium sand. Also TALLQVIST (2001) did not find a significant difference between burial time of *Macoma balthica* in sandy sediment and mud. According to SAKURAI et al. (1996), the burial velocity of *Pseudocardium sachalinensis* (Schrenck, 1865), *Mactra chinensis* Philippi 1846, and *Ruditapes philippinarum* (Adams & Reeve, 1850) was found not to differ significantly between sediments varying from very fine sand with 10% silt to medium sand. ALEXANDER et al. (1993), who measured the burrowing times of 21 species of bivalves on several sorted substrates, reported that although the maximum BRI occurs in the sediment which the species most commonly inhabits, most of bivalve species tested showed the highest burrowing rates in medium to fine sand. According to DE LA HUZ et al. (2002) the burrowing time of *Donax trunculus* Linnaeus, 1758 was shorter in fine and medium sand than in coarse sand to gravel. Also, *D. serra* Röding,1798 and *D. sordidus* Hanley, 1845 presented the fastest burial times in fine and medium sediments (NEL et al., 2001). Coherent with these results, the mixed sand and native sediment used in our experiments presented a higher percentage of grain sizes in the range from very fine sand to medium sand (59.3%, in mix mixed sand; 58.1%, in native sediment).

A wide variation in burrowing times and consequently in BRI is found in the literature both within and between species (see TRUEMAN, 1983; ALEXANDER et al., 1993 : HULL et al., 1998). In *A. trapesialis*, burrowing times in mixed sand and native sediment are among the longest measured in bivalves, corresponding to the smallest BRI. A slow burrowing may be disadvantageous since once exhumed the animal is exposed to predators for a long time and may be carried away by currents. Nevertheless, as *A. trapesialis* is adapted to a still water environment (HEBLING, 1976), it is seldom dislodged from the substrate and once buried it probably remains in the same place for a long time. Thus, these animals probably did no suffer a selection pressure to develop a faster burrowing. Consistent with this statement, recent results from our laboratory have revealed that *A. trapesialis* from a lentic site (dam) where the water level is stable, burrows in mixed sand slower yet than those from a lotic site (river) whose water level lowers during the dry period of the year, exposing the mussel to unfavorable conditions (CÂNDIDO & ROMERO, 2006).

Our results have revealed that although the burrowing times did not differ between the two substrates, the total number of digging cycles performed by *A. trapesialis* in mixed sand was significantly greater than that in native sediment. According to NEL et al. (2001), *D. sordidus* exhibited a decrease in the number of digging cycles needed to achieve complete burial in medium and coarse sediments, in the range in which burrowing was possible, whereas this number appears to be unaffected by grain size in *D. serra*. LEWIS and REIBEL (1984) reported that in the mussel *Lampsilis radiata* (Gmelin, 1791) the depth burrowed in 30min was greater in mixed sand than in gravel but the number of burrowing cycles was the same on the two substrata, whereas in *Elliptio complanata* and *Anodonta grandis* Say, 1829, more burrowing cycles were required in the gravel to reach the same depth as in sand. These results indicate that each cycle performed in sand, a more compact sediment, produces a greater deepening than that obtained in gravel.

The foot of *A. trapesialis* is wide and adapted to digging (HEBLING, 1976) and, according to our observations, burrowing is based essentially on the muscular activity of the foot which must perform a suitable penetration into the substrate. Nevertheless, the firmness of anchor of the foot may determine how much the shell penetrates into the sediment at each digging cycle. According to TRUEMAN et al. (1966), the holding power of the anchorage of the foot is related to the resistance of the substrate to the penetration of the shell. As an increase in grain size decreases compactness (PETTIJOHN, 1956 *in* DE LA HUZ et al., 2002) while increasing penetrability, a suitable substrate must have an optimum relation between these two parameters. Since cohesiveness increases among grains finer than 100µm (ALEXANDER et al., 1993), the native sediment (41%<65µm), while penetrable, is more cohesive than mixed sand (0.28%<62µm), affording a better purchase of the foot and thus increasing its power to drag the shell into the sediment on each digging cycle. Thus each digging cycle in native sediment probably produces a greater deepening than in mixed sand and so a smaller number of digging cycles is needed to finish burrowing. This characteristic confers an adaptive advantage on the animal, since it supports a reduction in energy expenditure during burrowing and saves endogenous fuel reserves and thus may lead to maximization of survival time during periods of unfavourable environmental conditions.

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Channallabes sanghaensis sp. n., a new anguilliform catfish from the Congo River basin, with some comments on other anguilliform clariids (Teleostei, Siluriformes)

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ABSTRACT. *Channallabes sanghaensis* sp. n., an anguilliform clariid from the vicinity of Ntchouo (Congo River drainage) is distinguished from other Congolese anguilliform clariids by the following combination of characters : a large foramen on the fourth post-Weberian vertebra; two large lateral processes on the second dorsal fin ray pterygiophore; a small supraorbital process on infraorbital IV, not reaching the rostral border of the eye; a fenestra between the scapulo-coracoid and the cleithrum; an interdigitation zone between the quadrate and the entopterygoid; serrations on both sides of the pectoral spine; 86–89 vertebrae; 121-125 dorsal fin rays and 104-124 anal fin rays. An updated identification key is given for the six species of *Channallabes*.

KEY WORDS : *Channallabes,* new species, biometry, osteology

INTRODUCTION

The freshwater clariids are one of the 37 catfish families within the Siluriformes (Sabaj et al., 2004). Although they occur in Syria, southern Turkey and large parts of Southeast Asia, they are most diverse and specious in Africa (Teugels, 1996; Teugels & Adriaens, 2003). This richness is demonstrated by the presence of 12 genera with up to 74 species (Teugels, 1996, Teugels & Adriaens, 2003). Some of the generalised, fusiform species show a large geographic distribution, whereas all anguilliform species, belonging to the genera *Channallabes, Gymnallabes, Dolichallabes, Platyclarias* and *Platyallabes*, occur in a small area, occupying a more specialized, burrowing niche. They can only be found in swampy areas in the Nilo-Sudan (Niger delta), Lower Guinea (Woleu, Ogowe, Ivindo River basin) and the Zaire (Congo River basin) ichthyological provinces (Poll, 1957; Roberts, 1975; Teugels, 1986; Teugels et al., 1990). Obviously, anguilliform clariids are morphologically specialized. Besides the body elongation, a whole set of morphological changes are observed, such as loss of the adipose fin, continuity of unpaired fins, reduction of paired fins, reduction of several skull bones, reduction of the eyes and hypertrophy of the jaw muscle complex (Cabuy et al., 1999; Devaere et al., 2001; 2004).

During an ongoing revision of the alpha–level taxonomy of catfishes from the Congo drainage, a new species of *Channallabes* was discovered and it is the aim of this study to describe the new species and to give an overview on the Congolese anguilliform clariids.

MATERIALS AND METHODS

Material examined is listed below in the species accounts. For this study, we used available museum material from the Royal Museum of Central Africa, Tervuren; British Museum of Natural History, London; Museum of comparative Zoology, Harvard; Musée National d'Histoire Naturelle, Paris; Musée d'Histoire Naturelle de Genève and the Naturhistorisches Museum, Vienna. Institutional abbreviations are listed in Leviton et al. (1985).

For all specimens, 36 measurements were taken point to point using a digital callipers with an accuracy of +/- 0.1mm following Devaere et al. (2004). Measurements of bilaterally paired structures were taken on the left side. Not all specimens were preserved well enough to make all meristic counts. Six specimens were cleared and stained following Taylor & Van Dyke (1985).

The data obtained was submitted to a Principal Components Analysis, using Statistica 6.0 (StatSoft Inc.). Morphometric and meristic data were independently submitted to a PCA, using the covariance matrix for 28 log transformed measurements and the correlation matrix of five meristics. The obtained PC- scores for the meristic data were combined with the PC-scores of the metric data in one plot (Boden et al., 1997). The first principle component was not used as it showed to be a size factor (Bookstein et al., 1985; Teugels et al., 1999). Qualitative and absence/presence characteristics were not included in the analyses but help to further identify and differentiate the species. Between groups variance was tested by Two-way analysis of variance (Statistica 6.0, Statsoft). Basic statistics included the Kolmogorov-Smirnov test and the non-parametric Mann-Whitney U-test.

RESULTS

In the intitial analysis all anguilliform clariids from the Congo River basin are included, inclusive type material of *Platyclarias machadoi* Poll, 1977*, Platyallabes tihoni* (Poll, 1944)*, Gymnallabes nops* Roberts & Stewart, 1976*, Dolichallabes miscrophthalmus* Poll, 1942 and *Channallabes apus* (Günther, 1873).

The PCA's were performed, using the covariance matrix for 28 log transformed measurements and correlation matrix of 5 meristics. Fig. 1 combines the second factor scores for the measurements PCA with the first factor scores for the meristic PCA. We obtain three clear groups. One group, located in the upper left quadrant, contains the type material of *Platyallabes tihoni*. Closely located to that group is the holotype of *Gymnallabes nops*, indicating the questionable systematic status of this species (see discussion). Type material of both species and additional specimens all come from the Lower Congo Rapids, the Lower Congo and the Pool Malebo freshwater ecoregions. A second group located in the upper right corner, contains all type material from *Platyclarias machadoi*. All specimens come from the Kasai freshwater ecoregion. The group, in the lower right quadrant, contains the type material of *Dolichallabes microphthalmus* and *Channallabes apus*. The factor loadings are shown in Table 1. The second principal component for the metric characters mainly reflects the distance between the origin of the dorsal fin and the occipital process and the caudal peduncle depth; while the first principal component of the meristic counts corresponds mainly to the total number of vertebrae and the number of precaudal vertebrae.

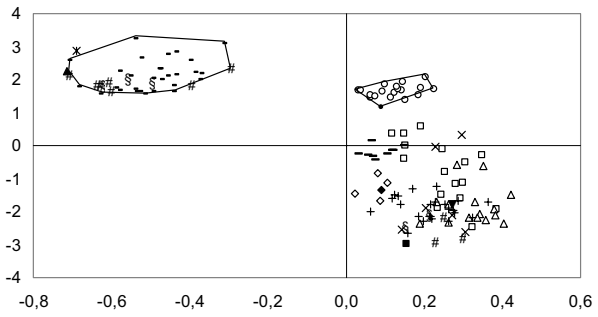

Fig. 1. – Combined plot of all specimens, including type material of *Channallabes apus, Platyallabes tihoni, Platyclarias machadoi, Dolichallabes microphthalmus* and *Gymnallabes nops,* of the scores of the second component (factor 2) taken from a principle components analysis of log-transformed metric variables versus the first principal component (factor 1) taken from a principle components analysis of meristics. × Upper Congo Rapids; □ Central Congo; △ Sudanic Congo; — Sangha; + Kasai; ▾ Tumba; § Pool Malebo; – Lower Congo Rapids; # Lower Congo; ■ holotype of *Channallabes apus*; ◆ holotype of *Dolichallabes microphthalmus*; ◇ paratypes of *Dolichallabes microphthalmus*; ● holotype of *Platyclarias machadoi*; o paratypes of *Platyclarias machadoi*; ▲ holotype of *Platyallabes tihoni*; * holotype of *Gymnallabes nops*.

TABLE 1

Factor loadings for the combined PCA; PCII of a PCA carried out on 28 log-transformed morphometric variables and PCI of a PCA carried but on five meristic variables.

	Factor 2		**Factor 1**
preanal length	0,170124	number of ribs	-0,478006
prepectoral length	0,209874	precaudal vertebrae	-0,508840
distance between the occipital process and the dorsal fin	0,663950	number of non-rib bearing precaudal vertebrae	0,013902
caudal peduncle depth	0,238794	caudal vertebrae	-0,492487
abdominal body depth	0,197651	total number of vertebrae	-0,519476
maxillary barbel	-0,200894		
external mandibular barbel	-0,224091		
internal mandibular barbel	-0,155758		
nasal barbel	-0,191597		
skull length	-0,005581		
preorbital length	-0,037684		
supraoccipital spine length	0,065308		
skull width	-0,147273		
supraoccipital spine width	0,127108		
inter orbital distance	-0,127932		
anterior nostril interdistance	-0,067250		
posterior nostril interdistance	-0,152486		
rostral skull width	-0,144574		
orbital skull width	-0,160837		
skull height	0,085116		
eye diameter	0,049640		
snouth height	-0,027317		
orbital skull height	0,045248		
prehyoid length	-0,033215		
internal mandibular interdistance	-0,177385		
external mandibular interdistance	-0,160344		
mouth width	-0,168569		
skull roof width	0,069837		

The results of a separate analysis on the cluster around the type material of *Dolichallabes microphthalmus* and *Channallabes apus* (meristic and log-transformed metric data) are shown in Fig. 2. Again three groups can be recognized. The first group (lower left quadrant) includes the type material of *Dolichallabes microphthalmus,* all coming from the Sudanic Congo (Oubangui) freshwater ecoregion. The second group in the upper left corner does not contain any type material and includes all the specimens from the Sangha freshwater ecoregion included in this study. The third group includes the type material of *Channallabes apus* and contains specimens from all ecoregions, except the Sangha (see above). The factor loadings are shown in Table 2. The dominant characters for the second principal component for the metric characters are the length and width of the occipital spine, width of the skull roof, mouth width and the snout height; while for the first principal component of the meristic counts, total number of vertebrae and the number of caudal vertebrae are the most important. The Sangha specimens clearly represent a population that is metrically and meristically distinct from the rest, to which no currently known type–specimen can be assigned. This information, as well as additional, distinctive osteological data (see below), supports the hypothesis that this represents a natural group, and thus a new species.

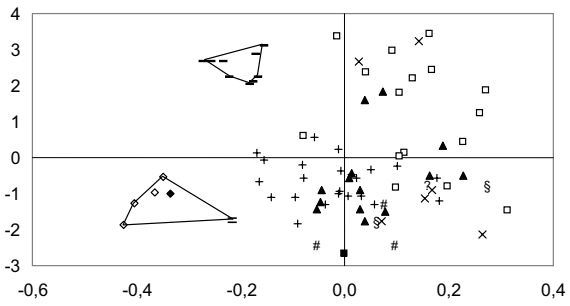

Fig. 2. – Combined plot, including type material of *Channallabes apus* and *Dolichallabes microphthalmus,* of the scores of the second component (factor 2) taken from a principle components analysis of log-transformed metric variables versus the first principal component (factor 1) taken from a principle components analysis of meristic counts. × Upper Congo Rapids; □ Central Congo; △ Sudanic Congo; — Sangha; + Kasai; ▾ Tumba; § Pool Malebo; # Lower Congo; ■ holotype of *Channallabes apus*; ◆ holotype of *Dolichallabes microphthalmus*; ◇ paratypes of *Dolichallabes microphthalmus*; = specimen of *Dolichallabes microphthalmus*.

TABLE 2

Factor loadings for the combined PCA; PCII of a PCA carried out on 28 log-transformed morphometric variables and PCI of a PCA carried out on five meristic variables.

	Factor 2		**Factor 1**
preanal length	-0,039298	number of ribs	-0,202709
prepectoral length	0,046373	precaudal vertebrae	-0,511543
distance between the occipital process and the dorsal fin	0,060023	number of non-rib bearing precaudal vertebrae	-0,207890
caudal peduncle depth	0,014936	caudal vertebrae	-0,557968
abdominal body depth	0,067776	total number of vertebrae	-0,585394
maxillary barbel	-0,028298		
external mandibular barbel	-0,091223		
internal mandibular barbel	-0,209065		
nasal barbel	-0,179787		
skull length	0,010614		
preorbital length	0,030991		
supraoccipital spine length	0,443546		
skull width	-0,014993		
supraoccipital spine width	0,556500		
inter orbital distance	0,026394		
anterior nostril interdistance	-0,025638		
posterior nostril interdistance	-0,064973		
rostral skull width	-0,089030		
orbital skull width	-0,076638		
skull height	0,099443		
eye diameter	0,228413		
snouth height	-0,290028		
orbital skull height	-0,123188		
prehyoid length	-0,035749		
internal mandibular interdistance	-0,077767		
external mandibular interdistance	-0,074561		
mouth width	-0,336050		
skull roof width	0,297431		

Next, we turn to the remaining large group on the right of Fig. 2, including the type material of *Channallabes apus*. Again, a combined PCA was plotted (Fig. 3). A subdivision can be observed, splitting the remaining specimens into two groups. The group in the upper part of the plot includes the type material of *Channallabes apus* (group I). The most important factor for recognizing these groups are the total number of vertebrae and the number of caudal vertebrae. In Fig. 4, the total number of vertebrae of these specimens is than plotted against the SL. Since the total number of vertebrae could be counted on a larger dataset, more specimens (n = 123) could be included. The two same groups can again be separated, with the cluster including *C. apus* having a higher number of vertebrae. The non-parametric Mann-Whitney U-test showed a p-level of $1.5e^{-15}$ ($p<0.05$), thus rejecting the null-hypothesis of equal means. This shows that the two group are significant different. No other differences (morphometric, meristic, …), however, could be found (see discussion).

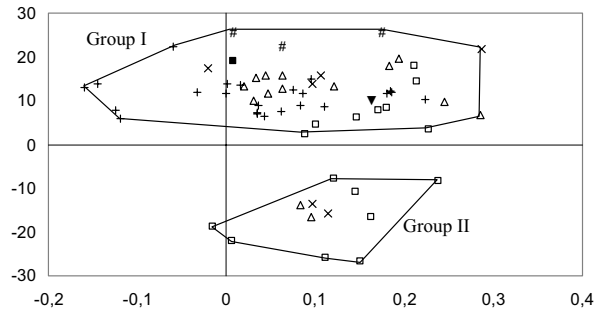

Fig. 3. – Combined plot, including type material of *Channallabes apus*, of the scores of the second component (factor 2) taken from a principle components analysis of log-transformed metric variables versus the first principal component (factor 1) taken from a principle components analysis of meristic counts. × Upper Congo Rapids; □ Central Congo; △ Sudanic Congo; + Kasai; ▼ Tumba; # Lower Congo; ■ holotype of *Channallabes apus*.

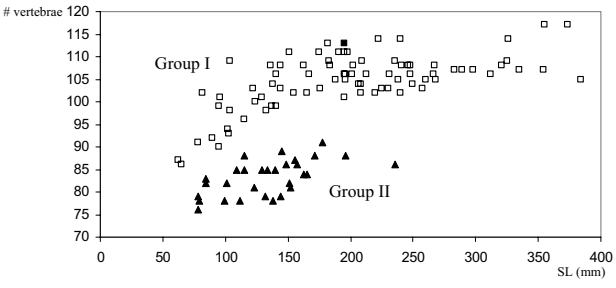

Fig. 4. – Scatterplot of the standard length against total number of vertebrae. ■ holotype of *Channallabes apus*; □ specimens with a high number of vertebrae (Group I); ▲ Specimens with a low number of vertebrae (Group II).

Key to the species of *Channallabes* (updated from DEVAERE et al., 2007)

1a Small supraorbital process on infraorbital IV, not reaching the rostral border of the eye (Fig. 5a); interdigitation between entopterygoid and quadrate 2

b Large, well-pronounced supraorbital process present on infraorbital IV, reaching the rostral border of the eye (Fig. 5b); no contact between entopterygoid and quadrate 3

2a Fenestra present between the cleithrum and scapulocoracoid, large foramina at the bases of the parapophyses of the first post-Weberian vertebrae (2^{nd} to 10^{th}) (Fig. 6a), second dorsal fin pterygiophore with two large lateral processes (Fig. 7a). . .*C. sanghaensis*

b No fenestra present between the cleithrum and scapulo-coracoid, small foramina at the bases of the parapophyses of the first post-Weberian vertebrae (2^{nd} to 10^{th}) (Fig. 6b), second dorsal fin pterygiophore with two small lateral processes (Fig. 7b) . . . . . .*C. apus*

3a Spot present on skull roof between anterior and posterior fontanel, low number of dorsal (98-116) and anal (75-105) fin rays. 4

b No spot present on skull roof, high number of dorsal (118-160) and anal (105-155) fin rays . . . . *C. alvarezi*

4a Serrations only on the posterior edge of the pectoral spine . . . . . *C. ogooensis*

b Serrations only on the anterior edge of the pectoral spine . . . . . *C. teugelsi*

c Serrations on both edges of the pectoral spine . . . . . *C. longicaudatus*

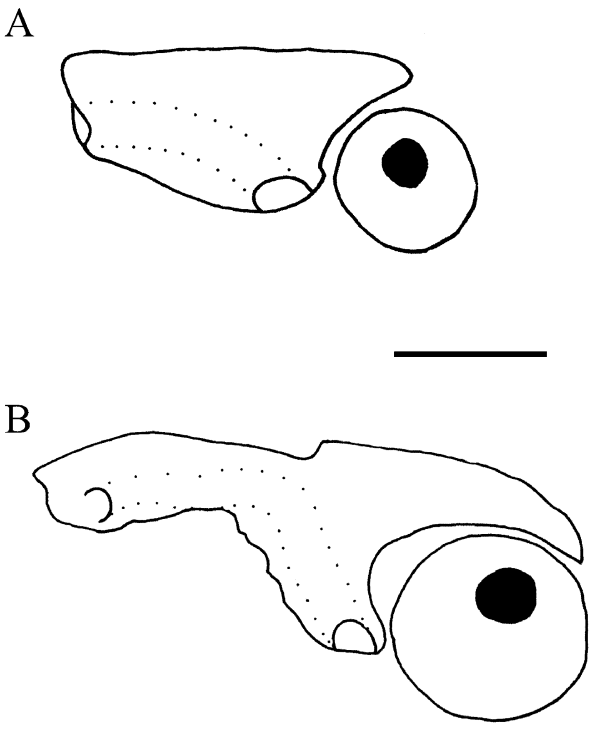

Fig. 5. – Illustration of the extent of the supraorbital process of infraorbital IV. **a** : supraorbital process not reaching the rostral border of the eye; **b** : supraorbital process reaching the rostral border of the eye (scale = 1mm).

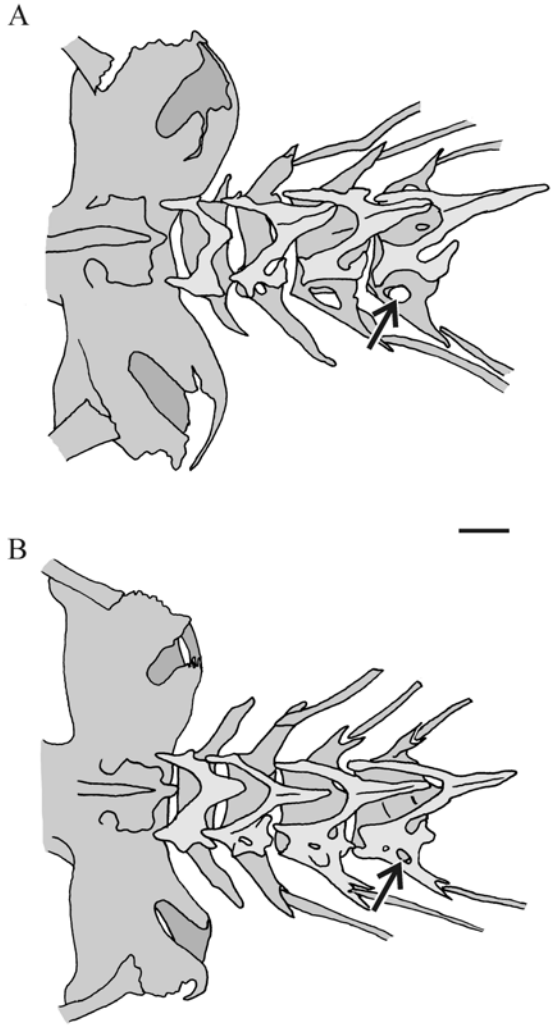

Fig. 6. – Illustration of the size of the foramen at the bases of the parapophyses of the 4th post-Weberian vertebra in **a :** *C. sanghaensis* and **b :** *C. apus* (dorsal view) (scale = 1mm).

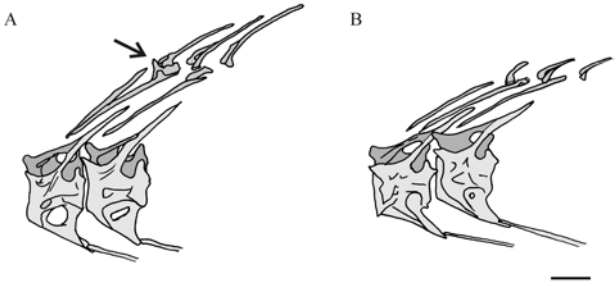

Fig. 7. – Illustration of the size of the lateral processes on the second dorsal fin ray pterygiophore (arrow) in **a :** *C. sanghaensis* and **b :** *C. apus* (dorsal oblique view) (scale = 1mm).

Channallabes sanghaensis n. sp.

(Fig. 8)

Holotype. MRAC A4-31-P-171-183, 114mm SL, Republic of the Congo; River Mbessy, close to the village of Ntchouo, (0° 46'N-14° 19'E), S. Devaere, D. Adriaens and A. Herrel, September 2000.

Paratypes. Total of 12 specimens. S. Devaere, D. Adriaens and A. Herrel, September 2000. 114-221mm SL, MRAC A4-31-P-171-183, in the vicinity of Ntchouo, River Mbessy, Republic of the Congo (0° 46'S-14° 32'E).

Differential diagnosis. *Channallabes sanghaensis* differs from *C. alvarezi, C. longicaudatus, C. teugelsi* and *C. ogooensis* in having a small supraorbital process on infraorbital IV, not reaching the rostral border of the eye and in the presence of an interdigitation zone between the quadrate and the entopterygoid. Further, *C. sanghaensis* can be distinguished from *C. apus* in the presence of a fenestra between the scapulo-coracoid and the cleithrum, the presence of large foramen at the bases of the parapophyses of the first post-Weberian vertebrae (2nd to 10th) (Fig. 6a) and the presence of two large lateral processes on the second dorsal fin ray pterygiophore (Fig. 7a).

Description. Measurements and meristics for holotype and paratypes are given in Table 3. The standard length ranges from 114 to 221mm. *C. sanghaensis* has an elongated body (Fig. 8) (ABD 4.6-6.4% of SL, mean : 5.6%), with a preanal length of 27.5% up to 33.2% of SL (mean : 30.1%). Due to the extreme elongation of the body, there is a very small skull length (10.0-12.1% SL). Skull width is 58.2-71.5% of skull length. The very narrow skull roof, width 16.5-24.4% of maximal skull width, remains clearly visible between the bulging jaw muscles. Although the eyes are small, they remain clearly visible. Tube-like anterior nostrils are present, although small.

The fleshy dorsal, anal and caudal fins form a continuous finfold. The pectorals fins are always present, length 4.0-5.3% of SL and are always preceded by a pectoral spine with a length of 2.3-3.3% of SL. The pectoral spine is serrated on both sides. Pelvic fins present in only one specimen; in the other specimens no evidence of pelvic fins. The total number of vertebrae is 86-89 (mode = 87), ribs 12-14 (mode = 13). Branchiostegal rays 8, dorsal fin rays 121-125, anal fin rays 104-124.

The skull in *C. sanghaensis* shows reduced ossifications and a narrow skull roof. This reduction is shown in the reduced lateral plate of the frontal; from a ventral view this lateral plate is only a little wider than the outlines of the orbitosphenoid (Fig. 9a). A plate-like outgrowth is present on the posttemporo-supracleithral bone. The posterior border of the mesethmoid is indented, which makes that the anterior part of the anterior fontanel lies within the mesethmoid (Fig. 9b). One suprapreopercular bone is present. On the prevomer, one long posterior process is present. The entopterygoid rostro-dorsally encloses the metapterygoid. For the interdigitation with the neurocranium, the hyomandibula has two sets of three processes. Oral teeth are present on dentary, premaxilla and prevomer.

Colour. Alcohol preserved specimens gradually fade from dark brown on the dorsal side to whitish brown on the ventral side. Both sides are separated by a white line, representing the lateral line. The skin on the jaw muscles shows a somewhat paler brownish colour than the surrounding skin of the head. The barbels and nostrils have a similar coloration.

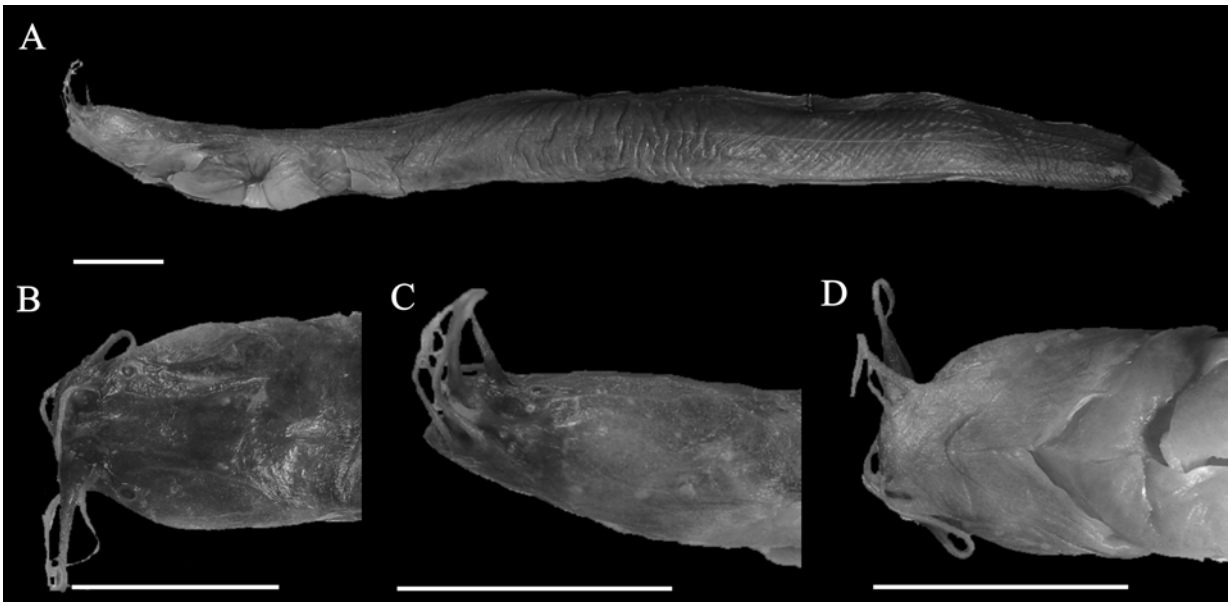

Fig. 8. – Holotype of *Channallabes sanghaensis* (114mm SL), **a** : lateral view, **b :** dorsal view of the head, **c** : lateral view of the head and **d** : ventral view of head. (scale = 10mm); (Photographs : S. Devaere).

TABLE 3

Measurements and meristic data for *C. apus* and *C. sanghaensis*

	C. apus						***C. sanghaensis***					
	holo-type	**n**	**min**	**max**	**mean**	**SD**	**holo-type**	**n**	**min**	**max**	**mean**	**SD**
Total Length (mm)	205.0	191	42.0	416.0			123.0	12	123.0	238.0		
Standard Length (mm)	195.0	191	38.0	398.0			114.0	12	114.0	221.0		
Measurements in % standard length												
preanal length	27.3	191	21.4	35.4	27.4	3.1	28.9	12	27.5	33.2	30.1	1.7
prepectoral length		113	5.3	15.3	10.0	1.8	10.0	12	10.1	11.8	11.1	0.6
prepelvic length		4	22.2	32.9	29.6	5.0		1			29.8	
predorsal length	16.0	191	8.4	26.3	18.6	2.7	23.0	12	19.4	23.0	20.6	1.2
distance between the occipital process and the dorsal fin	7.5	191	4.6	20.0	9.2	1.9	9.5	12	7.8	11.5	8.9	1.1
pectoral fin length		113	0.2	5.3	1.9	1.3	5.3	12	4.0	5.3	4.6	0.4
pectoral spine length		113	0.2	3.6	1.1	0.6	2.9	12	2.3	3.3	2.7	0.3
pelvic fin length		4	2.2	4.4	3.2	1.2		1			3.2	
caudal peduncle depth	1.4	191	1.1	5.4	2.2	0.7	3.3	12	2.6	3.3	3.0	0.2
abdominal body depth	4.4	191	2.7	9.6	5.1	1.1	6.0	12	4.6	6.4	5.6	0.6
inter pectoral distance		113	3.8	10.1	5.9	1.2	5.9	12	6.3	7.7	6.9	0.4
inter pelvic distance		4	0.5	2.1	1.4	0.8						
skull length	9.7	191	6.9	17.8	9.8	1.9	11.4	12	10.0	12.1	11.3	0.6
Measurements in % head length												
preorbital length	75.2	191	10.5	40.5	29.2	4.0	30.3	12	26.1	33.1	31.0	1.8
supraoccipital spine length	19.0	191	6.0	24.8	16.0	3.3	13.5	12	9.7	15.1	12.3	1.6
skull width	50.9	191	41.6	71.5	58.7	5.0	70.2	12	58.2	71.5	64.2	4.1
supraoccipital spine width	17.6	191	12.1	45.0	23.3	6.0	16.5	12	13.5	18.6	16.0	1.4
inter orbital distance	28.6	191	18.6	37.0	30.6	3.0	32.3	12	28.3	37.1	32.3	2.5
anterior nostril interdistance	9.1	191	7.4	19.0	13.0	2.3	15.8	12	10.4	15.9	12.4	1.6
posterior nostril interdistance	21.2	191	14.5	35.2	24.4	3.1	27.4	12	21.9	27.4	24.4	1.4
rostral skull width	30.2	191	24.4	49.8	32.5	3.7	35.1	12	28.3	39.8	34.2	3.4
orbital skull width	38.2	191	28.5	52.9	44.4	3.8	46.6	12	42.5	50.9	46.7	2.6
skull height	14.0	191	29.0	60.3	45.6	6.1	40.6	12	33.3	48.2	39.0	4.5
eye diameter	4.0	191	4.0	11.6	7.2	1.4	5.7	12	4.6	7.7	6.0	1.0
snouth height	14.0	191	6.0	21.5	14.4	2.9	17.4	12	16.1	20.9	18.5	1.5

TABLE 3

Measurements and meristic data for *C. apus* and *C. sanghaensis*

	C. apus						*C. sanghaensis*					
	holo-type	**n**	**min**	**max**	**mean**	**SD**	**holo-type**	**n**	**min**	**max**	**mean**	**SD**
orbital skull height	27.4	191	12.7	41.8	27.7	4.8	29.5	12	23.8	34.4	29.9	3.1
prehyoid length	24.0	191	16.6	41.2	27.2	3.7	28.5	12	25.0	31.9	28.8	1.7
internal mandibular interdistance	20.2	191	13.6	27.8	20.3	2.9	21.0	12	19.7	25.8	22.4	2.2
external mandibular interdistance	27.8	191	20.9	41.1	32.2	4.1	34.6	12	31.1	44.2	34.7	3.3
mouth width	23.3	191	13.9	36.9	25.2	4.0	35.4	12	27.9	38.4	33.7	3.5
skull roof width	17.7	191	12.0	29.0	21.2	3.2	24.4	12	16.5	24.4	20.8	2.1
Meristic counts					**mode**						**mode**	
total number of ribs	17	79	10	17	14		13	11	12	14	13	
total number of vertebrae	113	108	76	117	106		87	11	86	89	87	
number of dorsal fin rays	140	20	86	154			123	5	121	125		
number of anal fin rays	126	20	82	130			115	5	104	124		

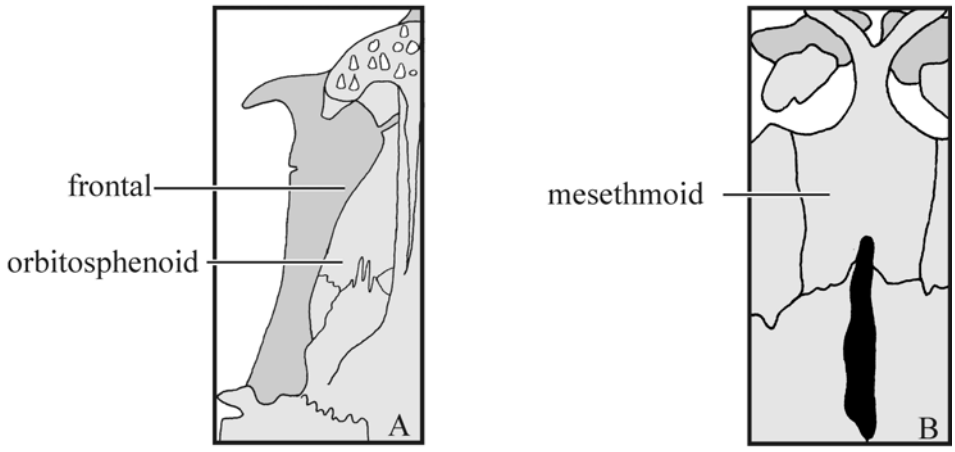

Fig. 9. – Illustration of **a** : the extent of the lateral plate of the frontal in relation to the orbitosphenoid (ventral view of neurocranium); **b** : the dorsal view of the indented, posterior margin of the mesethmoid.

Distribution. Currently known from the Congo River system, in the vicinity of Ntchouo (Fig. 10). All known sampling sites were characterised by shallow, muddy, still water.

Etymology. From the Sangha freshwater ecoregion (THIEME et al., 2004) where the species was found.

DISCUSSION

Based on several genera diagnosis (DEVAERE et al., 2001; 2004; 2005a; b; 2007) and on this species diagnosis, the new species should be included in the genus *Channallabes*. This genus is characterized by reduced infraorbital and suprapreopercular bones, with small plate-like extensions on the posterior most bone in both series; small lateral plates on the frontals and the first dorsal fin pterygiophore is situated posterior to the sixth post-Weberian vertebrae.

Besides this new species, ten other elongated clariids are known, designated to five genera. The new species can be clearly discerned from each one of the other anguilliform species. *Platyallabes tihoni* (Poll, 1944) has a very small distance between the origin of the dorsal fin and the supraoccipital process (2.2-6.6% of SL) (DEVAERE et al., 2005a). *Platyclarias machadoi* Poll, 1977 has an extremely flattened skull (SkH 22.9-37.1% of Skl) (DEVAERE et al., 2006). *Dolichallabes microphthalmus* Poll, 1942 is characterized by the presence of only one fontanel on the skull roof (DEVAERE et al., 2004). *Gymnallabes nops* Roberts & Stewart, 1976 shows a large reduction in the infraorbital series in size and number (two instead of five) (DEVAERE et al., 2005b). *Gymnallabes typus* Günther, 1867 is characterized by well developed skin folds, bordering the side wall of the mouth (CABUY et al., 1999 : Fig.1).

The remaining five anguilliform species are all in the genus *Channallabes*. The differences present between "the Congo basin" species (*C. apus, C. sanghaensis*) and the "Lower Guinea" species (*C. alvarezi, C. longicaudatus, C. teugelsi, C.ogooensis*) are reflected in both genetic (JANSEN et al., 2006) and morphological (DEVAERE et al.,

in press) differences. Finally, the differences between *C. sanghaensis* and *C. apus* are the presence of two clear, lateral processes on the second dorsal fin pterygiophore and the presence of a large foramen at the bases of the parapophyses of the first post-Weberian vertebrae. Although, this foramen is larger than in most other anguilliform clariids, it is not as large as in *Platyallabes tihoni*, where it is one of the diagnostic features for the genus and species (DEVAERE et al., 2005a).

Although, Figs 3 & 4 show two clear groups, which were tested significantly different, no new species is currently recognized. There is no evidence for size-related changes of the meristics in the anguilliform clariids species studied here. Moreover, in fishes in general, several authors, as for instance LANDRUM & DARK (1968), have reported on the independence of the total vertebrae number from the length of the fish within the same species. Thus, the apparent different growth series in Fig. 4 cannot be considered as such. Besides the difference in total vertebra number, no other differences (morphometric, osteological, …) were found between these groups, indicating the large similarity of these two groups.

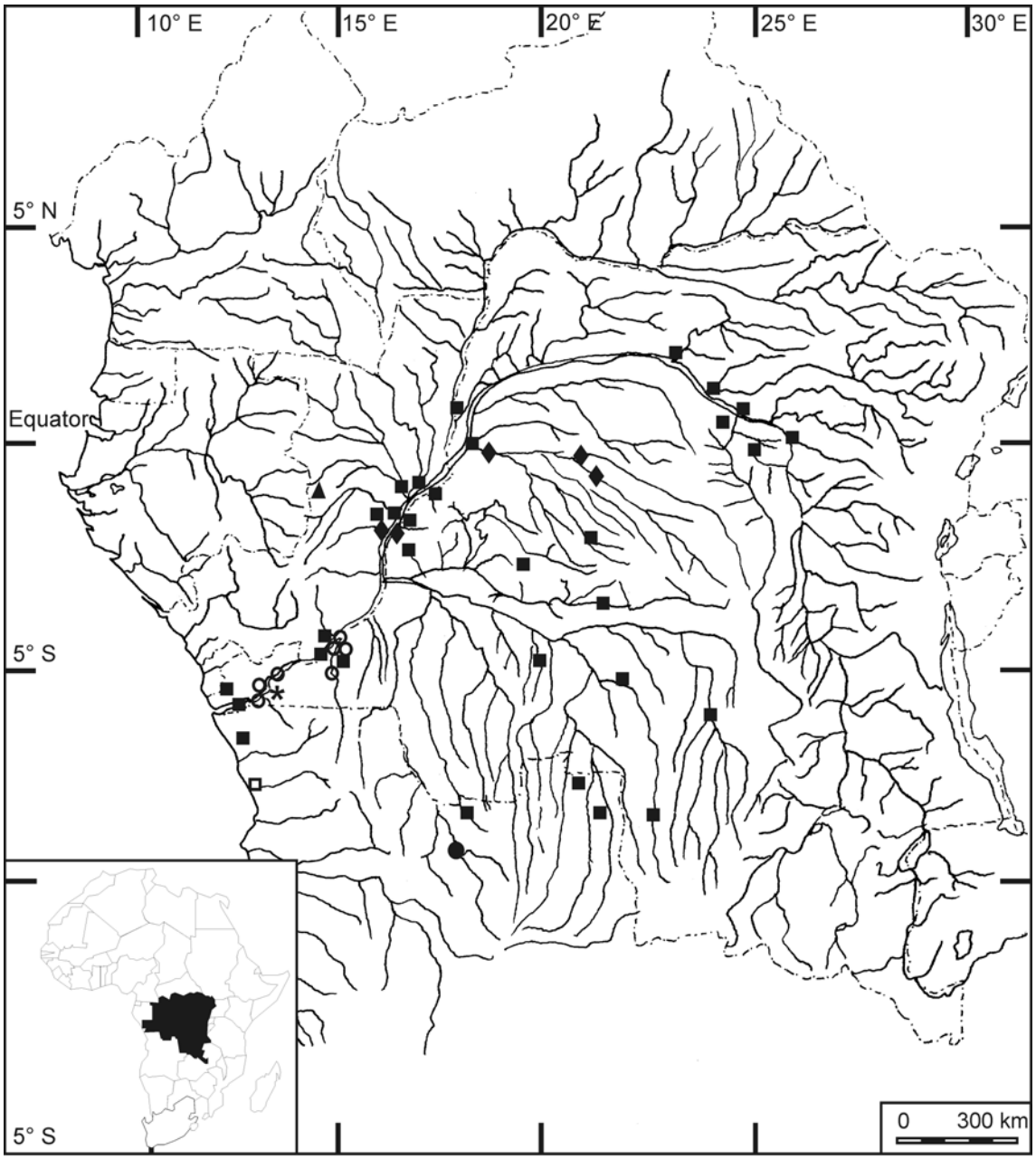

Fig. 10. – Geographic distribution of *C. apus, C. sanghaensis, P. machadoi, P. tihoni, D. microphthalmus* and *G. nops* based on the localities of the examined specimens. □ Holotype of *Channallabes apus*; ■ specimens of *Channallabes apus*, ▲ specimens of *Channallabes sanghaensis;* ◆ specimens of *Dolichallabes microphthalmus*; ● specimens of *Platyclarias machadoi*; o specimens of *Platyallabes tihoni;* * holotype of *Gymnallabes nops.*

Fig. 1 also shows the great similarity between *Platyallabes tihoni* and *Gymnallabes nops*. This large similarity, as well as morphological correspondences, was already shown by DEVAERE et al. (2005b). This similarity is based on both meristic and osteological characteristics. The additional metric data, presented in this paper, could be an extra argument for a systematic shift of *Gymnallabes nops* to the genus *Platyallabes*; however, further data is required.

The geographic distribution shows that *C. apus* and *C. sanghaensis* occur in two different parts of the Congo River system (Fig. 10). While *C. sanghaensis* only occurs in the border region with Gabon, close to the watershed of the Southern West Coastal Equatorial (Ogowe and Ivindo River), *C. apus* is found in the largest part of the Congo basin, from the mouth up to the Upper Congo Rapids. The group with the low number of vertebrae (Group II in Fig. 4) is only found in the Kasai, Upper Congo Rapids, Sudanic Congo, Central Congo and Lower Congo freshwater Ecoregions.

ADDITIONAL MATERIAL EXAMINED

Museum abbreviations are listed in LEVITON et al. (1985).

Channallabes apus. Angola. Ambriz, BMNH 1873.7.28.16 (holotype); Dem. Rep. Congo. Bokalakala, MRAC 175247-270 (n = 10); Kinshasa, MRAC 97-056-P-0001-0003 (n = 2); Bumba, MRAC 88-25-P-2192-227 (n = 36); Boma, MRAC 939; Riv. Lula, Bushimaie, MRAC 153505; Kelé, MRAC 1491; Stanleyville, MRAC 30893-30900 (n = 8), MRAC 88-01-P-1976-1992 (n = 17); Riv. Ruki, Eala, MRAC 14747-49 (n = 3); Lake Tumba swamp area, MRAC 46299; Katanga, MRAC 39480; Riv Botota, keseki, MRAC 67763-77 (n = 15); Mwilambongo, MRAC 72886-887 (n = 2); Dekese, Riv. Lofu, Anga, MRAC 153352; Yangambi, MRAC 68700; Riv. Oubangui, Imfondo, MNHN 1922-0029; Loango, MNHN 1924-0079, MNHN 1924-0080; Sangha, MNHN 1925-0137; Mogende, MNHN 1926-0155-59 (n = 5); Riv Congo, MNHN, 1937-0124-25; Stanleypool, Bamu, MNHN 1958-0111; Boloko, Riv. Likouala, MNHN 1962-0401 (n = 7); Mossaka, Riv. Likouala, MNHN 1963-0402 (n = 2); Riv. Loadjili, Songolo, MNHN 1967-0143 (n = 6); Mangala, BMNH 1896.3.9.17; Riv. Lebuzi, Kaka Muno, BMNH 1912.4.1411-12 (n = 2); Lower Congo, BMNH 1887.1.13.8-9 (n = 2); Stanley Falls, BMNH 1889.11.20.5; New Antwerp, Upper Congo, BMNH 1899.2.20.16; Siala-Ntoto Swamps, BMNH 99.11.27.92; Bangyville, Ubangi, BMNH 1907.12.26.34; Kashi, Lulua, MHNG 1248.3; Banana, NMW 47240-42; Mollunda, NMW 47245 (n = 4), NMW 47246. Congo. Yangala Youbi, MNHN 1967-0146; Djembo, Kouilou, MNHN 1967-0147; Cayo, MNHN 1989-0527; Riv. Nanga, between Boukou-Zassi and Kouilou swamp area, MRAC 90-57-P2315; Sintou, Riv. Kibombo, Kouilou, MNHN 1967-0144; Riv. Loadjili, Songolo, Pointe Noire, MNHN 1967-0145 (n = 6); Riv. Youbi, Noumbi. Angola. Caungula, Mabete, Riv. Uamba, MRAC 162088; Riv. Camuconda, Tchimenji, MRAC 162089, MRAC 162090-094 (n = 5), MRAC 162095-100 (n = 6); Riv Ganga-Ludchimo, MRAC162083-086 (n = 4).

Platyallabes tihoni. Dem. Rep. Congo. Kingabwa, Stanley pool, MRAC 13307 (holotype); Kinsuka, MRAC 73-68-P-143, MRAC 138698-699 (n = 2), 125345-349 (n = 4), MRAC 73-22-P-3127 (n = 3); Bulu, Luozi, BMNH 1976.5.21.30-39 (n = 9), MCZ 50239 (n = 13); Inga, MCZ 88947, MCZ 50537 (n = 15); Tadi, Kibunzi, MCZ 50297 (n = 5).

Platyclarias machadoi. Angola. Cuango, Cafunfo, Borio River, MRAC 78-6-P-1345, (holotype), MRAC 78-6-P-1348-364, 78-6-P-1346, 78-6-P-1366-1367 (76-180mm SL) (21 paratypes).

Dolichallabes microphthalmus. Dem. Rep. Congo. Kunungu, MRAC 44655, adult male, (holotype), MRAC 44656-659 (n = 3) and 62407, 188mm SL (paratypes), MRAC 57662, MRAC 18850; Boende swamps, MRAC 101843, MRAC 176123-124 (n = 1), 68mm SL; Bokuma, MRAC 79093, MRAC 93774, 66mm SL; Bokuma – Tchuapa, MRAC 79258-260 (n = 3); Ndwa (Boloko), MRAC 78808-810 (n = 3); Inonge, MRAC 96672; Maylimbe, Tshela, MRAC 66721.

Gymnallabes nops. Dem. Rep. Congo. Tadi, Kibunzi, Congo River, MCZ 50298, 57mm SL (holotype).

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Effect of Cypermethrin Exposed Hosts on Egg-Adult Development Time, Number of Offspring, Sex Ratio, Longevity, and Size of *Apanteles galleriae* Wilkinson (Hymenoptera : Braconidae)

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ABSTRACT. The effects of sub-lethal doses of cypermethrin onto the larval host *Achoria grisella* Fabr. (Lepidoptera : Pyralidae) were evaluated on egg-adult development time, number of offspring produced, sex ratio, longevity, and size of the larval endoparasitoid *Apanteles galleriae* Wilkinson (Hymenoptera : Braconidae). Overall time to adult eclosion increased by more than 50% when *Ap. galleriae* was reared on cypermethrin-treated host larvae and the development time of the wasp increased in a dose-dependent manner. Adult longevity and the number of surviving offspring produced decreased with increasing insecticide dose. The number of surviving offspring decreased more than 50% at the lowest dose of the insecticide. Neither sex ratio nor adult body sizes were altered by cypermethrin exposure when compared to untreated wasps. This work suggests that sublethal doses of the insecticide could limit the development, survival, and growth of parasitoid wasps due to possible metabolic, hormonal, and nutritional deficiencies. The potential adverse effects that cypermethrin has on the natural enemy of the pest can impact on the success of IPM programs.

KEY WORDS : *Apanteles galleriae*, cypermethrin, biological control, risk assessment, non-target insect

INTRODUCTION

Insecticides frequently disrupt the balance between a host and its natural enemy (Van Driesche & Bellows, 1996; Xu et al., 2001). Several studies have shown that insecticides applied to insect pests cause various sublethal effects on parasitoids, such as changes in development and emergence rates, and sex ratio (Krespi et al., 1991; Willrich & Boethel, 2001; Saber et al., 2005) either by direct chemical contact or by ingestion of treated prey (Wells et al., 2001). The use of insecticides may have an adverse effect on the life cycle of beneficial nontarget insects, and this may subsequently result in an outbreak of pest numbers (Tomberlin et al., 2002).

Apanteles galleriae Wilkinson (Hymenoptera : Braconidae) is a koinobiont, solitary, larval endoparasitoid of several lepidopterans including the pyralid wax moths, *Galleria mellonella* L., *Achoria grisella* Fabr., *Ac. innotata* Walker, and *Vitula edmandsae* (Packard) (Watanabe, 1987; Shimamori, 1987; Whitfield et al., 2001). Caterpillars of these host species are pests in beehives because they feed on pollen and generally destroy the combs. *Ap. galleriae* adults feed on honey, fruit nectar, and host larvae in nature. Therefore, adult wasps are likely to be exposed to residues of insecticides used against these pests and that accumulate on honeycomb, fruit trees and host larvae.

Pyrethroids are among the most commonly used insecticides worldwide, accounting for more than 30% of global use (Shukla et al., 2002). Cypermethrin (CYP) [α-cyano-3-phenoxybenzyl (1 RS)-cis-, trans-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate] is a synthetic pyrethroid (Shukla et al., 2002) which is used widely in the control of various agricultural pests belonging to the orders Lepidoptera, Coleoptera, Diptera, and Hemiptera (Cox, 1996; Liu et al., 1998; Suh et al., 2000). Like all pyrethroids, cypermethrin kills insects by disrupting normal functioning of the nervous system (Vijverberg & Van Den Bercken, 1990). Even sublethal doses of insecticides can have profound effects on parasitoids (Smilanick et al., 1996), thereby greatly reducing populations of indigenous natural enemies, as well as hindering biological control efforts. Evaluation of the impact of insecticides on non-target insects, like parasitoids, is of great importance for success in biological control applications, and in any integrated pest management program utilizing chemical control. Therefore, this research was undertaken to determine if feeding by a host on a sublethal cypermethrin-treated diet adversely affected emergence time, number, sex ratio, longevity, and size of *Ap. galleriae* offspring.

MATERIALS AND METHODS

Insects. Laboratory colonies of *Ac. grisella* and *Ap. galleriae* were established from adults, which were collected from several beehives located in the vicinity of Ardeşen, Turkey. *Ac. grisella* was reared on honeycomb at 25 ± 1°C, 60 ± 5% RH under a photoperiod of 12 : 12h (L :D). Adults of *Ap. galleriae* were fed a 30% (w/v) honey solution and kept at the same rearing conditions with the host species. For details of the biology and rearing of the parasitoid and the host, see Uçkan & Gülel (2000) and Uçkan & Ergin (2003).

Insecticide Application. Cypermethrin (Imperator, 250g/liter EC, Zeneca Ltd., İzmir, Turkey) was used in all bioassays as water source and prepared in distilled water as parts per million of active ingredient. To ensure survival of host larvae, preliminary tests were carried out on small groups so that an appropriate range of doses (below 50% mortality range) for cypermethrin could be selected. Various doses (10, 20, 50, and 100ppm) of cypermethrin-treated distilled water were incorporated to the synthetic diet of host larvae. Host diet including crumbled honeycomb, bran, honey, glycerin, and distilled water was prepared by the method of BRONSKILL (1961) and SAK et al. (2006).

Bioassays. An individual mating pair of *Ac. grisella* (1- to 2-day-old at 25ºC) was placed in 250ml jars containing 1g honeycomb to provide a mating and oviposition substrate. The adults were removed from the jars on the fifth day. Early instars of one female equivalent host larvae, which is produced by a mated host female in five days (25 to 57 larvae), were exposed to 5g of host diet treated with the selected doses of the cypermethrin in each jar. Host larvae were exposed to parasitization by placing an individual mating pair of adult parasitoids (1- to 2-day-old at 25ºC) in jars two days later. Parasitoid adults were fed a 50% (w/v) honey solution soaked in cotton balls and removed from the jars after five days. The jars were maintained in another rearing room under the same conditions mentioned above for the stock cultures. Control groups were also prepared with the same methodology, but untreated synthetic diet including only distilled water instead of cypermethrin solution was used.

All jars were observed daily for date of adult eclosion. The time required for completion of parasitoid development from egg to adult eclosion was recorded, as was the total number of progeny and the sex of each eclosing adult. Longevity of newly emerged adult female and male wasps was assessed by placing an individual mating pair (n = 5 pairs) in 80ml jars each containing a piece of cotton ball soaked with a 50% (w/v) honey solution. Jars were held under the environmental conditions mentioned above for the stock cultures. Food supplement was replenished at 2d intervals until all parasitoids died. Adult body sizes (length) of cypermethrin-treated wasps and controls were determined by selecting random samples (5 females, 5 males) of wasps for each experimental and control group. However, adult size could only be obtained from a total of 11 females at 100ppm. Adults were measured from the head to the tip of the abdomen using an Olympus S2X 12 stereodissecting microscope with a calibrated eyepiece micrometer. All experiments were repeated three times.

Statistics. Variations due to cypermethrin doses in egg-adult development time, number of viable offspring developing to adulthood, sex ratio, longevity and size were inferred using one-way analysis of variance (ANOVA). Subsequently, means were separated using Tukey's Honestly Significant Difference (HSD) test (SPSS, 1999). Data for adult longevity were also subjected to two-way ANOVA (SPSS 1999) to determine the main effects of cypermethrin dose, sex, and their interaction on adult longevity. An arcsine square-root transformation was performed on percentage values of fecundity before analysis. Results were considered statistically significant when $P<0.05$.

RESULTS

Egg-adult development time of *Ap. galleriae* reared on *Ac. grisella* larvae exposed to different doses of cypermethrin was significantly longer than those parasitoids that developed on untreated hosts (Table 1; $F = 24.594$; df = 4, 10; $P = 0.000$). Wasp development from egg to adult emergence at 25ºC normally requires 27-35d. However, parasitoids reared on hosts exposed to any dose of insecticide tested required 12 to 25d longerthan controls to complete devlopment (Table 1).

TABLE 1

Effect of different sub-lethal doses of cypermethrin-treated *Ac. grisella* larvae on the egg-adult development time of *Ap. galleriae*.

CYP (ppm)	Egg-adult development time (day)	
	Range	($\bar{x}$ ± SE)[a]
C	27 – 35	31.3 ± 2.3a
10	44 – 50	47.0 ± 1.7b
20	49 – 51	50.0 ± 0.6b
50	50 – 60	55.0 ± 2.9b
100	53 – 59	56.0 ± 1.7b

a. Numbers in column followed by the same letter are not significantly different (P>0.05, Tukey's HSD test). CYP : Cypermethrin doses, C : Control group.

Similarly, cypermethrin treatment lowered the number of offspring produced by *Ap. galleriae*. For example, the number of progeny surviving to adulthood by a single parasitoid female throughout its adult life was on average 108 ± 13.2 when the host was fed on an insecticide-free diet. However, when host larvae were treated with cypermethrin, the number of offspring developing to adulthood was significantly lower in all experimental groups in contrast to the controls ($F = 30.615$; df = 4, 10; $P = 0.000$). This decline in total number of offspring was dose-dependent between 10-100ppm cypermethrin (Table 2). Though the number of offspring developing to adulthood was reduced by pyrethroid-treatment, the sex ratio of emerging adults was not disturbed : The sex ratio of adults was always male biased in treated and untreated wasps ($F = 1.505$; df = 4, 10; $P = 0.273$) (Table 2).

The effect of cypermethrin on adult longevity was dose and sex dependent, and the relationship between insecticide dose and adult longevity was not significantly influenced by gender (Table 3). Mean longevity of cypermethrin-treated females and males decreased at all doses of insecticide >10ppm tested with respect to controls ($F = 23.648$; df = 4; $P = 0.000$) (Table 4).

Adult body sizes of male and female parasitoids appeared to decrease with increasing dose of cypermethrin (Table 5), but these differences were not found to be significant for either sex ($F = 2.498$; df = 4, 66; $P = 0.051$ for females and $F = 1.280$; df = 4, 70; $P = 0.286$ for males).

TABLE 2

Effect of different sub-lethal doses of cypermethrin-treated *Ac. grisella* larvae on the number of surviving offspring developing to adulthood and female sex ratio of *Ap. galleriae*.

CYP (ppm)	Number of offspring and sex ratio					
	Female		Male		Total number ($\bar{x}$ ± SEM)[a]	Female sex ratio (%)[a]
	Range	($\bar{x}$ ± SEM)	Range	($\bar{x}$ ± SEM)		
C	19 – 73	40.3 ± 16.6	59 – 84	67.7 ± 8.2	108.0 ± 13.2a	37.6a
10	12 – 13	12.7 ± 0.3	28 – 47	35.3 ± 5.9	48.0 ± 6.0b	26.4a
20	7 – 13	10.3 ± 1.8	16 – 23	19.3 ± 2.0	29.7 ± 3.7bc	34.8a
50	10 – 15	11.7 ± 1.7	12 – 14	13.3 ± 0.7	25.0 ± 2.1bc	46.7a
100	3 – 4	3.7 ± 0.3	4 – 10	7.7 ± 1.9	11.3 ± 2.2c	32.4a

a. Numbers in columns followed by the same letter are not significantly different (P>0.05, Tukey's HSD test). CYP : Cypermethrin doses, C : Control group.

TABLE 3

ANOVA of the effects of cypermethrin dose, sex, and their interaction on adult longevity of *A. galleriae* reared on *Ac. grisella* larvae treated with cypermethrin ($r^2 = 0.451$).

Source	df	MS	F	P
Dose	4	1,234.128	23.648	0.000
Sex	1	395.438	7.577	0.007
Dose*Sex	4	21.143	0.405	0.805
Error	126	52.188		

TABLE 4

Effect of different sub-lethal doses of cypermethrin-treated *Ac. grisella* larvae on the adult longevity (day) of *Ap. galleriae*.

CYP (ppm)	Female		Male		Both sexes
	Range	($\bar{x}$ ± SEM)	Range	($\bar{x}$ ± SEM)	($\bar{x}$ ± SEM)[a]
C	27 – 51	39.1 ± 1.8	31 – 57	40.5 ± 2.0	39.8 ± 1.4ab
10	30 – 50	39.3 ± 1.6	36 – 57	43.9 ± 1.5	41.6 ± 1.2a
20	16 – 44	32.8 ± 2.5	17 – 49	37.6 ± 2.6	35.2 ± 1.8b
50	14 – 37	26.9 ± 1.6	17 – 38	28.6 ± 1.7	27.7 ± 1.1c
100	18 – 29	23.3 ± 1.6	22 – 31	28.3 ± 1.1	25.6 ± 1.1c

a. Numbers in column followed by the same letter are not significantly different (P>0.05, Tukey's HSD test). CYP : Cypermethrin doses, C : Control group.

TABLE 5

Effect of different sub-lethal doses of cypermethrin-treated *Ac. grisella* larvae on the adult size (mm) of *Ap. galleriae*.

CYP (ppm)	Female			Male		
	n	Range	($\bar{x}$ ± SEM)[a]	n	Range	($\bar{x}$ ± SEM)[a]
C	15	2.1 – 2.3	2.8 ± 0.1a	15	2.0 – 2.8	2.4 ± 0.1a
10	15	2.2 – 3.1	2.6 ± 0.1a	15	1.8 – 2.6	2.3 ± 0.1a
20	15	2.2 – 2.8	2.6 ± 0.1a	15	1.8 – 2.8	2.3 ± 0.1a
50	15	2.2 – 2.9	2.5 ± 0.1a	15	1.8 – 2.7	2.2 ± 0.0a
100	11	2.0 – 2.9	2.5 ± 0.1a	15	1.8 – 2.4	2.1 ± 0.0a

a. Numbers in columns followed by the same letter are not significantly different (P>0.05, Tukey's HSD test). CYP : Cypermethrin doses, C : Control group.

DISCUSSION

Our results indicated that the overall time to adult eclosion increased by more than 50% when *Ap. galleriae* was reared on cypermethrin-treated host larvae. This result is in agreement with other reports on the effects of some insecticides on the larval and pupal developmental time of lepidopterous species (GAABOUB et al., 1985; BIDDINGER & HULL, 1999). However, we could find no report on insecticide-dependent delay in adult parasitoid eclosion although a number of studies noted reduced emergence rates as a result of insecticide treatment for various parasitoid species (SCHNEIDER et al., 2003; 2004; SABER et al., 2005). There was a considerable decline in

the number of wasps emerging from the cypermethrin-treated host larvae. Even at the lowest dose (10ppm), the number of adult wasps decreased more than 50% and declined more so at higher doses. The fact that some larvae that were parasitized by *Ap. galleriae* died from cypermethrin should also be taken into consideration for this drastic decrease. However, none of the insecticide doses tested in our study affected the female sex ratio in wasp progeny. The insignificant impact of insecticides on the progeny sex ratio of parasitoids was also previously reported by SUH et al. (2000) and SABER et al. (2005). These results may suggest that insecticides are nonselective toward developing larvae, females and males of parasitoid wasps. Examining the effect of cypermethrin on longevity of adults revealed that insecticide treatment significantly affected longevity of *Ap. galleriae* and the response was both dose- and sex-dependent. Comparisons of the longevity of wasps at four cypermethrin treatments showed that the disruptive effect of cypermethrin on longevity was higher at 50 and 100ppm with respect to 20ppm. Cypermethrin was both highly toxic to female and male wasps in terms of longevity. However, longevity of females exposed to cypermethrin tended to decrease more drastically relative to males with increasing dose. The difference is thought to be related partly to differences in size and physiology between the sexes.

General stress responses in arthropods are known to be energetically demanding events (KORSLOOT et al., 2004). The organisms may consume more energy to repair mechanisms and pathological effects may deplete energy reserves. Therefore, the decrease in energy storages of in the host, and subsequently parasitoid larvae resulting from cypermethrin-induced stress may prolong the growth and development of parasitoid progeny. The emergence rate of parasitoids may be reduced due to organ malformations in the larvae or other perturbations. SCHNEIDER et al., (2004) reported a decrease in emergence from parasitized hosts after exposure to spinosad in the endoparasitoid, *Hyposoter didymator* (Thunberg) (Hymenoptera : Ichneumonidae), due to the incapacity for the larvae to produce silk for spinning his cocoon. The neurotoxic effects of cypermethrin may suppress juvenile hormone levels in the host (OPPENOORTH, 1985). Parasitoid larvae synchronizing development with the host by making use of host hormones may have been affected by the changes in the hormonal milieu of the host and display a delay in larval developmental time. The delay in immature development of this parasitoid may also be attributed to the cypermethrin-induced decline in diet quality and to the potency of cypermethrin as an antifeedant (TOMLIN, 2000), resulting in an interference of sufficient food supply from the host.

Because insect behaviour is affected by both the nervous system and hormones, insecticides that attack the nervous system and disrupt the hormonal balance and/or metabolic process in insects can affect behaviour and physiology at levels that do not lead to direct mortality (HAYNES, 1988). Therefore, insecticides decrease the production of offspring because of behavioural modifications in mate location, courtship, and oviposition, or due to physiological effects on egg fertilization, oogenesis, ovulation, spermatogenesis, and sperm motility (HAYNES, 1988). Studies with parasitoids have also shown deleterious effects on reproduction with sublethal doses of insecticides (SUH et al., 2000; TAKADA et al., 2001; XU et al., 2001). It has been reported that malathion applied orally to *Pimpla turionellae* L. (Hymenoptera : Ichneumonidae) females have decreased the hatching rate of wasp eggs (ÖZKAN & EMRE, 1997). As a result, sublethal doses of insecticides can affect the population density and may further inhibit continuity of the generation of wasps in nature by preventing eggs from hatching. This, in turn, may disrupt the effectiveness of parasitoid species in integrated pest management programs.

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Current status of the Balkan chamois (*Rupicapra rupicapra balcanica*) in Greece : Implications for conservation

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ABSTRACT. In this study, we carried out direct and indirect surveys in 30 Greek areas to clarify the current status of the Balkan chamois (*Rupicapra rupicapra balcanica)*. It is an important flagship species for conservation in Greece, protected by both National and European legislation. New chamois populations are recorded for the first time in 3 areas, direct sightings of chamois are found in 11 areas, and presence is reported by locals in a further 4 areas. The overall potential distribution area of the species is 1.663km^2. The chamois is considered extinct in 6 areas and its presence is doubtful in the remaining 6 areas. Chamois have a fragmented dispersal pattern in Greece and three blocks of populations are distinguished : Pindus, Sterea Ellada and Rhodopi populations. Population sizes do not usually exceed 30 individuals in each area, and the maximum population size recorded is 120-130 individuals (Mt. Timfi). Our preliminary estimate of the total Greek population size is between 477 and 750, which is slightly higher than previous estimates. Although most sites are within established reserves, protected by the Natura 2000 network, there is an urgent need for further conservation measures. Poaching is considered to be the major threat to this species, therefore effective protection is urgently needed, through the enhancement of guarding system against poaching, the control of roads usage within its core range, and the creation of protected natural corridors between chamois populations.

KEY WORDS : Chamois, conservation, distribution, habitat, management, poaching, flagship species.

INTRODUCTION

Choosing threatened and charismatic species to raise public interest in nature protection, and thus facilitating conservation decision and application, is a common practice in conservation policy (SIMBERLOFF, 1998). Besides, formal appraisal of the status of species of primary conservation interest (Annex II of Habitat Directive) (COUNCIL OF EUROPE, 1992) is a requirement across European countries. In this context the Balkan chamois (*Rupicapra rupicapra balcanica)*, a charismatic ungulate with an endangered status in Greece, could be used as an excellent flagship species for the conservation of mountainous ecosystems in this country. The chamois occurs in the Balkan peninsula, on sub-alpine meadows, in proximity with cliffs and rocky formations during summer, whereas it moves at lower altitudes in forested zones in winter. It is listed in the Lower Risk category of the IUCN Red List of Threatened Animals with a population size exceeding 17.000 individuals, and a stable or decreasing population trend (SHACKLETON, 1997). The status of the species is safe in the countries of former Yugoslavia (Bosnia-Herzegovina, Croatia, F.Y.R.O.M., Slovenia, Federal Republic of Yugoslavia) (>11.000 individuals), vulnerable in Albania (1.050 individuals), rare in Bulgaria (1.600 individuals) and endangered in Greece (400-500 individuals) (ADAMAKOPOULOS et al*.,* 1997; GIACOMETTI et al., 1997; GJIKNURI, 1997; KRYSTUFEK et al*.,* 1997; SPIRIDONOV & GENOV, 1997).

In Greece, the Balkan chamois is protected by both European and National legislation : it is listed in the Annexes II and IV of the Habitat Directive (COUNCIL OF EUROPE, 1992), classified as a rare species in the Red Data Book of Threatened Vertebrates of Greece (KARANDINOS & PARASCHI, 1992) and its shooting has been prohibited since 1969. Despite the endangered status of the species and its declining population trend (ADAMAKOPOULOS et al., 1997), data concerning its distribution pattern and population size in Greece are poor and based mainly on short surveys carried out in the 1980's (HATZIRVASSANIS, 1991; PAPAIOANNOU, 1991).

In this study (1994-2003), we surveyed all the documented sites known to be occupied by chamois as well as additional areas of high altitude that include steep rock formations and could therefore fulfil the main habitat requirements of the species (ELSNER-SCHACK, 1985; ADAMAKOPOULOS-MATSOUKAS, 1991; MICHALLET et al., 1999; PAPAIOANNOU, 2003). Our main objectives were to investigate the presence of the species in these 30 areas and to produce the first distribution map of the species in Greece. Moreover, we briefly described its habitat, estimated population size in each area and evaluated the current threats on the species, thus providing baseline data for future management purposes.

MATERIALS AND METHODS

We visited thirty areas during 1994-2003 (Fig. 1) scanning them with the help of Nikon and Minolta binoculars (10 x 50 & 8-20 x 50) and a Swarowski telescope (20 x 60). Foot surveys were conducted, which were inevitably limited by accessibility (trails and footpaths) and access to vantage-points. The geographical points where we recorded the animals, their droppings or tracks, were plotted on 1 :50.000 maps (supplied by the Geographic Service of the Greek Army). The direct surveys entailed 2.928

man-hours. Various factors such as the suitability of the habitat for chamois, the difficulty of conducting transects, and the existence of historical data regarding chamois presence, determined the time spent in each area. Species presence was considered verified when we recorded chamois sightings, tracks or droppings. In three cases (pops.1, 4 and 28) more than two groups of researchers conducted simultaneous population counts in order to estimate the population density. Seven groups of two researchers simultaneously scanned Mt. Timfi (pop. 4) for three days during the autumn of 2002 (PAPAIOANNOU, 2003) and two groups of two researchers conducted a population count in Mt. Grammos (pop. 1) and Fracto forest (pop. 28) for 10 and 8 days respectively in autumn 1998.

Since we recognised that direct surveys were inevitably limited, we also carried out 297 interviews with permanent residents of the areas visited (mainly shepherds, hunters and loggers) (Table 1). Interviews were targeted to determine current chamois presence, the estimated local population size, its current and previous distribution, and its survival threats on a local scale. Reported sightings by locals were plotted on maps of scale 1 :50.000.

All the mapped points that indicated current chamois presence were enclosed by a boundary line giving the potential distribution of the species in each area (Fig. 1). Regarding those populations which are extinct, the boundary line indicates the previous chamois distribution as reported by local inhabitants.

Concerning all the cases of verified presence, the minimum population size refers to the individual count during our direct surveys, whereas the estimated population size refers to reports by local inhabitants or bibliographic data. In cases of unverified presence, the population size was estimated using local inhabitant's reports or bibliographic sources (HATZIRVASSANIS, 1991; PAPAIOANNOU, 1991; SFOUGARIS et al., 1999). When local people provided contrasting information, the presence of chamois was considered as doubtful (minimum population = size 0).

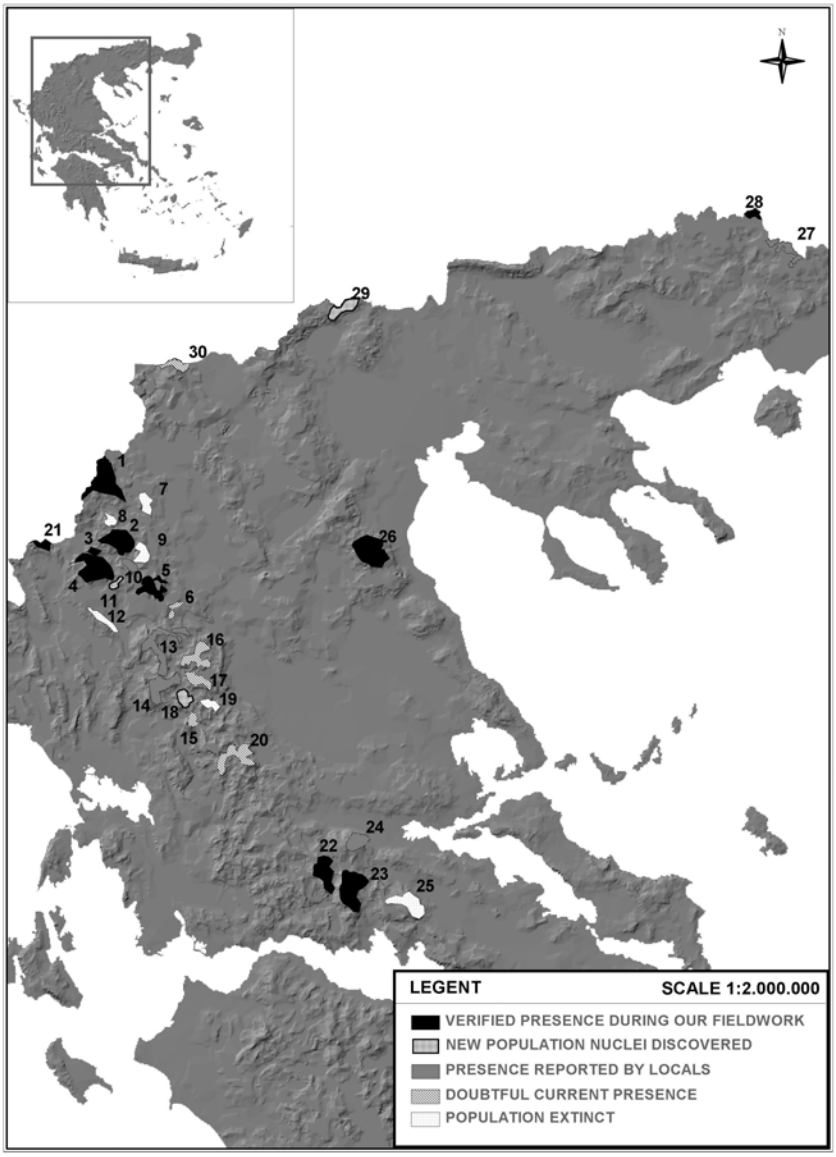

Fig. 1. – Distribution pattern of chamois populations throughout Greece.

TABLE 1

Sampling effort during direct (man-hours) and indirect surveys (number of local people interviewed), and year of sampling in the 30 mountains visited.

Site	Mountain	Direct surveys (hours)	No. of locals interviewed	Year of sampling
1.	Grammos	440	23	1994-95, 1997-2000
2.	Smolikas	120	16	1994-95, 1999- 2000
3.	Trapezitsa	48	7	1997-98, 2002
4.	Timfi	1280	43	1994-98, 2001-02
5.	Ligos-Tsouka Rossa	32	14	1997-98, 2000-03
6.	Zigos	0	6	2002
7.	Voio	0	6	1997-98
8.	Tambouri	48	5	1994-95
9.	Vassilitsa	16	7	1994-95, 1999-2000
10.	Kleftes- Flabouro	32	8	1994-95, 1997-98, 2001
11.	Central Zagori	144	12	1994-95, 1997-98, 2000-01
12.	Mitsikeli	0	4	1994-95
13.	Peristeri-Kakarditsa-Stefani	24	13	1994-95, 1998
14.	Tzoumerka-Pahtouri	24	11	1998
15.	Kokkinolakka	0	5	1998
16.	Trigia	0	9	1998
17.	Avgo	16	6	1998
18.	Hatzi	16	7	1998
19.	Axladias-Tsoukes	0	2	1998
20.	Agrafa	0	5	1999
21.	Nemertsika	16	7	1998
22.	Vardoussia	64	12	1999
23.	Giona	136	11	1999
24.	Iti	16	5	1999
25.	Parnassus	0	5	1999
26.	Olympus	112	16	1994, 1999
27.	Gyftocastro-Haidou	8	5	1998
28.	Fracto forest	232	9	1997-98
29.	Tzena-Pinovo	96	13	1999
30.	Varnountas	8	5	1994
	Total	2928	297	

RESULTS

Population status

New chamois populations were recorded for the first time in three areas (Central Zagori, Hatzi and Tzena-Pinovo) covering an estimated area of 132 km^2. The species presence was verified in 11 areas (1.183 km^2) and in 16 areas where the species was previously considered present, no individuals were recorded. In the latter case, local inhabitants reported chamois as present in four areas (349 km^2), provided contrasting reports for six areas (351 km^2), where current chamois presence was therefore regarded as doubtful, and reported the species as extinct in 6 areas (318 km^2) (Fig. 1 and Table 2).

The current estimate of the total chamois population size in Greece is between 477 and 750 individuals. Specific population density data are available for the three areas that were surveyed in detail (Timfi, Grammos and Fracto forest) (Table 2). Of the areas surveyed only one population exceeds 120 individuals (pop. 4) and only three populations exceed 60 individuals (pops. 23, 26, 28), whereas 14 populations comprise less than 15 individuals (Fig. 2).

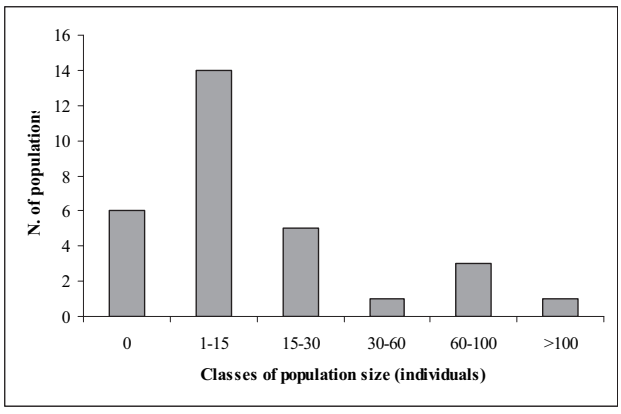

Fig. 2. – Distribution of population sizes of chamois in Greece. Extinct populations are referred as zero.

TABLE 2

Catalogue of Balkan chamois populations in Greece, potential local dispersal area, minimum and estimated population sizes, years of report, proportion of chamois area under protection status, number and type of protected areas.

N	Mountain	Presence	Potential dispersal area (km^2)	Year of last report	Minimum population size	Estimated population size	Population density	Year of population estimation	Protected chamois area (%)	Protection status
1.	Grammos	●	184	2002	40	50	0.24	1998	25-50	4 W, Na
2.	Smolikas	●	129	2001	20	30		B	25-50	2 W, Na
3.	Trapezitsa	●	17	2002	10	20		1998	>75	1 W, NP, Na
4.	Timfi	●	169	2003	120	130	0.74	2002	50-75	3 W, NP, Na
5.	Ligos-Tsouka Rossa	●	93	2003	15	30		B	50-75	4 W, NP, Na
6.	Zigos	?	24	80s	0	10		B	0	–
7.	Voio	E	47	70s				1998	< 25	2 W
8.	Tambouri	E	25	70s				1995	< 25	1 W
9.	Vassilitsa	E	52	60s				1999	< 25	1 W, Na
10	Kleftes- Flabouro	+	30	1998	1	10		1998	0	–
11.	Central Zagori	●N	19	1995	1	10		1995	0	Na
12	Mitsikeli	E	45	60s				1992	0	Na
13	Peristeri-Kakarditsa-Stefani	+	145	1998	5	15		1998	< 25	2 W, Na
14	Tzoumerka-Pahtouri	+	109	1998	5	15		1998	0	1 W
15	Kokkinolakka	?	27	1998	0	5		1998	< 25	–
16	Trigia	?	89	80s	0	15		B	>75	1 W, H
17	Avgo	?	55	1992	0	10		1992	50-75	H
18	Hatzi	●N	45	1998	15	20		1998	25-50	1 W
19	Axladias-Tsoukes	E	33	70s				1999	< 25	1 W
20	Agrafa	?	116	1999	0	15		1999	< 25	3 W
21	Nemertsika	●	27	1994	15	15		B	0	–
22	Vardoussia	●	114	1999	10	10		B	25-50	H, Na
23	Giona	●	176	1999	70	100		B	50-75	2 W, H, Na
24	Iti	●	65	1999	10	30		B	25-50	NP, Na
25	Parnassus	E	116	70s				1999	25-50	1 W, NP, Na
26	Olympus	●	184	1999	60	100		B	50-75	2 W, NP, Na
27	Gyftocastro-Haidou	+	65	1998	10	30		1998, B	25-50	1 W, Na
28	Fracto forest	●	25	1998	60	65	2,5	1998	>75	1W,PNM,Na
29	Tzena-Pinovo	●N	68	1999	10	15		1999	>75	1 W, Na
30	Varnountas	?	41	1994	0	5		1994	0	–

●, verified presence during direct surveys; ●N, new population nuclei discovered; +, presence reported by locals; ?, doubtful current presence; E, population extinct, B, bibliographical source referring data of 80s. H, controlled hunting area; Na, Natura network; PNM, protected natural monument; NP, national park; W, wildlife reserve.

Habitat

Extensive steep rock formations and sub-alpine plateaus with open pastures above the timberline (1.600-2.000 m) mostly characterize the chamois distribution area in the mountains of Pindus, Sterea Ellada and Olympus. The northern part of Mt. Pindus (pops. 1-12) comprises oak woodlands up to 1.000 m, pure forests of fir *(Abies borisii-regis)*, black pine (*Pinus nigra)* and beech *(Fagus sylvatica*) from 1.000-1.600 m, and forests of Bosnian pine *(Pinus leucodermis*) at altitudes usually up to 2.000 m. Extensive forests of pure fir and beech woods at altitudes of 1.000-2.000 m characterize the southern part of Mt. Pindus (pops. 13-20). The chamois distribution area in Sterea Ellada (pops. 22-25) consists of similar habitat types to those in southern Pindus, with the difference that Greek fir *(Abies cephalonica)* is the dominant tree species above 1.000 m. The chamois populations in Rhodopi mountain range (pops. 27-28) occur in quite different habitats, including lower altitudes and smoother forested slopes of beech, pure fir, black pine, Scottish pine *(Pinus sylvestris)* and Norway spruce *(Picea abies)*.

Threats

Interviewed local inhabitants reported a decline in chamois population size and a restriction of its distribution area in all the 24 mountains the species inhabits or may inhabit since the 1950's. They also reported that the main reason for this decline was legal shooting, which occurred until the 1960's, and poaching carried out after this date. In most cases, residents reported that poaching increased after the construction of a dense mountain road network that gives easier access to chamois habitats. Tourism and livestock breeding were not considered as threats for chamois survival.

During our nine year survey, we noted only one possible case of sarcoptic mange infection (Mt. Timfi, 1998). Besides, the local inhabitants interviewed never reported the presence of infected chamois by any parasitic disease during the last 30 years. In addition, we never found carcasses of chamois that were dead by natural causes or diseases. On the other hand, we have quite often found remains of chamois shot by poachers (e.g. hide).

Protection status

Chamois hunting is prohibited throughout Greece. Considering as protected areas those where hunting activity is prohibited or is under strict control (National Park core areas, natural monuments, wildlife reserves and controlled hunting areas), we compare the map of protected/unprotected areas (after TRIANTAFILAKOS, 1998, 2001, 2002 a, b) with he map of the chamois distribution (Fig. 1). We find that chamois populations are completely unprotected in seven mountains, they are poorly protected in seven additional mountains (less than 25% of the established range overlaps with protected areas), while in a further seven mountains less than half (25-50%) of the occupied chamois range fell within a protected area. Finally, in five of the mountains surveyed, chamois area is protected (50-75%) and in four mountains the greatest part of the occupied area is protected (>75%) (Table 2).

DISCUSSION

Population status

Based on our estimates, Greece has a total population of 477-750 chamois, the smallest national population size in the Balkans. This estimate is greater than previous counts (300-500 individuals estimated by ADAMAKOPOULOS et al., 1997; HATZIRVASSANIS, 1991), but we attribute this to our more extensive survey rather than to actual population increase. Likewise, we consider that the new populations discovered pre-existed in the three areas and didn't colonise them recently.

The distribution pattern of Balkan chamois in Greece is rather fragmented (Fig. 1) and chamois usually occur in small, scattered, populations of less than 30 individuals (n = 18), thus pinpointing the vulnerability of the species throughout the mainland. However, a limited gene flow may occur, mostly in the form of wandering males (HAMR, 1984), within three populations groups which we therefore consider as three potential metapopulations : Pindus (pops. 1-20), Sterea Ellada (pops. 22-24) and Rhodopi mountains (pops. 27-28).

The northern part of Pindus mountain range (pops. 1-12) is the most important area for the conservation of the Balkan chamois in Greece, as it hosts approximately 40% (207-290 ind.) of the national population and includes the largest populations. On the contrary, urgent conservation action should be taken in Southern Pindus (pops. 13-20), where few small populations occur (about 15 individuals each) and are directly threatened with extinction, even if a gene flow exists between these and the Northern Pindus populations. The mountains of Sterea Ellada (90-140 individuals) host the second largest Greek chamois population (Mt. Giona), and two smaller populations in neighbouring mountains. Finally, Mt Rhodopi (pops 27-28) contains 70-95 individuals, and the population of Mt. Olympus is relatively large (60-100 individuals) but geographically isolated.

Regarding the chamois populations on the borderlines of northern Greece, Mt. Nemertsika (pop. 21) may have a larger population size than that recorded, given that the largest part of this mountain lies in Albania. Likewise, Mt Grammos (pop. 1) may have a slightly larger population size, because chamois are recorded in the Albanian part of Mt Grammos as well (GJIKNURI, 1997). The chamois population in Mt. Varnountas (pop. 30) is probably part of a larger neighbouring population in Mt. Pelistel (KRYSTUFEK et al., 1997) in F.Y.R.O.M. Finally, the distribution range of the populations of Mt. Tzena-Pinovo (pop. 29) and Mt Rhodopi (pops 27-28) extends probably over the Greek borders, but no populations are reported near the bordeline in the neibouring countries (KRYSTUFEK et al., 1997; SPIRIDONOV & GENOV, 1997).

Threats

Although sarcoptic mange, a parasitic disease transferred by livestock, is one of the most important short-term mortality factors for chamois in Europe (FERNANDEZ-MORAN et al., 1997; PENCE & UECKERMANN, 2002), the disease does not seem to affect substantially the chamois population dynamics in Greece. Likewise, this disease has never been reported to infect Balkan chamois in former Yugoslavia (e.g. KRYSTUFEK et al., 1997). Livestock breeding is not currently a serious threat for chamois populations, especially given its decreasing trend during the last decades. However, we observed that chamois withdrew to more remote habitats after the arrival of transhuman livestock in summer, on Mts Timfi and Grammos.

The major threat to chamois survival in Greece is considered to be poaching, enhanced by the dense mountain road network constructed either for livestock breeding activities or logging. Some of the local inhabitants interviewed were poachers themselves and in Mt. Mitsikeli they reported to have killed the last animal. Poachers were aware of the illegal nature of their activity, but not of the endangered status of Balkan chamois in Greece. Chamois populations in Europe have a higher population density in areas where hunting is prohibited compared with those where hunting is allowed. Examples of this are noted in the National Park of Ecrins in the French Alps (CORTI et al., 1985), the National Park of Ordesa in the Pyreneans (GARCIA-GONZALEZ & HIDALGO, 1988), and

the National Park of Grand Paradiso in Italy (FRAMARIN, 1985). However, in Greece poaching occurs both inside and outside protected areas, as there is no efficient system to eliminate illegal activities in the country's National Parks. We assume however that in tourist areas such as the National Parks of Mt. Olympus (Olympus National Park) and Mt. Timfi (Vikos-Aoos National Park), the presence of hikers occasionally discourages poaching activity.

Further potential threats to the chamois could be the continuing development of adventure tourism activities, given their increasing trend in Greece and their known negative impact on chamois behaviour and activity (CEDERNA & LOVARI, 1985; GANDER & INGOLD, 1997; SCHNIDRIG et al., 1992).

Conservation implications

Formal attention should be drawn to the need to eliminate poaching, which is the main threat to the survival of the Balkan chamois in Greece. Although a significant proportion of chamois habitats are included in protected areas where hunting is theoretically prohibited or controlled, no efficient enforcement system exists in these areas. Access to the mountain road network in chamois habitats should also be controlled. The main conservation measure we propose for the survival of the species is the funding of a national body of rangers to guard the Greek reserve network and to control road use.

The construction of new mountain roads in close proximity to chamois habitats above the timberline or within winter habitats should be avoided. New hunting-prohibited corridors should be established under the form of wildlife reserves to guarantee the undisturbed movements of chamois within the three metapopulations in Pindus, Sterea Ellada and Rhodopi. This action would hopefully combat the potential local extinction of small populations due to geographic and genetic isolation.

Further conservation measures should involve : the establishment of a permanent monitoring programme on chamois population trends, the control of tourist activities within chamois distribution areas, the creation of environmental education programmes targeting local communities, and the funding of conservation-oriented research on chamois population density, home range and genetics.

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Fitness-heterozygosity associations differ between male and female winter moths *Operophtera brumata* L.

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ABSTRACT. The association between heterozygosity and fitness is positive but weak on average and varies between studies. Inbreeding has been invoked as the driving force between the positive heterozygosity-fitness associations, yet in spatio-temporally stable environments a negative correlation is expected. Furthermore, different patterns can arise because of the effects of natural selection on different loci and variation can be expected among groups of individuals that experience different levels of stress. In this paper we report on fitness-heterozygosity associations in the winter moth for six allozyme loci. The relationship is estimated for males and females separately, in four areas differing in their degree of fragmentation, and variation among loci is modelled. We introduce a linear mixed model framework to achieve this analysis. This approach differs from more traditional (multiple) regression analyses and allows testing specific interactions. We show that fitness, as estimated by body size, is negatively correlated with heterozygosity, but only so in females. This association does not vary significantly among loci and the four areas. We speculate that a trade-off between fitness-consequences of inbreeding and outbreeding at different stages of the winter moth life cycle could explain the observed patterns.

KEY WORDS : fitness, heterozygosity, inbreeding, outbreeding, natural selection, genetic drift, winter moth, Operophtera

INTRODUCTION

The extent to which the environment interacts with the genotype affecting fitness can influence population dynamics and even extinction risks of small endangered populations. Yet, this issue remains poorly investigated and often ignored in ecology and evolutionary biology (COULSON et al., 1998a). One aspect of the association between genotype and fitness that has received relatively much attention in the literature are correlations with degree of heterozygosity or inbreeding. Yet, the predicted positive association between genetic diversity and fitness is far from general (BRITTEN, 1996; MITTON, 1997), and little is know about the factors that affect this association (but see e.g., PALMER, 1996; MITTON, 1997; LESBARRÉRES et al., 2005). Recently, LESBARRÉRES et al. (2005) found heterogeneity in fitness-heterozygosity associations among geographic areas and SACCHERI et al (2005) found indications for differences between the two sexes.

How an association between heterozygosity and fitness can become established is a subject of many debates. Observed individual heterozygosity may reflect genome-wide heterozygosity and thus the overall levels of inbreeding and may reduce fitness (CHARLESWORTH & CHARLESWORTH, 1987). Yet, a large number of loci should be scored to obtain a reliable estimate of genome-wide heterozygosity drastically reducing power to detect a fitness-heterozygosity association. Failing to do so drastically reduces the power to detect an association. Since many studies do find an association with few loci, rather natural selection phenomena on single loci, or effects at tightly linked areas on the chromosome, are often thought to be responsible for the observed fitness-heterozygosity association (MITTON, 1997). It is, however, unlikely that a single mechanism explains such a widespread phenomenon. Indeed, some studies using presumed selectively neutral microsatellite markers also found positive fitness-heterozygosity associations (COLTMAN et al., 1998; COULSON et al., 1998b; LESBARRÉRES et al., 2005) favouring the hypothesis of genome-wide effects of inbreeding over the selection theory. On the other hand, some detailed studies of the kinetics of specific metabolic enzymes have shown clear effects of selection on single locus genotypes (see MITTON, 1997 for references and details). HALDANE (1954) proposed that heterozygotes at enzyme loci would be more efficient than homozygotes at controlling flux in metabolic pathways. Under this hypothesis it is assumed that different genotypes have different properties under different environmental conditions and that heterozygotes would be superior when conditions fluctuate between those favouring the different homozygotes (MITTON, 1997). When environmental differences are persistent in space, however, genetic differentiation (either through the effects of genetic drift or natural selection) between the environments is expected to evolve, and mating between genetically distant individuals becomes disadvantageous as it disrupts local adaptation (i.e. outbreeding depression, e.g. STRAUSS & KARBAN, 1994; COULSON et al., 1998b). Therefore, in spatio-temporally stable selective environments, a negative association between fitness and heterozygosity is expected. In theory it should be possible to differentiate effects due to drift and selection. Genetic drift is expected to affect different loci in a similar way whereas the effects of natural

selection are more likely to differ among loci. It is therefore important to compare fitness-heterozygosity associations among loci.

Environmental stress may increase the expression of heterozygote advantage although the underlying mechanisms are only poorly understood (PALMER, 1996; MITTON, 1997). Fitness-heterozygosity associations may therefore differ among areas and/or groups of individuals that experience different levels of stress. Here we report on fitness-heterozygosity associations in the winter moth *Operophtera brumata* L. at 6 allozyme loci. The winter moth inhabits oak forests that differ in quality (degree of isolation and surface area) and levels of stress differ between males and females as they differ in their respiration levels and degree of weight loss during pupation. Males loose much more weight compared to females and suffer higher mortality during pupation (GRADWELL, 1974). We study four areas that differ with respect to area and degree of isolation (see below for description). Earlier results confirmed that these factors influence different aspects of the population structure and fitness in particular (VAN DONGEN et al., 1994; 1998a; VAN DONGEN, 1997). Considering the importance of comparing associations among loci, areas and sex we introduce a statistical framework based on linear mixed models to perform these analyses with maximal power.

MATERIALS AND METHODS

Study species

Winter moth egg hatching is to some extent locally synchronised to the individual-specific budburst dates of pedunculate oak *Quercus robur* L. (VAN DONGEN et al., 1997). Within single study plots, neighbouring trees consistently differ by up to 4 weeks in budburst date creating a highly heterogeneous environment on a very small geographical scale (CRAWLEY & AKHTERUZZAMAN, 1988; VAN DONGEN et al., 1997). This local adaptation is the result of the strong negative fitness effects on caterpillars hatching out of synchrony (FEENY, 1968; 1970; GRADWELL, 1974; WINT, 1983; VAN DONGEN et al., 1997). Because egg-hatching date has a genetic basis (SPEYER, 1938; 1941) dispersal of locally adapted individuals is likely to be disadvantageous and outbreeding depression and negative fitness-heterozygosity associations are expected to occur in this species. The winter moth is a particularly interesting species to study the fitness-heterozygosity associations because males and females are subject to different levels of stress during pupation. Males consume much more oxygen, loose more weight and suffer higher mortality during pupation than females (GRADWELL, 1974). If stress has an effect on the fitness-heterozygosity association, males and females will show a different association. Furthermore, oak forests have become highly fragmented in N-Belgium, as in other parts of the world. Fragmentation has been shown to affect winter moth fitness/weight in our study area (VAN DONGEN et al., 1994; 1998a).

Sampling and electrophoresis

Male and female winter moths were collected in copula on individual trees (N = 520 couples) at night by hand during November 1994 in four different areas near Antwerp, Belgium. All study sites were dominated by pedunculate oak that showed up to four weeks of consistent variation in budburst date. Still, areas differed considerably with respect to area, isolation and structure : area1 (51°16 N, 4°30 E) : part of a forest-parkland complex of well over 200 ha on the suburban fringe of Antwerp (N = 105 couples); area2 (51°07 N, 4°32 E) : a small oak forest fragment of 1.6ha with a low degree of isolation (N = 334 couples); area3 (51°09 N, 4°32 E) : a small oak forest fragment of 0.5ha with a relative high degree of isolation (N = 41 couples); and area4 (51°08 N, 4°30 E) : an oak lane of 400m length (N = 40 couples). After collection, moths were stored at –80°C for electrophoretic analysis. A total of six enzyme loci with relative high degree of genetic variation were scored [peptidase (PEP : E.C. 3.4.11.*, with leucylalanine as substrate), phosphoglucomutase (PGM : E.C. 5.4.2.2), glucose-phosphate isomerase (GPI : E.C. 5.3.1.9), 6-phosphogluconate dehydrogenase (PGD : E.C. 1.1.1.44, detectable in males only), b-hydroxibutyrate dehydrogenase (HBDH : E.C. 1.1.1.30), alkaline phosphatase (ALP : E.C. 3.1.3.1, detectable in females only) with vertical polyacrylamide gel electrophoresis (details in VAN DONGEN et al., 1994; 1998a). Bodyweight after log-transformation (to obtain approximate normality) was used as an estimate of individual fitness. Weight is closely correlated to other measures of body size and reflects several components of fitness such as female fecundity, male and female mating success, adult lifespan, and pupal survival (FEENY, 1970; GRADWELL, 1974; WINT, 1983; VAN DONGEN et al., 1997; 1998b; 1999). Body weight could also be obtained most reliably after moths were frozen and defrosted again. We also wanted to avoid as much time as possible during the preparations of the samples for electrophoresis to assure that gels could be reliably scored.

Statistical analysis

To analyse the association between weight and heterozygosity a mixed model approach (LAIRD & WARE, 1982) was performed in SAS (ver. 6.12) used to evaluate variation among loci, areas and gender. In this analysis, each observed individual weight (logtransformed) was used five times, corresponding to the five different loci for each sex. In this way, the factor locus and relevant interactions could be added to the model as random effects. This approach differs from traditional regression analyses. The advantage is that it allows addressing particular hypotheses directly by testing specific interactions, rather than making inference indirectly from presence or absence of associations (e.g. LEARY, KNUDSEN & ALLENDORF, 1983). Because each individual weight is used five times, residual values are correlated within individuals. To assure that the degrees of freedom and consequently the type-I error rate were not inflated due to this statistical dependency, the correlation between these residual values was modelled explicitly and degrees of freedom were

approximated by Satterthwaites procedure (LITTELL et al., 1996; VERBEKE & MOLENBERGHS, 1997). This was achieved by adding the factor individual as a random effect to the model (LITTELL et al., 1996; VERBEKE & MOLENBERGHS, 1997). The Satterthwaite procedure makes use of this correlation to adjust the degrees of freedom and to assure that the Type-I error rate equals the nominal level of 5%. In case there was no indication of any locus-effect the analysis was continued by relating individual weight to heterozygosity averaged over the five loci. In this way, each individual was used only once in the analysis so that the data were statistically independent. For some individuals, some loci could not be scored unambigiously. To avoid potential bias, only individuals for which all genotypes were known (N = 571) were included. Nevertheless, analyses inclusing all available data showed similar patterns (data not shown).

Next to the random locus effect, area and relevant interactions were added as random effect, in order to model how the weight-heterozygosity relationship varied among the four study areas. As fixed effects, heterozygosity, sex and their two-way interaction were included. Fixed effects were tested by F-tests while significances of the random effects were obtained from likelihood ratio tests (as outlined in VERBEKE & MOLENBERGHS, 1997).

RESULTS

There appeared to be no detectable variation in the weight-heterozygosity association among the six loci because all variance component estimates equalled zero (Table 1). Therefore, the analysis was continued with the average individual heterozygosity. Neither the weight-heterozygosity relationship nor adult weight varied among the four study areas as judged from the non-significant area related random effects (Table 1). There was a significant relationship between weight and heterozygosity, and this relationship differed between males and females (Table 1) : weight decreased significantly with heterozygosity in females but not in males (Fig. 1). The residuals of the finally selected model were approximately normally distributed. We also examined whether some alleles were specifically associated with weight variation, yet after correction for multiple testing, none of these tests were statistically significant. However, due to the high number of comparisons, statistical power was probably low.

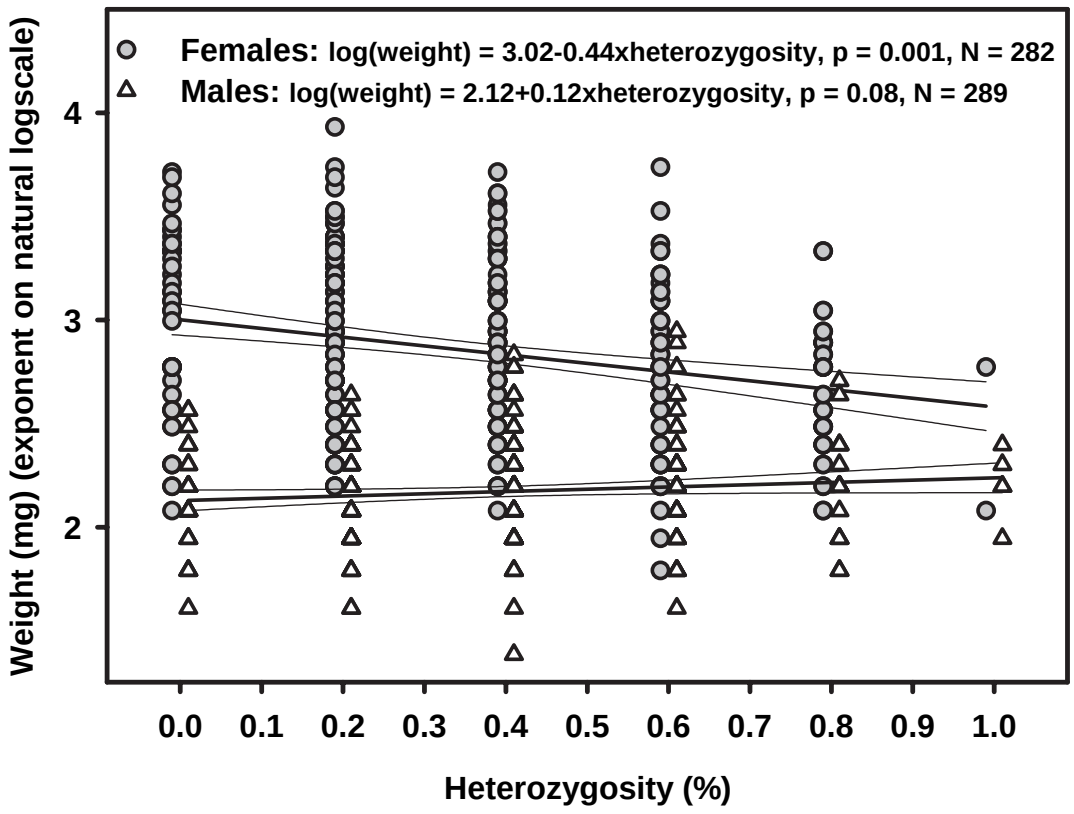

Fig. 1. – Relationship between individual heterozygosity as estimated from five polymorphic enzyme loci and individual weight (on a logscale) for males and females. Data from different areas and loci were pooled because the relationship did not differ between the different levels of these factors (see results and Table 1). Regression lines and their 95% confidence intervals are indicated for both sexes.

TABLE 1

Summary of the fixed and random effects in the mixed model relating male and female body-weight to heterozygosity at six allozyme loci in four areas.

Fixed effects	F-test statistic[a]	d.f. 1	d.f. 2	p-value
Sex	199.0	1	568	0.0001
Heterozygosity	5.0	1	568	0.03
Sex × heterozygosity	15.6	1	568	0.0001
Random effects	**σ^2**	**χ^2 test statistic[b]**	**d.f.**	**p-value**
Area	0.003	0.2	1	0.95
Area × sex	0	–	–	>0.05
Area × heterozygosity	0.001	0.1	1	0.97
Area × sex × heterozygosity	0	–	–	>0.05
Locus	0	–	–	>0.05
Locus × sex	0	–	–	>0.05
Locus × heterozygosity	0	–	–	>0.05
Locus × sex × heterozygosity	0	–	–	>0.05

a. Fixed effects were tested by means of traditional F-tests with d.f. approximated by Satterthwaites procedure.

b. Random effects were tested by likelihood ratio test when the estimated variance component was larger than zero.

DISCUSSION

In contrast to many other studies we find a negative relationship between fitness and heterozygosity, a correlation that appeared constant across loci and areas, but only present in females. This negative association suggests the presence of outbreeding depression. Local adaptation of winter moth egg hatching to host tree budburst may have resulted in a genetic differentiation of the loci under investigation, i.e., a balanced polymorphism. Mating between individuals that are adapted to trees with different budburst phenology will then result in more asynchronous offspring with lower fitness and higher heterozygosity. Unfortunately, this presumed genetic differentiation could not be quantified accurately because of low number of moths collected on individual trees. Yet, deviations from Hardy-Weinberg equilibrium (heterozygote deficit) at the level of the study site, as reported earlier by VAN DONGEN (1997), suggest the presence of such a genetic substructuring.

We can only speculate about the reasons for the observed differences in males and females. Adult size is determined by two factors : the amount of food a caterpillar accumulates and thus its degree of synchrony with the individual host (VAN DONGEN, 1997), and the weight loss during pupation (GRADWELL, 1974). We argued that heterozygosity could be associated with larval synchrony (VAN DONGEN, 1997), an association that is unlikely to differ between males and females during the caterpillar stage. Thus, the significant difference in the weight-heterozygosity association between males and females is likely to result from effects occurring during pupation. Males suffer higher mortality, more weight loss and consume more oxygen during pupation than females (GRADWELL, 1974). Because heterozygous individuals have been shown to have a higher metabolic efficiency in several species (MITTON, 1997), it is possible that heterozygous males, although lighter at the start of pupation due to lower synchrony, lose less weight during pupation, reversing or counteracting the earlier established negative weight-heterozygosity association. In addition, the higher heterozygosity in males than in females (VAN DONGEN, 1997) is in agreement with this hypothesis. To test this hypothetical explanation formally, it is necessary to investigate weight-heterozygosity associations in caterpillars, to monitor weight loss during pupation for individuals differing in their degree of heterozygosity and/or to compare the observed associations with those for a selectively neutral marker (e.g. microsatellite loci).

The observed associations between fitness and heterozygosity can potentially affect several aspects of the population structure. The presumed higher mortality in less heterozygous males not only affects the sex ratio and effective population size, but may also limit the evolution of local adaptation of egg hatching to host budburst phenology. The synchrony between the latter two is statistically significant, yet relatively weak (r^2<32%, VAN DONGEN et al., 1997) or even absent in some years or areas (VAN DONGEN, unpublished results). This could be explained by the lower fitness-benefits of synchrony for males as it results in lower heterozygosity and consequently higher pupal mortality.

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A head with a suckermouth : a functional-morphological study of the head of the suckermouth armoured catfish *Ancistrus* cf. *triradiatus* (Loricariidae, Siluriformes)

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ABSTRACT. The neotropical loricariid catfishes are highly specialized for adhering to substrates, and can continue breathing and even scrape food from these surfaces while using the mouth for suction. A detailed study integrating bones, muscles and ligaments was performed on *Ancistrus* cf. *triradiatus*, using cleared and stained specimens, dissections and manipulations on fresh specimens, serial sections, and histological examination of key tissues. A limited kinematic study using high-speed video was performed as well.

The suspensorium is a rather rigid structure; the hyoid is more movable and associated muscles are more substantial; it appears to be more important in the buccal pump system. The transverse orientation of the hyohyoideus abductor suggests it can't open the branchiostegal membrane. This movement might be passive. Apart from divisions inserting on the lower and upper jaws, a medial adductor mandibulae division, the retractor veli, inserts on the oral valve. The retractor tentaculi[1] and the lateral part of the completely subdivided extensor tentaculi move the maxillary barbel, a structure that allows controlled inspiration preventing failing of the suction system. Rotational movements of the lower and upper jaws result in scraping the substrate. Antagonistic muscles for the adductor mandibulae divisions inserting on the lower and upper jaws might be a part of the protractor hyoidei and the medial part of the extensor tentaculi. The lower jaws are most mobile, not being linked to the hyoid arch medially. A medial cartilage plug acts as a supporting and gliding device for the lower jaws.

KEY WORDS : Feeding, function, morphology, myology, osteology, respiration.

INTRODUCTION

Many fishes have adopted a behaviour in which adhesion to substrates proves to be advantageous. Three typical features of the body plan that seem to have an adaptive value can be discerned (HORA, 1930) : firstly, a depressed body shape reduces drag of torrential water when lying on a substrate. Paired fins that are closely appressed to the substrate can aid in maintenance of this close contact. Secondly, frictional devices may evolve, such as spines or odontodes on the ventral side of the body and fins, making it less likely an adhering fish is washed away. Thirdly, a suction apparatus can attach a fish more firmly, irrespective of the substrate inclination or the current direction. The latter two mechanisms are also of great value in non-flowing water systems, and (especially the third) allow the adhesion to inclined substrates.

Examples of the latter mechanism in recent teleosts are numerous. Thoracic discs providing both friction and suction, as well as paired fins with adhesive surfaces have been described in Sisoridae and Erethistidae (HORA, 1930; SAXENA & CHANDY, 1966; TILAK, 1976). A suction disc is formed by the pelvic fins in various families, such as Gobiidae (HORA, 1930), or by the ventral surface of the lower lip in the cyprinid *Garra* (HORA, 1930; SAXENA & CHANDY, 1966). Gyrinocheilidae have a modified gill opening, providing both an inspiratory and an expiratory canal. Consequently, when adhering to a substrate, the mouth can continue to work as a sucking cup (SMITH, 1945; JAYARAM, 1950; BENJAMIN, 1986; ROBERTS & KOTTELAT, 1993; NELSON, 1994). A small ventral mouth with thick, papillated lips, as well as tentacles and minute spines on fin rays ensure adhesion in certain Amphiliidae (HORA, 1930; DIOGO et al., 2000). A ventral suckermouth is also well developed in members of the Mochokidae and Astroblepidae. The latter group can even use the suckermouth for terrestrial movements (JOHNSON, 1912).

Also, in some fishes the coupling of a suckermouth to structural transformations in the jaws or the lips has allowed an evolutionary development of a substrate-scraping device. The single largest group, in which the combination of oral suction and a scraping feeding apparatus is found, is the family of the South American Loricariidae, or suckermouth armoured catfishes. The family is characterized by remarkable features such as a bony armor, a ventrally oriented suckermouth, ventrally tilted lower jaws and new and unique muscle configurations increasing jaw mobility (SCHAEFER & LAUDER, 1986). Not

[1] This muscle, however, is not homologous to the retractor tentaculi of related and non-related siluriforms. Therefore it has been renamed levator tentaculi in a recent paper (in submission).

only this morphology, but also their status as the largest catfish family [more than 670 species according to a recent count (FERRARIS et al., 2003)] has drawn focus to the group. Among the most important papers discussing loricariid cranial morphology are ALEXANDER (1965), HOWES (1983), SCHAEFER (1987; 1988), SCHAEFER & LAUDER (1986; 1996) and ARMBRUSTER (2004). Accounts on postcranial morphology were given by SHELDEN (1937) and SCHAEFER (1987; 1997), while ALEXANDER (1964) and CHARDON (1968) treated the loricariid Weberian apparatus.

Cranial osteology and myology of the loricariid *Otocinclus vittatus* have been described by SCHAEFER (1997), with a strong systematic and phylogenetic interest. The aim of the present paper is to describe in detail the cranial osteology and myology of one loricariid species, and to discuss those parts of the head involved in suction, respiration and feeding, with an emphasis on functionality of structures rather than their use as systematic characters. The anatomical results are coupled to a limited kinematic data set.

MATERIALS AND METHODS

Ancistrus cf. *triradiatus* Eigenmann 1918, a bristlenose suckermouth armoured catfish, was chosen for this study, as we consider it a generalized loricariid (medium sized, with an intermediate body depth and length). Specimens of both sexes of *A.* cf. *triradiatus* were commercially obtained. Osteology was studied on *in toto* cleared and stained specimens (TAYLOR & VAN DYKE, 1985). Dissections were performed for the study of both hard and soft tissues. Whenever necessary, visualisation of muscle fiber arrangement was enhanced by the use of iodine (BOCK & SHEAR, 1972). Examination of the specimens was done using an Olympus SZX9 stereoscopic microscope, equipped with a camera lucida for drawing. One subadult specimen was selected for serial sectioning. The obtained toluidine stained 5µm sections (Technovit 7100 embedding, cut with a Reichert-Jung Polycut microtome) were studied using a Reichert-Jung Polyvar light microscope. Selected tissue samples (both lips, oral valve and tissue connecting it to the upper jaws, cartilage plug between lower jaws) of another specimen were histologically studied; the 10µm paraffin cut sections were stained with Verhoeff-Van Gieson's stain for elastin and collagen (PEARSE, 1985).

Examined specimens : clearing and staining : 4 (male : 88mm SL, 90mm SL; female : 44mm SL; gender unknown : 36mm SL); dissection : 3 (male : 94mm SL; female : 68mm SL, 74mm SL); serial sections : 1 (gender unknown : 33.5mm SL); histological study : 1 (female : 71mm SL). Some specimens of *Farlowella acus*, *Otocinclus vestitus* and *Pterygoplichthys lituratus* were studied for comparison.

Live observations were carried out in aquaria (including experiments with milk used as dye to visualize water flows during respiration). Filming of three specimens (in lateral, ventral and oblique ventrolateral view) was done with a Redlake Motionscope digital video camera at 200 frames per second. Only each eight frame was analysed (25 frames per second). Observations are qualitative; no markers or length measurements were included. An extensive biomechanical study was not aimed for; such study on *Pterygoplichthys lituratus*, using X-rays and electromyography, is in progress.

RESULTS

Except where noted, osteological terminology follows SCHAEFER (1987), and myological terminology follows WINTERBOTTOM (1974).

Neurocranium (Figs 1-2-3-4)

The anterior half of the long mesethmoid is almost cylindrical and gives the snout region rigidity. Anterolateral cornua are absent, and there is an expanded ventral disc projecting ventrally (Fig. 2). The large lateral process of the lateral ethmoid [antorbital process of SCHAEFER (1987)] has an articular facet for the autopalatine anteriorly, and an articular facet with a supporting ridge for the metapterygoid ventrally.

The anterior part of the frontal is relatively narrow, while the posterior part is broader, reaching the orbit. The sphenotic has a prominent lateral process, enclosing the infraorbital canal. Posterior to the orbit, the sphenotic contributes to the articulation with the hyomandibular (Fig. 2B).

In the skull floor the toothless prevomer is a narrow bone, without well developed lateral wings. It sutures deeply with the mesethmoid (anteriorly) and the parasphenoid (posteriorly), which sutures with the basioccipital as well. The parasphenoid forms a longitudinal protruding ridge on the ventral side of the neurocranium. It has a pair of small lateral wings. A major part of the skull wall and floor lateral to the parasphenoid is occupied by the orbitosphenoid. Together with the pterosphenoid and the prootic it forms the border of the sphenotic fenestra (Fig. 2B). The prootic contributes to the posterior skull floor and, to a lesser degree, to the neurocranium wall, where it bears almost half of the articulation facet for the hyomandibular.

The basioccipital is fused posteriorly with the ossified Baudelot's ligaments into a T-shaped bone (Fig. 4A). Laterally, a certain degree of fusion has occurred with the exoccipital. The ossified Baudelot's ligaments are vertical bony ridges protruding ventrally from the skull floor. They extend toward, and suture with, ventral flanges of the compound pterotics, which are the continuation of this transverse ventral ridge.

A substantial part of the posterior skull roof, skull floor and caudolateral wall of the brain case is taken by the pterotic. Thus it is a double-layered bone, providing ample insertion space for the opercular muscles. Where it contacts the sphenotic and prootic, it forms the caudal edge of the hyomandibular articulation. The pterotic is fused to the more ventrocaudal supracleithrum (or even posttemporo-supracleithrum) of the pectoral girdle. The true nature of this fused bone complex, as well as its relation to the ossified Baudelot's ligament, has not been unambiguously resolved (LUNDBERG, 1975; FINK & FINK, 1996); its development is part of a forthcoming paper. It

will be further referred to as compound pterotic. The epioccipital [epiotic of SCHAEFER (1997)] is a small element composing part of the caudal neurocranium wall (Fig. 3). Finally, the parieto-supraoccipital lacks a pronounced posterior process. Posteroventrally, a *V*-shaped medial ridge is fused with the fused neural arch of the second and third vertebrae. Moreover, the posterior tip is fused with the neural spine of the sixth vertebra, as in *Hypostomus plecostomus* (ALEXANDER, 1964) (Fig. 3). There is no dorsomedial crest.

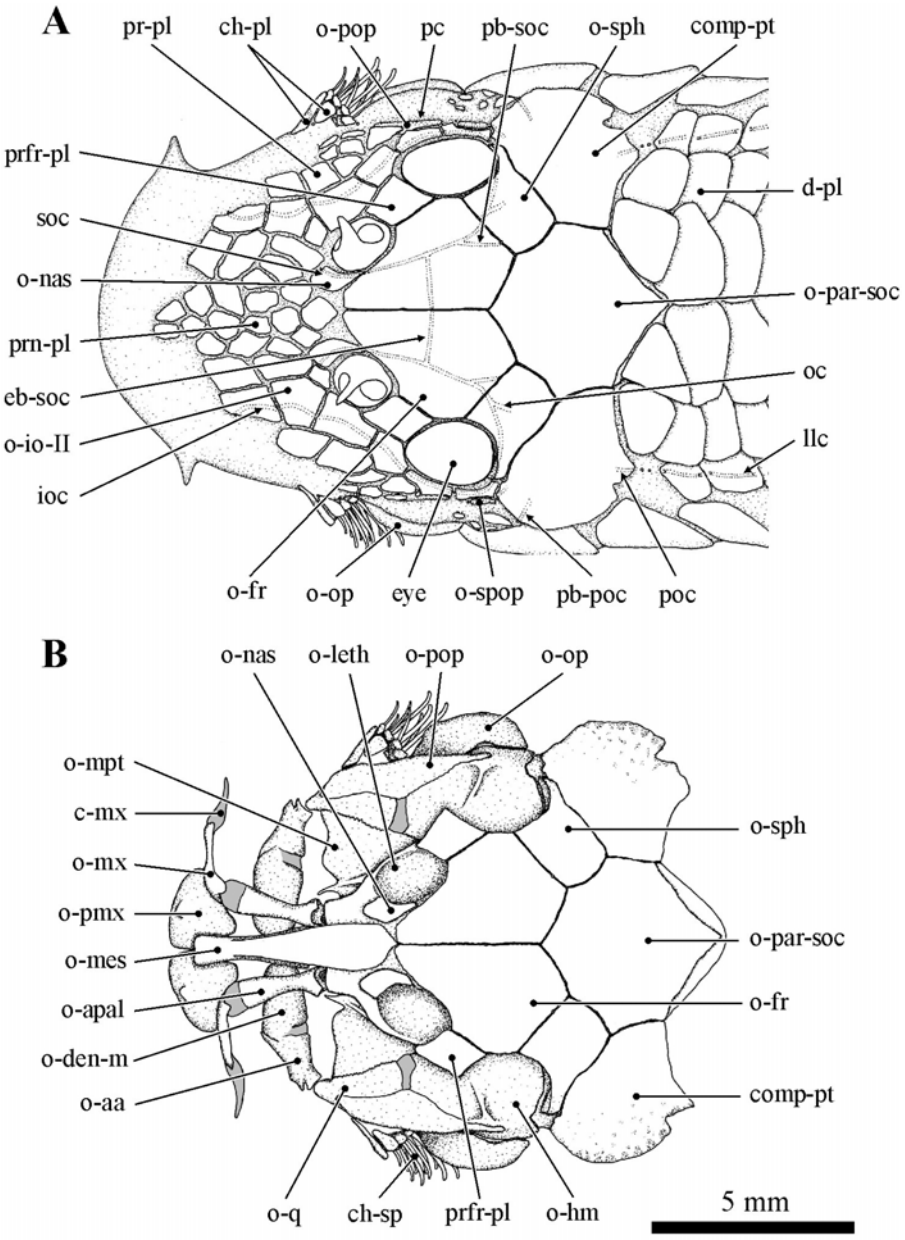

Fig. 1. – A, Dorsal view of the skull of *Ancistrus* cf. *triradiatus* (44mm SL); B, Skin and most dermal plates removed. Cartilage is indicated by grey shading. c-mx, cartilago maxillaris; ch-pl, cheek plates; ch-sp, cheek spines; comp-pt, compound pterotic bone; d-pl, dermal plate; eb-soc, epiphysial branch of supraorbital canal; ioc, infraorbital canal; llc, lateral line canal; o-aa, os anguloarticulare; o-apal, os autopalatinum; o-den-m, os dentomentomeckelium; o-fr, os frontale; o-hm, os hyomandibulare; o-io-II, os infraorbitale II; o-leth, os latero-ethmoideum; o-mes, os mesethmoideum; o-mpt, os metapterygoideum; o-mx, os maxillare; o-nas, os nasale; o-op, os operculare; o-par-soc, os parieto-supraoccipitale; o-pmx, os praemaxillare; o-pop, os praeoperculare; o-q, os quadratum; o-sph, os sphenoticum; o-spop, os suprapraeoperculare; oc, otic canal; pb-poc, pterotic branch of postotic canal; pb-soc, parietal branch of supraorbital canal; pc, preopercular canal; poc, postotic canal; pr-pl, postrostral plate; prfr-pl, prefrontal plate; soc, supraorbital canal.

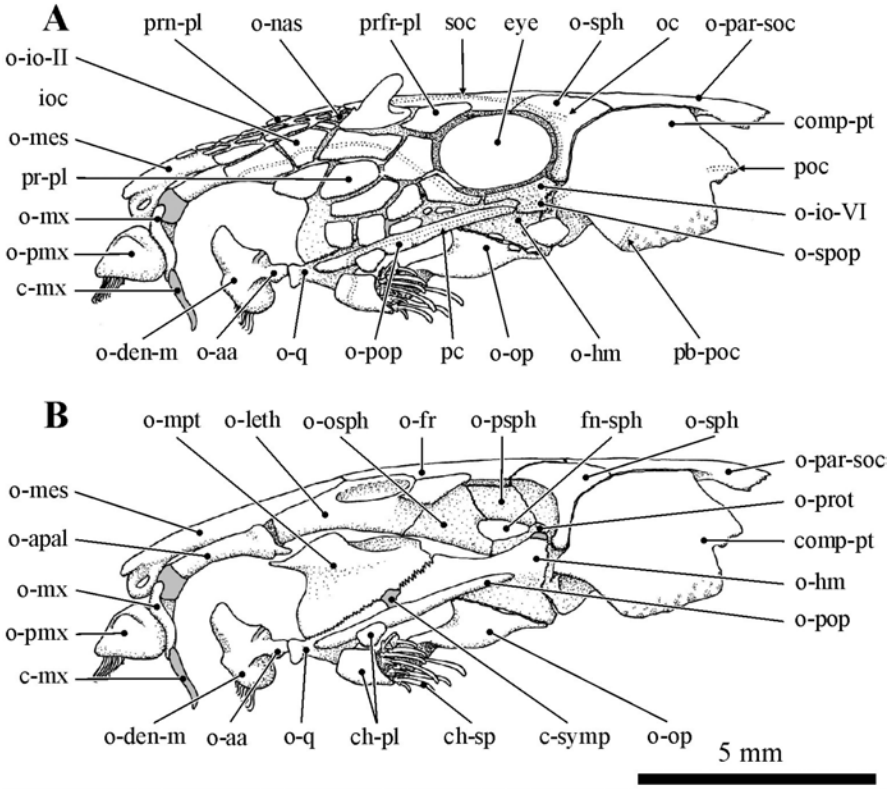

Fig. 2. – A, Lateral view of the skull of *Ancistrus* cf. *triradiatus* (44mm SL); B, Most dermal plates removed. Cartilage is indicated by grey shading. c-mx, cartilago maxillaris; c-symp, cartilago symplecticum; ch-pl, cheek plates; ch-sp, cheek spines; comp-pt, compound pterotic bone; fn-sph, fenestra sphenoidea; ioc, infraorbital canal; o-aa, os anguloarticulare; o-apal, os autopalatinum; o-den-m, os dento-mentomeckelium; o-fr, os frontale; o-hm, os hyomandibulare; o-io-II/VI, os infraorbitale II/VI; o-leth, os latero-ethmoideum; o-mes, os mesethmoideum; o-mpt, os metapterygoideum; o-mx, os maxillare; o-nas, os nasale; o-op, os operculare; o-osph, os orbitosphenoideum; o-par-soc, os parieto-supraoccipitale; o-pmx, os praemaxillare; o-pop, os praeoperculare; o-prot, os prooticum; o-psph, os pterosphenoideum; o-q, os quadratum; o-sph, os sphenoticum; o-spop, os suprapraeoperculare; oc, otic canal; pb-poc, pterotic branch of postotic canal; pc, preopercular canal; poc, postotic canal; pr-pl, postrostral plate; prfr-pl, prefrontal plate; prn-pl, prenasal platelet; soc, supraorbital canal.

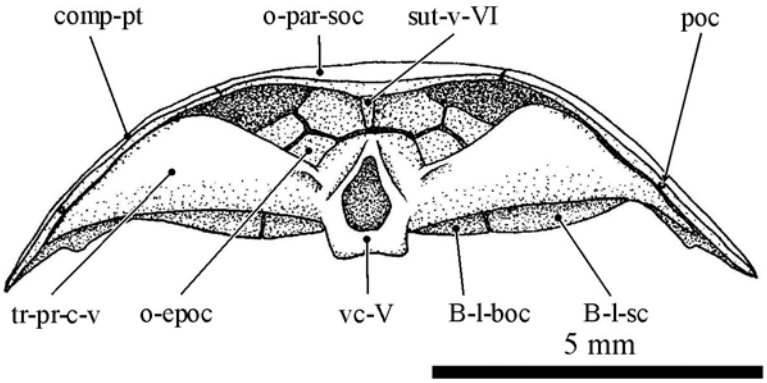

Fig. 3. – Caudal view of the neurocranium of *Ancistrus* cf. *triradiatus* (44mm SL). Cartilage is indicated by grey shading. B-l-boc, Baudelot's ligament pars basioccipitalis; B-l-sc, Baudelot's ligament pars supracleithris; comp-pt, compound pterotic bone; o-epoc, os epioccipitale; o-par-soc, os parieto-supraoccipitale; poc, postotic canal; sut-v-VI, occipital suture with neural spine of sixth vertebra; tr-pr-cv, transverse process of complex vertebra; vc-V, fifth vertebral centrum.

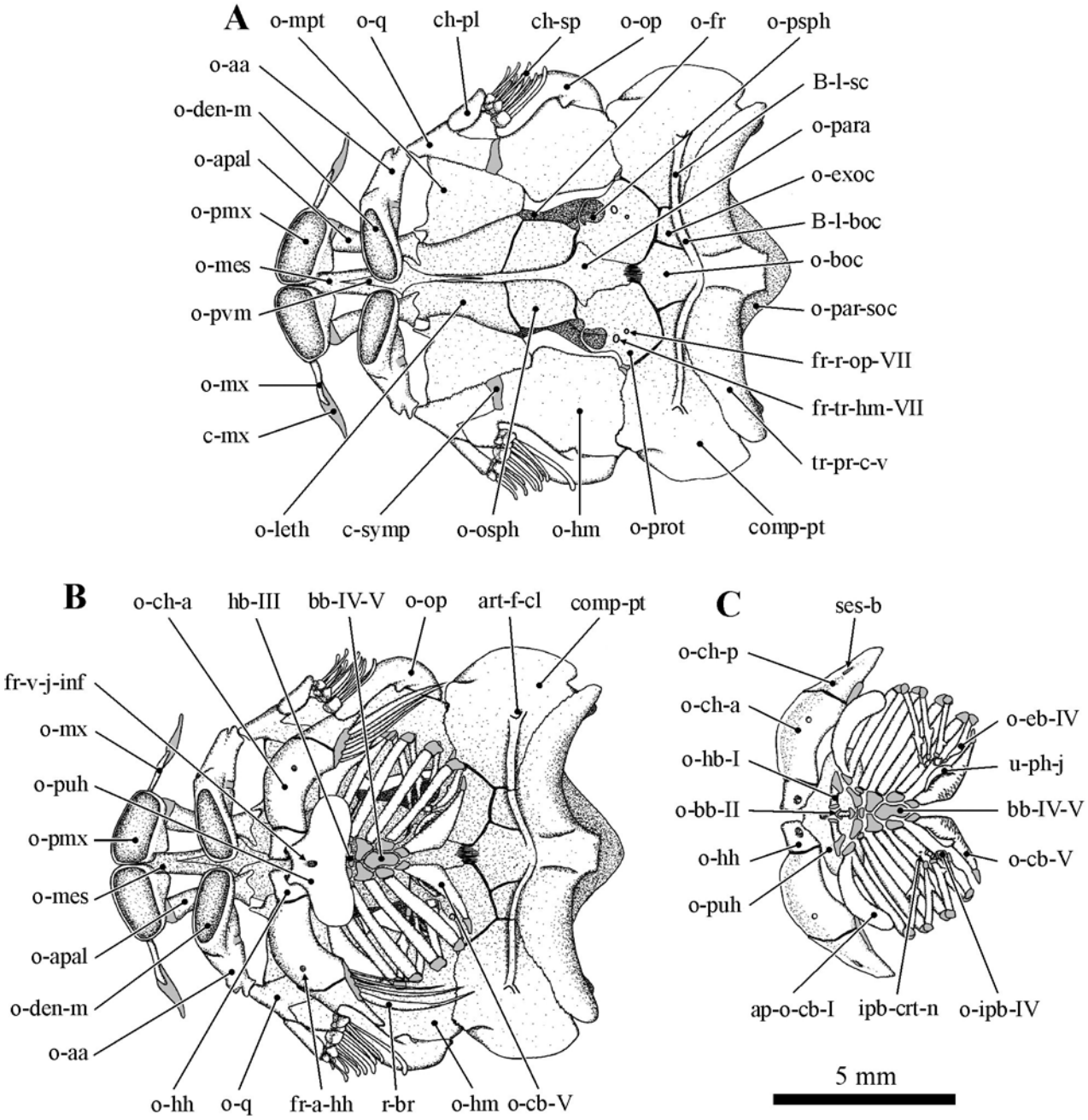

Fig. 4. – A, Ventral view of the skull of *Ancistrus* cf. *triradiatus* (44mm SL), hyoid and branchial arches removed; B, With hyoid and branchial arches; C, Dorsal view of hyoid and branchial arches. Cartilage is indicated by grey shading. ap-o-cb-I, anterior process of os ceratobranchiale I; art-f-cl, articulation facet for cleithrum; bb-IV-V, basibranchialia IV-V (posterior copula); B-l, Baudelot's ligament; c-mx, cartilago maxillaris; c-symp, cartilago symplecticum; ch-pl, cheek plates; ch-sp, cheek spines; comp-pt, compound pterotic bone; fr-a-hh, foramen for artery supplying hyohyoideus inferior; fr-v-j-inf, foramen vena jugularis inferior; hb-III, hypobranchiale III; ipb-crt-n, infrapharyngobranchial-like cartilage nucleus; o-aa, os anguloarticulare; o-apal, os autopalatinum; o-bb-II, os basibranchiale II; o-boc, os basioccipitale; o-cb-V, os ceratobranchiale V; o-ch-a, os ceratohyale anterior; o-ch-p, os ceratohyale posterior; o-den-m, os dento-mentomeckelium; o-eb-IV, os epibranchiale IV; o-exoc, os exoccipitale; o-fr, os frontale; o-hb-I, os hypobranchiale I; o-hh, os hypohyale; o-hm, os hyomandibulare; o-ipb-IV, os infrapharyngobranchiale IV; o-leth, os latero-ethmoideum; o-mes, os mesethmoideum; o-mpt, os metapterygoideum; o-mx, os maxillare; o-op, os operculare; o-osph, os orbitosphenoideum; o-para, os parasphenoideum; o-par-soc, os parieto-supraoccipitale; o-pmx, os praemaxillare; o-prot, os prooticum; o-psph, os pterosphenoideum; o-puh, os parurohyale; o-pvm, os praevomerale; o-q, os quadratum; r-br, radius branchiostegus; ses-b, sesamoid bone; tr-pr-c-v, transverse process of complex vertebra; u-ph-j, upper pharyngeal jaw.

Six infraorbital canal-bearing bones are present on either side. These bones, as well as the canal-bearing nasal, are part of the dermal plating of the snout and cheek region (Figs 1A, 2A). Between both infraorbital series, a total of 24 prenasal plates were counted in the specimen illustrated in the figures (this number varies somewhat among the examined specimens). Ventrolateral to the infraorbitals a group of postrostral plates is present, also varying in number and size, even between both sides of the same specimen (nine at left, eight at right side of drawn specimen; Figs 1A, 2A). The paired, square-shaped prefrontal plate partly covers the lateral process of the lateral ethmoid, bordering the nostril and the orbit.

Splanchnocranium (Figs 1-2-3-4)

The paired autopalatine articulates with the anterior end of the lateral ethmoid. Ventral to the articulation facet (which is directed caudodorsally) two processes are present (Fig. 4A). The tendons of the pars medialis and lateralis of the extensor tentaculi muscle insert on these medial and lateral processes respectively. Anteriorly a large cartilaginous head is present on the autopalatine, on which the bar-shaped maxilla articulates by means of a double medial process, providing two articulatory surfaces. The autopalatine splint, as seen in some other loricariids (SCHAEFER, 1987; 1997), is rudimentary in *Ancistrus* cf. *triradiatus*, fused to the anterolateral side of the autopalatine. The slightly curved maxilla is flattened, providing abundant insertion space for the retractor tentaculi muscle posteriorly. The distal end is fused to the maxillary barbel cartilage, which supports a short barbel. The premaxillae are oval to rectangular basket-shaped and provide space for developing teeth rows. One row of functional teeth (number of teeth per jaw averaging 60 to 80 in adults) inserts on the inner rostral side.

The dento-mentomeckelium and anguloarticular of which the lower jaw consists, are strongly sutured rostrally; a long splint of the dento-mentomeckelium overlies the anguloarticular ventrally. Caudally, the small cartilaginous remnant of the Meckel's cartilage connects both bones. There is no separate coronomeckelian bone. The dento-mentomeckelium resembles the premaxilla in being basket-shaped, and bearing functional and developing teeth rows. As in the premaxilla, the functional teeth (60 to 85 on average) point ventrally, as the whole lower jaw is twisted ventrally and medially. The coronoid process of the dento-mentomeckelium, and, more importantly, the high dorsal ridge of the dento-mentomeckelium and the anguloarticular serve as ample insertion surface for the adductor mandibulae muscle (Fig. 2). Laterally, each anguloarticular articulates with the quadrate; medially, both dento-mentomeckelian bones are loosely embedded in soft tissue containing a cartilaginous plug (Figs 5D-E, 8B). This configuration allows both lower jaws to move independent from each other. The cartilage plug acts as a supporting device for the free medial end of the dento-mentomeckelian bones.

The long, triangulate suspensorium is a very sturdy structure. The quadrate and the hyomandibular are tightly sutured, as well as reinforced by the preopercle that overlies their lateral surfaces, tightly coalesced to both bones (Figs 1B, 2B). Dorsally the small symplectic cartilage is situated between both bones. The hyomandibular articulates with the neurocranium at the point where the prootic, sphenotic and compound pterotic meet. The combined hyomandibular crest for the adductor mandibulae and levator arcus palatini is very conspicuous (Fig. 1B). The restricted mobility of the suspensorium is largely caused by an additional, long and almost suture-like articulation between the posterior edge of the hyomandibular and the compound pterotic (Figs 2B, 4A). Both quadrate and hyomandibular are loosely sutured to the metapterygoid (Fig. 2B). Dorsally this latter bone articulates with the lateral ethmoid, the long articulation being most rigid in the posterior half. The body of the metapterygoid and a dorsolateral lamina extending toward the autopalatine provide a groove-like housing for the pars lateralis of the extensor tentaculi muscle (Fig. 2B). Although the entire suspensorium is rather rigid, some bending along the joints and sutures allows a certain degree of movement toward the medial, while elasticity seems to return it to the resting position.

The hyoid (Fig. 4B-C) consists of paired (ventral) hypohyals, and anterior and posterior ceratohyals [anterohyal and posterohyal of SCHAEFER (1987; 1997)]. The anterior ceratohyal has synchondral joints with the hypohyal and the posterior ceratohyal. The latter joint is reinforced by means of a suture between the anterior laminae of both bones. The posterior ceratohyal has a long hinge with the medial face of the hyomandibular : halfway along the hyomandibular it has a cartilaginous articulation; at the rostral end of the hyomandibular, near the symplectic cartilage, a second, more movable ligamentous connection is present, with ligaments from the posterior ceratohyal to the preopercle and to the symplectic cartilage too. In the latter ligaments a minute sesamoid bone is found (Fig. 4C). An interhyal is not present. The rostral margin of the anterior ceratohyal adheres to the quadrate and metapterygoid with still longer ligamentous fibers. The resulting articulation between the hyoid and the suspensorium is strongest posteriorly. The articulatory configuration restricts the mobility of the hyoid to a, still considerable, oblique dorsoventral movement, reducing or enlarging the oral cavity. The anterior and posterior ceratohyals share a large, cartilaginous ventrocaudal process, with which four branchiostegal rays articulate (Fig. 4B).

The compound parurohyal bone connects the sternohyoideus muscle to the hyoid arch. It bears two rostral articulatory processes, each fitting into a cavity of one hypohyal (Fig. 4B-C). Strong ligaments keep these elements well connected. A mediodorsal ridge of the parurohyal increases the insertion surface for the sternohyoideus muscle.

In *Ancistrus* cf. *triradiatus* only the second and third basibranchials are ossified (ossification of the third is inconspicuous and only present in the largest specimens). Basibranchial I can't be distinguished, and basibranchials IV and V are fused and remain cartilaginous. Basibranchial II is connected to the parurohyal ligamentously. Hypobranchials I and II are separate from their corresponding ceratobranchials, whereas III and IV are fused to them. Hypobranchial V is reduced to such an extent that it can't be discerned from ceratobranchial V. Only

hypobranchial I is ossified. The ossified ceratobranchials I-IV are long and bar-shaped; V is flattened, has gill filaments on the anterior side only, and bears about 35 conical pharyngeal teeth. Ceratobranchial I bears an accessory process, as large as the bone itself (Fig. 4C). This process is loosely attached to the hyomandibular, and bears gill rakers on its posterior side. The process, present in many loricariids, is unique among siluriforms (SCHAEFER, 1987; ARMBRUSTER, 2004). All epibranchials except the fourth bear posterior uncinate processes, of which the third is the largest. Epibranchial I bears an additional small anterior process. Ossified infrapharyngobranchials III and IV are present, articulating with epibranchials II-IV, the prootic and the upper pharyngeal jaws; the latter being paired elongated dermal bones that bear about 25 conical teeth each. Infrapharyngobranchial III is a short bar, whereas IV is more square-formed. In front of infrapharyngobranchial III a minuscule cartilage nucleus can be seen (Fig. 4C). Whether this corresponds to infrapharyngobranchial I or II could not be determined.

Of the opercular bones, only the opercle itself is a separate structure in *Ancistrus* cf. *triradiatus*. The suprapreopercle, a simple canal bone with a ventral flange, fuses with the sixth infraorbital in large specimens, resulting in an apparently "double" canal bone (Fig. 2A). As mentioned above, the preopercle rigidly connects the quadrate and the hyomandibular in all examined specimens. It is an elongated, flattened canal bone overlying the lateral margin of these suspensorial bones, attaching them to the armoured skin. There is no interopercle, nor an interoperculo-mandibular ligament (there is, however, a lateral mandibulo-hyoid ligament). The opercle has a complex shape. It has a long joint with the suspensorium, consisting of a main articulation with a cartilaginous hyomandibular head and opercular socket, a rather stiff, bony articulation more posteriorly, and a tooth-like fortification of the joint more anteriorly (see also GEERINCKX & ADRIAENS, 2006). Movements along this hinge have an effect on the ventral process of the opercle (Fig. 2) that will push the large cheek spines, a set of very large odontodes, to a lateral, erect position. These spines insert on small bony platelets, which are embedded in ligamentous tissue and so articulate with the opercular process. Two cheek plates are present. The anteriormost cheek plate is the larger, and is situated more ventrally. It articulates with the spine-bearing platelets and the quadrate. The resulting cheek-spine mechanism has been described and discussed recently (GEERINCKX & ADRIAENS, 2006).

Jaw musculature (Figs 6-7-8)

Loricariid jaw musculature is highly complex. In addition, the homology between the adductor mandibulae subdivisions as described in loricariids and A1, A2 and A3 sections has been a matter of debate (ALEXANDER, 1965; WINTERBOTTOM, 1974; HOWES, 1983; SCHAEFER, 1997; DIOGO, 2005). Ideally, developmental studies, and a comparative examination of several loricarioid families should be done in order to clarify all possible homologies and de novo formations. At this moment it is most appropriate to describe muscle divisions according to their position, presumed function and (eventual) previous nomenclature in loricariids.

In the jaw muscle complex of *Ancistrus* cf. *triradiatus*, different muscle divisions can be discerned. The main part of the complex is the adductor mandibulae sensu stricto [muscle b of HOWES (1983)]. Two main subdivisions can be discerned. The longest, external bundle originates on the preopercle and on the lateral surface of the hyomandibular, anterior to, and on the prominent lateral hyomandibular ridge, and inserts on the high dorsal ridge of the dento-mentomeckelium and the anguloarticular of the lower jaw. The shorter, more ventral and interior part of the muscle, hidden below the external one, consists of two flat, distinct bundles (Fig. 6B) that originate on the quadrate, the hyomandibular and the metapterygoid. They insert dorsocaudally on the meckelian cartilage and the body of the anguloarticular. Experimentally pulling the adductor mandibulae results in an adduction of the mandible, but also rotates it, so that the teeth row swings anteriorly. This rotation appears to be due to the rather dorsal insertion on the lower jaw of the muscle.

Medial to the dorsal bundle of the adductor mandibulae lies the retractor premaxillae [muscle c of Howes (1983)], which also originates on the hyomandibular. Manipulation of the muscle pivots the premaxilla around its dorsal articulation. The result is analogous to that of the combined adductor mandibulae bundles on the lower jaw : the teeth scrape on the substrate where the fish is lying on.

The third part of the complex is the thin and strap-like retractor veli [muscle d of HOWES (1983); Figs 6B, 7C], originating posteriorly on the metapterygoid, and running medial to the retractor premaxillae. Almost a third of the muscle is composed of the thin aponeurosis, from which the fibers diverge in the oral valve (or velum). The presence of these collagen fibers running in both halves of the oral valve was shown by the Verhoeff-Van Gieson's stain methods. The collagen fibers intermingle at the midline where they enclose a band of elastic tissue running rostrocaudally (Fig. 5B-C), connecting the valve with the cartilage-like tissue between both premaxillae. The soft connective tissue between the autopalatine and the metapterygoid is loosely attached to the aponeurosis, but pulling the muscle has no apparent effect other than retracting the valve backward.

The dorsal retractor tentaculi [muscle a of HOWES (1983); Figs 6A, 7A] overlies the other muscles of the cheek region. It is a broad band-like muscle, originating from an anterior ridge on the ventral side of the lateral ethmoid. Two thirds of the muscle fibers run straight to the posterior face of the maxilla; the other third, comprising the lateroventral fibers, runs in a slightly more lateral direction, inserting closer to the distal tip of the bone. Contraction of the muscle appears to pull the tip of the maxilla in a caudodorsal direction. Due to the position of the bone and the maxillary barbel cartilage (where upper and lower lip meet) the lateral lip tissues are retracted toward the dorsal.

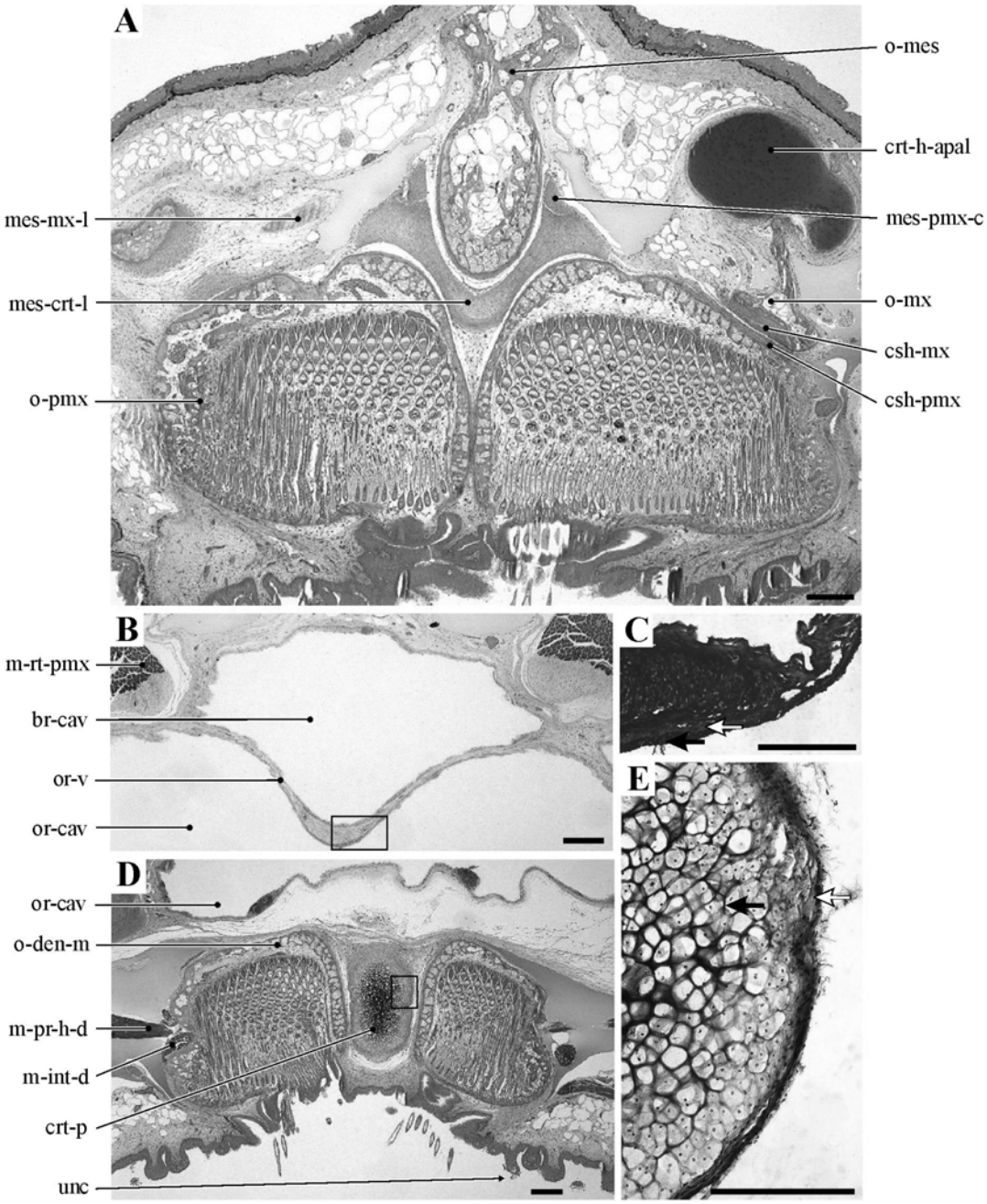

Fig. 5. – A, Section at the level of the premaxillae, showing the mesethmoid-premaxillary cartilage, some of the rostral ligaments and the tissue cushions between the premaxilla and the proximal head of the maxilla. Notice the close contact between both premaxillae. B, Section at the level of the rostral half of the oral valve, indicating the cartilage-like medial band, cut transversely. C, Detail of oral valve (cf. rectangle in fig. B), showing the elastic cartilage band (black arrow) and the collagen fibers (white arrow). D, Section at the level of the dento-mentomeckelian bones, indicating their relation to the cartilage plug attached to the hyoid. Notice the papillae with epidermal brushes or unculi on the lower lip. E, Detail of cartilage plug, showing the elastic cartilage (black arrow) and the thin sheet of perichondral collagen fibers (white arrow). (A, B, D, Technovit sections, toluidine blue stain. C, E, paraffin sections of other specimen, Verhoeff-Van Gieson's stains. Scale bars are 200 µm.) br-cav, branchial cavity; crt-h-apal, cartilaginous head of autopalatine; crt-p, cartilage plug; csh-mx, cushion on maxilla; csh-pmx, cushion on premaxilla; m-int-d, musculus intermandibularis pars dentalis; m-pr-h-d, musculus protractor hyoidei pars dentalis; m-rt-pmx, musculus retractor premaxillae; mes-crt-l, mesethmoid-cartilage ligament; mes-mx-l, mesethmoid-maxillary ligament; mes-pmx-c, mesethmoid-premaxillary cartilage; o-den-m, os dento-mentomeckelium; o–mes, os mesethmoideum; o-mx, os maxillare; o-pmx, os praemaxillare; or-cav, oral cavity; or-v, oral valve or velum; unc, unculi or unicellular keratinized brushes.

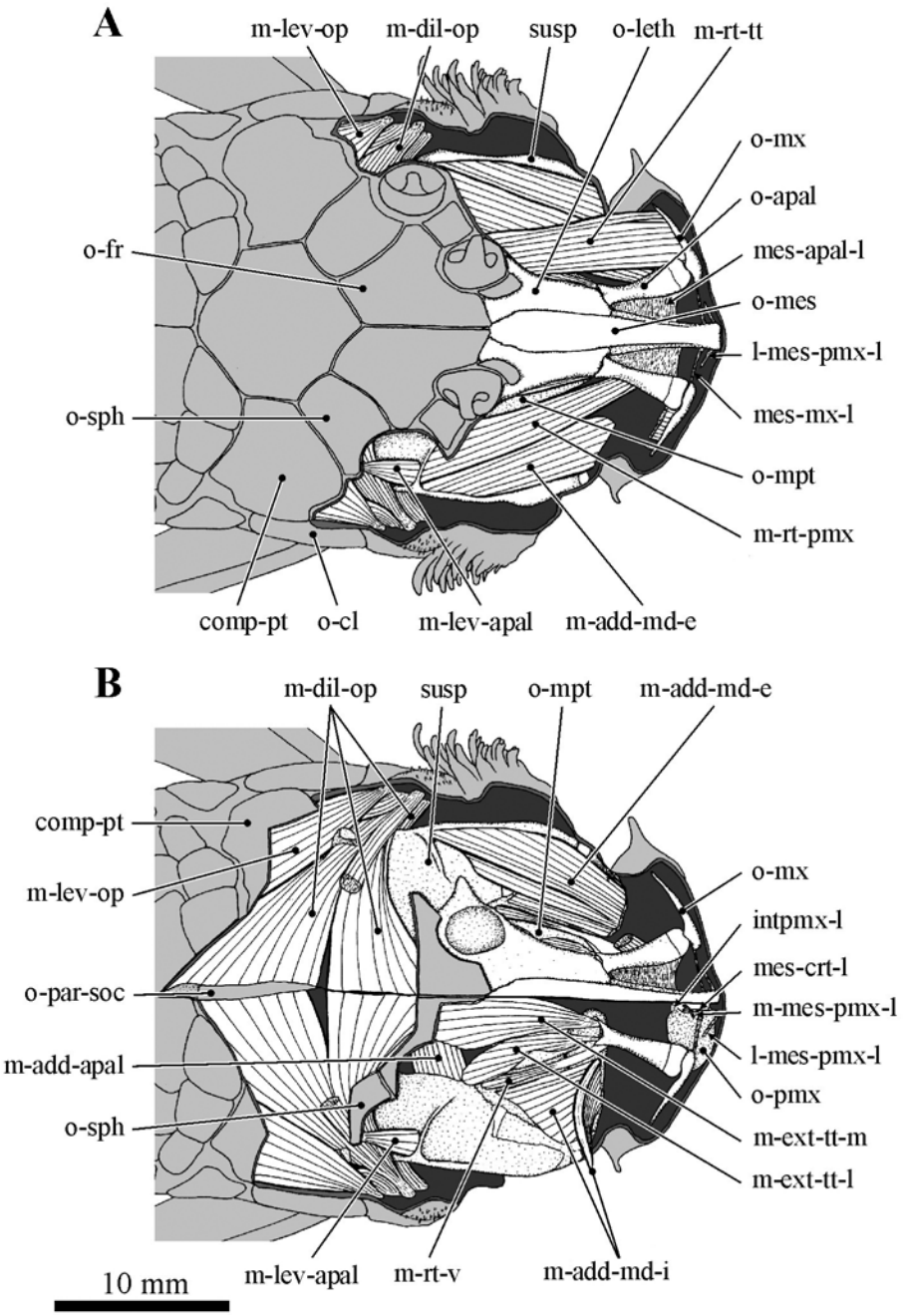

Fig. 6. – Dorsal view of cranial musculature of *Ancistrus* cf. *triradiatus* (94mm SL); A, Part of skin and dermal plates, and right retractor tentaculi muscle removed; B, Dissection showing deeper muscles (medial bundle of right adductor mandibulae muscle partly removed). comp-pt, compound pterotic bone; intpmx-l, interpremaxillary ligament; l-mes-pmx-l, lateral mesethmoid-premaxillary ligament; m-add-apal, musculus adductor arcus palatini; m-add-md-e, external part of musculus adductor mandibulae; m-add-md-i, internal part of musculus adductor mandibulae; m-dil-op, musculus dilatator operculi; m-ext-tt-l, musculus extensor tentaculi pars lateralis; m-ext-tt-m, musculus extensor tentaculi pars medialis; m-lev-apal, musculus levator arcus palatini; m-lev-op, musculus levator operculi; m-mes-pmx-l, medial mesethmoid-premaxillary ligament; m-rt-pmx, musculus retractor premaxillae; m-rt-tt, musculus retractor tentaculi; m-rt-v, musculus retractor veli; mes-apal-l, mesethmoid-autopalatine ligament; mes-crt-l, mesethmoid-cartilage ligament; mes-mx-l, mesethmoid-maxillary ligament; o-apal, os autopalatinum; o-cl, os cleithrum; o-fr, os frontale; o-leth, os latero-ethmoideum; o-mes, os mesethmoideum; o-mpt, os metapterygoideum; o-mx, os maxillare; o-par-soc, os parieto-supraoccipitale; o-pmx, os praemaxillare; o-sph, os sphenoticum; susp, suspensorium.

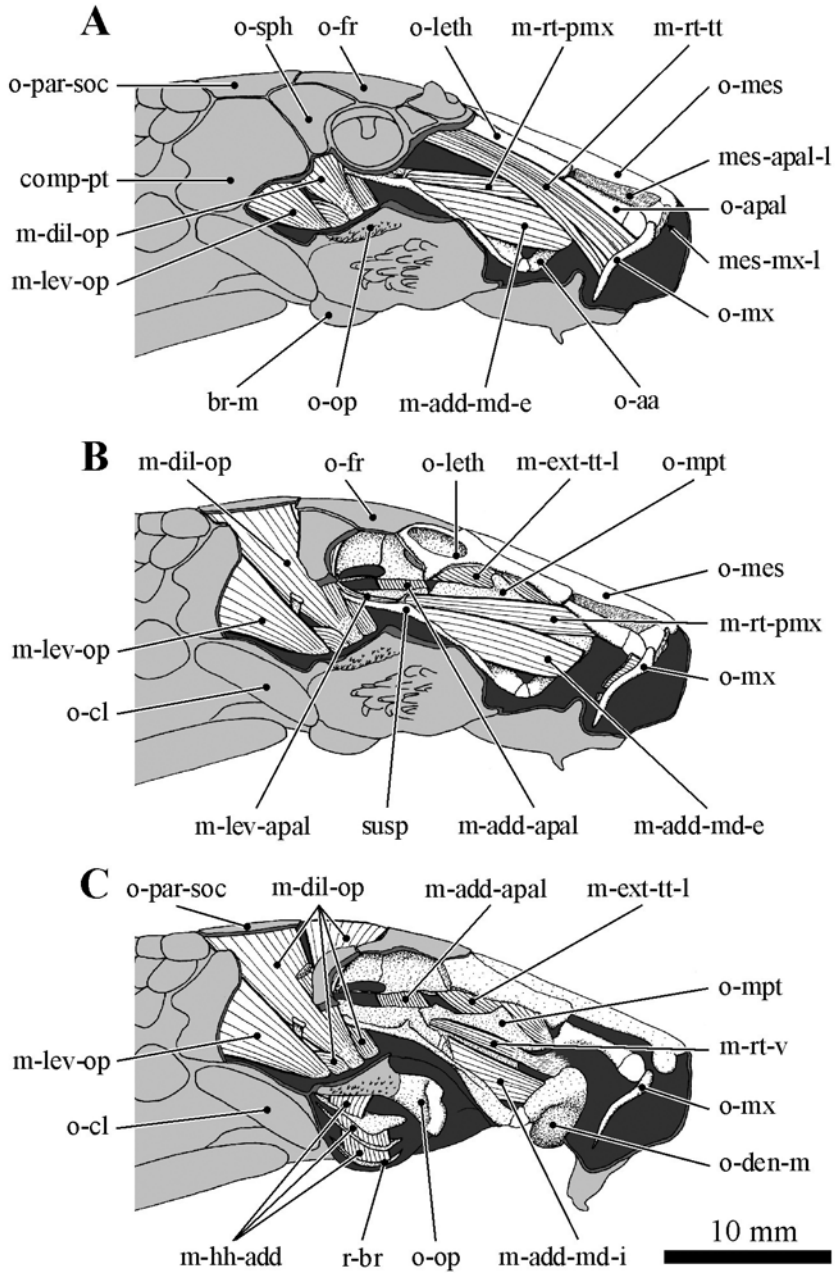

Fig. 7. – Lateral view of cranial musculature of *Ancistrus* cf. *triradiatus* (94mm SL); A, part of skin and dermal plates removed; B-C, Further dissections showing deeper muscles. br-m, branchiostegal membrane; comp-pt, compound pterotic bone; m-add-apal, musculus adductor arcus palatini; m-add-md-e, external part of musculus adductor mandibulae; m-add-md-i, internal part of musculus adductor mandibulae; m-dil-op, musculus dilatator operculi; m-ext-tt-l, musculus extensor tentaculi pars lateralis; m-hh-add, musculi hyohyoidei adductores; m-lev-apal, musculus levator arcus palatini; m-lev-op, musculus levator operculi; m-rt-pmx, musculus retractor premaxillae; m-rt-tt, musculus retractor tentaculi; m-rt-v, musculus retractor veli; mes-apal-l, mesethmoid-autopalatine ligament; mes-mx-l, mesethmoid-maxillary ligament; o-aa, os anguloarticulare; o-apal, os autopalatinum; o-cl, os cleithrum; o-den-m, os dento-mentomeckelium; o-fr, os frontale; o-leth, os latero-ethmoideum; o-mes, os mesethmoideum; o-mpt, os metapterygoideum; o-mx, os maxillare; o-op, os operculare; o-par-soc, os parieto-supraoccipitale; o-sph, os sphenoticum; r-br, radius branchiostegus; susp, suspensorium.

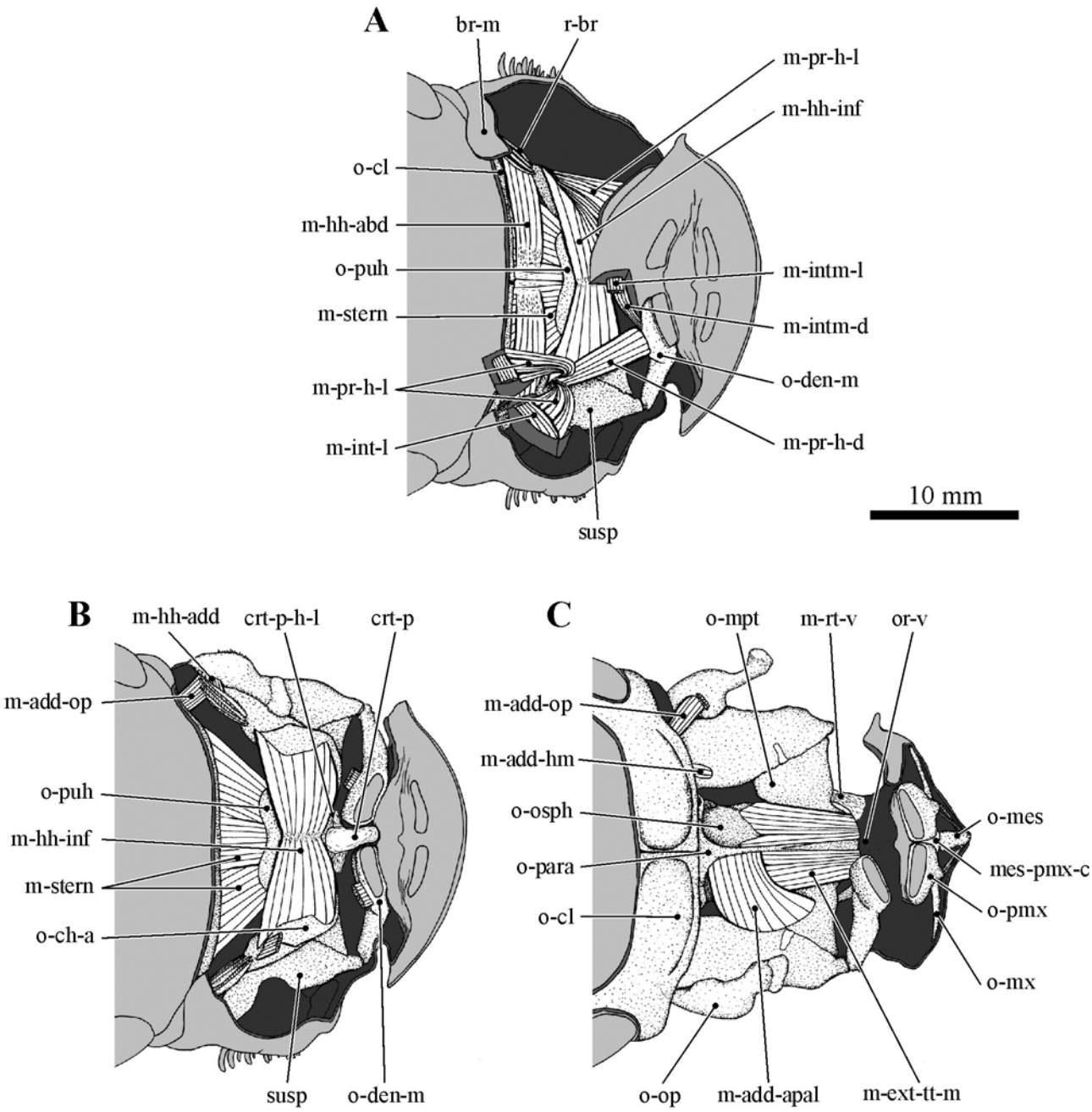

Fig. 8. – Ventral view of cranial musculature of *Ancistrus* cf. *triradiatus* (94mm SL); A, part of skin, left half of lower lip and associated muscles removed; B-C, Further dissections showing deeper muscles. br-m, branchiostegal membrane; crt-p, cartilage plug; crt-p-h-l, cartilage plug-hyoid ligaments; m-add-apal, musculus adductor arcus palatini; m-add-hm, musculus adductor hyomandibulae; m-add-op, musculus adductor operculi; m-ext-tt-m, musculus extensor tentaculi pars medialis; m-hh-abd, musculus hyohyoideus abductor; m-hh-add, musculi hyohyoidei adductores; m-hh-inf, musculus hyohyoideus inferior; m-intm-d, musculus intermandibularis pars dentalis; m-intm-l, musculus intermandibularis pars labialis; m-pr-h-d, musculus protractor hyoidei pars dentalis; m-pr-h-l, musculus protractor hyoidei pars labialis; m-rt-v, musculus retractor veli; m-stern, musculus sternohyoideus; mes-pmx-c, mesethmoid-premaxillary cartilage; o-ch-a, os ceratohyale anterior; o-cl, os cleithrum; o-den-m, os dento-mentomeckelium; o-mes, os mesethmoideum; o-mpt, os metapterygoideum; o-mx, os maxillare; o-op, os operculare; o-osph, os orbitosphenoideum; o-para, os parasphenoideum; o-pmx, os praemaxillare; o-puh, os parurohyale; or-v, oral valve or velum; r-br, radius branchiostegus; susp, suspensorium.

The intermandibularis muscle consists of two separate divisions (Fig. 8A). The dorsalmost, here called pars dentalis, connects the posterior aspects of both lower jaws, inserting in a groove along the teeth-bearing dento-mentomeckelian bones. Medially, where the contralateral halves meet in a slim raphe, they attach firmly to the inner tissue of the lower lip. The effect of this muscle is not clear, but it appears to pull the mandibles together and rotate them backward, which causes the lower lip to purse toward the substrate. The pars labialis of the intermandibularis is a separate division, attached to both lateral sides of the inner lower lip tissue ventral and caudal to the pars dentalis. Laterally, the fibers run into the connective tissue posterior to the basis of the maxillary barbel. Contrary to the pars dentalis, some muscle fibers do cross the midline, and there is no raphe. Manipulation of this muscle part leads to pursing of the lower lip. There is no muscular contact between the intermandibularis and the subdivisions of the protractor hyoidei.

Hyoid musculature (Fig. 8)

The protractor hyoidei muscle, often referred to as geniohyoideus (OSSE, 1969; SCHAEFER & LAUDER, 1986; SCHAEFER, 1997) consists of two completely separate subdivisions having different directions and insertions (Fig. 8A). Terminology in literature can be confusing, as the names often refer to the relative position of these subdivisions, but these can vary from taxon to taxon. Hence names referring to the insertions are given here. A separate, band-like subdivision is the protractor hyoidei pars dentalis [part a of HOWES (1983); lateral division of SCHAEFER (1997)], connecting the ventral side of the posterior ceratohyal to the ventrocaudal side of the dento-mentomeckelium. Pulling the pars dentalis retracts and rotates the mandibles around their axis and brings the teeth rows in a position ready for scraping; the movement of the mandibles automatically retracts the lower lip somewhat. The hyoid stays almost motionless. Another subdivision is the pars labialis [part b of HOWES (1983); medial division of SCHAEFER (1997)]. It runs between the posterior ceratohyal and the lower lip tissue. It diverges in several small bundles before reaching the lip. The effect appears to be on the lower lip only : it is flattened and retracted dorsally. Again, no significant movement of the hyoid is observed.

The largest ventral muscle is the unpaired hyohyoideus inferior (or hyohyoides inferior), lying ventral to the hyoid arch (Fig. 8B). It is narrower at the midline, where a medial raphe connects both halves. Fiber direction is from the medial raphe toward the lateral attachment on the ventral side of the anterior and posterior ceratohyals. As suggested by manipulation, any contraction elevates the hyoid, reducing the branchial cavity. An additional, indirect effect, probably caused mainly by the caudalmost fibers, appears to be on the proximal ends of the branchiostegal rays that articulate with the hyoid arch. As a result, the branchiostegal membrane is opened slightly.

The antagonist of the hyohyoideus inferior is the sternohyoideus muscle that diverges from the dorsal surface of the parurohyal to the anterior edge of the cleithrum of the pectoral girdle. The muscle is much broader than long. As the pectoral girdle is an almost immobile structure in *Ancistrus* cf. *triradiatus*, the only effect of manipulating the sternohyoideus is the retraction and depression of the hyoid arch. Although some movement of the cartilage plug anterior to the hyoid arch is observed, no effect is seen on the mandibles. The almost cylindrical plug of cartilage is connected to the ventral face of the hypohyals, projecting rostrally, between both dento-mentomeckelia. The cartilage is elastic, while no collagen is seen except for a very thin perichondral layer. No collagen fibers attach to the dento-mentomeckelian bones (Fig. 5D-E).

The two medialmost branchiostegal rays are kept close to each other by means of short ligamentous fibers. The second and the third, and the third and the fourth, respectively, are interconnected by short muscles, running from the lateral edge of the medial to the dorsal surface of the more lateral ray. From the ventral face of the fourth, lateralmost and broadest ray, an additional, narrowing muscle attaches to the medial aspect of the opercle. These three different muscles are collectively referred to as the hyohyoidei adductores (Figs 7C, 8B). They force the branchiostegal rays together and toward the opercle which results in an adduction of the branchiostegal membrane and, hence, the closure of the gill opening.

The first, medial branchiostegal ray provides insertion for the hyohyoideus abductor, a flat and straight transverse muscle projecting toward the midline, which it does not reach (Fig. 8A). At four fifths of its length it continues as a thin aponeurosis and connects to the ventral fascia of the sternohyoideus, thus only forming indirect contact with its counterpart. Pulling one or both abductors unambiguously closes the branchiostegal membrane.

Suspensorial musculature (Figs 6-7-8)

The levator arcus palatini is a minute muscle running obliquely from the sphenotic (medial to the emergence of the preopercular canal) to the hyomandibular, where it inserts on the posterior side of the ridge that also forms the attachment site for the adductor mandibulae. Attachment is muscular and relatively broad on the hyomandibular and tendinous and slim on the sphenotic (Figs 6B, 7B). These observations contradict HOWES' (1983) statement that the muscle joins the dilatator operculi in *Ancistrus*. The muscle is too small to allow manipulation, but probably can only slightly elevate the well-attached suspensorium.

A prominent adductor arcus palatini connects the medial face of the suspensorium (hyomandibular and metapterygoid) with the base of the neurocranium. Origin is on the ventral ridge of the parasphenoid as well as a large surface of the orbitosphenoid. Manipulation of the muscle brings the suspensorium to a more medial position.

In siluriforms the extensor tentaculi is a muscle derived from the adductor arcus palatini and the antagonist of the retractor tentaculi (WINTERBOTTOM, 1974). In *Ancistrus* cf. *triradiatus*, as in other loricariids (HOWES, 1983; SCHAEFER, 1997), the extensor tentaculi is divided in two completely separate elements, which we will refer to as the pars lateralis and pars dentalis (Fig. 8C). The extensor tentaculi pars lateralis [muscle e of HOWES (1983)] inserts on the ventrolateral process of the autopalatine. The muscle is circular in transsection and has an anterior aponeu-

rosis continuing halfway inside the muscle itself. Fibers radiate from this aponeurosis. Insertion space for this "circularly pinnate" muscle is provided by a bony canal formed by the ventral face of the lateral ethmoid and a groove formed by the lateral face and a lateral process of the metapterygoid. Pulling the muscle in fresh specimens results in a lateroventral swinging of the autopalatine and a corresponding ventral movement of the tip of the maxilla, as both are coupled through their articulation and the mesethmo-maxillary ligament. The maxillary barbel and the lateral parts of the lips are consequently pushed against the substrate.

The second segment, the extensor tentaculi pars medialis [muscle f of HOWES (1983)], is a more flattened muscle connecting the longer ventromedial process of the autopalatine with the lateral ethmoid, the orbitosphenoid and the lateral side of the ventral parasphenoid ridge. The tendon inserting on the autopalatine continues as a ventral aponeurosis to which the slightly dorsally oriented fibers attach. The effect of pulling the muscle is a ventral movement of the autopalatine. The autopalatine-maxillary articulation pushes the caudal edge of the premaxilla downward, pivoting the premaxilla around its ligamentous articulation with the mesethmoid. Due to the configuration of the articulation, effect on the distal tip of the maxilla itself is negligible. The abduction of the premaxilla brings the teeth in a position ready for scraping. The extensor tentaculi pars medialis can thus be considered the antagonist of the retractor premaxillae.

The small and extremely thin adductor hyomandibulae originates from the ventral floor of the compound pterotic (together with the adductor operculi), and loosely inserts on connective tissue at the medial side of the hyomandibular (Fig. 8C). Given its size, it is not easy to manipulate it, or to even speculate about its function. The name adductor hyomandibulae has been used for the adductor arcus palatini in loricariids (HOWES, 1983; SCHAEFER, 1997). This is erroneous, as both muscles are not homologous (WINTERBOTTOM, 1974; DIOGO & VANDEWALLE, 2003).

Opercular musculature (Figs 6-7-8)

The largest opercular muscle is the dilatator operculi, which has its origin on a large surface on the posterior part of the neurocranium and inserts via a thick aponeurosis on the dorsal side of the opercle, lateral to the articulation with the hyomandibular (Figs 6B, 7C). Its different bundles originate mainly on the compound pterotic, parieto-supraoccipital, sphenotic and the posterior margin of the hyomandibular. Due to the configuration of the hyomandibular-opercular articulation, any force exerted via the aponeurosis rotates the opercle dorsally, erecting the long cheek spines. In large adults some bones of the skull roof and walls show expanded ventral or medial laminae separating the dilatator operculi from the braincase.

Immediately posterior to the insertion of the dilatator operculi on the opercle, the short and sturdy aponeurosis of the levator operculi inserts. It has a broad origin on the compound pterotic. The effect of pulling this muscle is identical to that of the dilatator operculi. Experimental tension on both muscles indicates no role in the opening of the branchiostegal membrane.

The antagonist of these muscles is the adductor operculi (Fig. 8C). It has its tendinous origin on the ventral transverse rim of the compound pterotic contacting the Baudelot's ligament. It inserts muscularly on the medial side of the opercle just posterior to the insertion of the lateralmost division of the hyohyoidei adductores.

The musculature of the branchial basket is not discussed here. See SCHAEFER (1997) for a description of the branchial muscles in *Otocinclus* and FERNANDES et al. (1995) for a short account on the gill filament muscles in *Hypostomus* and *Rhinelepis*.

Ligaments in the rostral region

The mesethmoid, lacking the lateral cornua typical for most siluriforms but bearing a ventral disc-like process, influences the mobility of the upper jaw. The mesethmoid-premaxillary cartilage or meniscus rests on the ventral edge of the mesethmoid disc like a small cap and forms two articulatory cups for the premaxillae (Fig. 8C). It is *V*-shaped in transsection (Fig. 5A). Strong connective tissues and ligaments in the rostral region ensure the relative position of these elements. The mesethmoid-cartilage ligaments connect the rostral tip of the mesethmoid with both posterodorsal sides of the mesethmoid-premaxillary cartilage, running along both sides of the mesethmoid disc (Fig. 6B). The lateral mesethmoid-premaxillary ligaments attach to the anterodorsal side of both premaxillae and the rostral end of the mesethmoid, thus running more or less horizontally (Fig. 6A). The medial mesethmoid-premaxillary ligaments run vertically from the anterior face of the premaxillae to the mesethmoid disc right above it (Fig. 6B). The paired mesethmoid-maxillary ligament, as mentioned above, connects the maxilla, close to its ventral head, to the mesethmoid, immediately behind the other ligament attachments (Figs 6A, 7A). The short interpremaxillary ligament is an unpaired ligament, running transversely behind the mesethmoid-premaxillary cartilage. It keeps both premaxillae closely apposed to each other, restricting their relative movement. The mesethmoid-autopalatine ligament is a broad ligament between the mesethmoid shaft and the autopalatine; the anteriormost fibers are longest, and also contact the autopalatine-maxillary articulatory tissue (Figs 6A, 7A). The paired rostromaxillary and ventral labial ligaments as seen by Schaefer (1997) in *Otocinclus vittatus* are not observed in *Ancistrus* cf. *triradiatus*.

Kinematics (Figs 9-10)

Respiratory cycles while sucking onto a vertical glass wall took approximately 250 to 400ms (with a water temperature of 23°C). The fishes preferred to support themselves with their tail on the bottom of the aquarium. In a first phase the oral cavity expands by a depression of the hyoid (and lower jaw region), and a slight abduction of the suspensoria (Figs 9, 10A). During this expansion phase the oral valve is open, and the branchiostegal membrane is closed (Fig. 10A, E). At maximal expansion the oral valve is closing. Complete closure is observed only well after the onset of elevation of the hyoid and lower jaw region, and adduction of the suspensoria (Fig. 10F). Movements of these latter elements are synchronous. At maximal constriction of the oral cavity the oral valve is

still closed; it bulges out ventrally, probably due to the water pressure inside the mouth. It starts to open again only when a new expansion phase starts. During constriction the branchiostegal membrane opens, allowing water to flow out (Fig. 10B, F). This water flow could be visualized by the use of diluted milk. At maximal constriction the branchiostegal membrane starts to close again (Fig. 10C). The same milk experiment also shows the inflow of water through the narrow openings created in the lateral lip tissue by an elevation of the maxillary barbel. The opening and closure of this inflow opening coincides with the same movements of the oral valve (Fig. 10D). Sometimes only one of both sides of the lips is opening. During the whole cycle, opercular movements are restricted to a very small in- and outward movement, synchronous with the suspensorial movements.

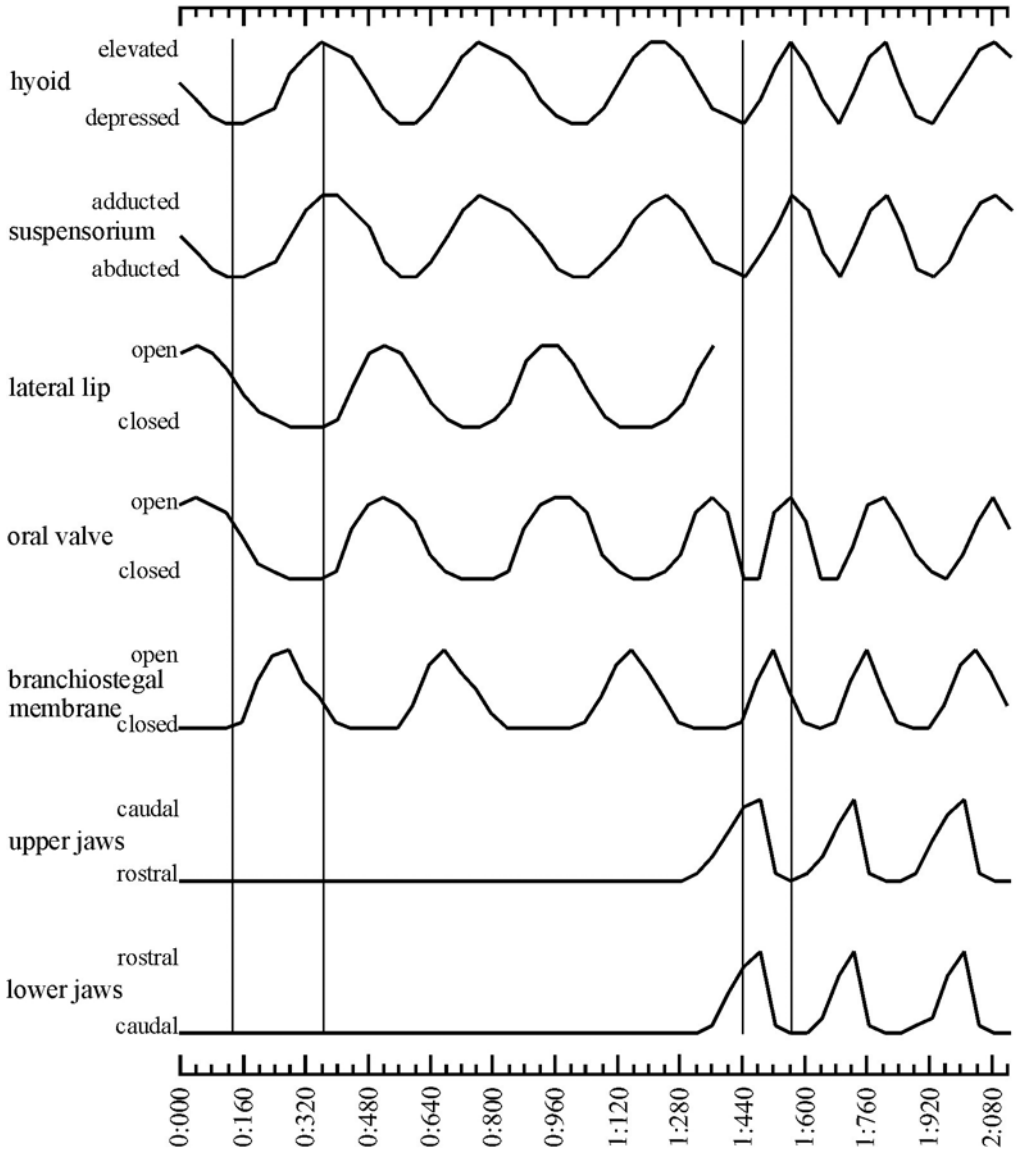

Fig. 9. – Graphs showing movements of some elements involved in respiration and feeding of *Ancistrus* cf. *triradiatus* during two seconds. Three respiratory cycles are followed by three cycles in which scraping occurs. Vertical reference lines indicate maximal oral expansion and constriction during first and fourth cycle. Graphs do not show distance of movements. Slight up and down motion of the lower jaws is not recorded; feeding movements inhibit unambiguous observation of lateral lip movements.

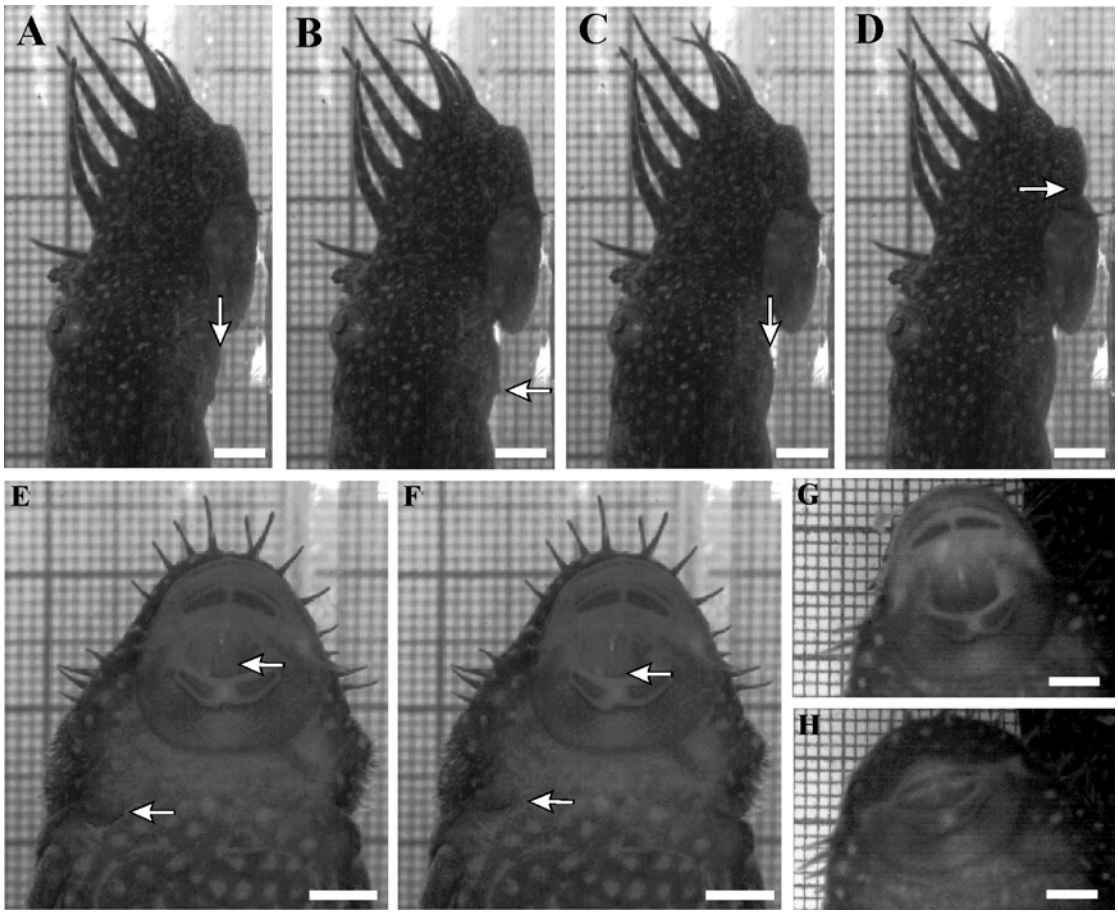

Fig. 10. – Images from respiratory and feeding cycles from two specimens (A-D; E-H). A-D, respiration (lateral view). A, maximal hyoid depression; B, complete opening of branchiostegal membrane; C, maximal hyoid elevation D, inflow via lip opening; E-F, respiration (ventral view). E, oral valve open (branchiostegal membrane closed); F, oral valve closed (moved caudally) (branchiostegal membrane open). G-H, feeding (ventral view). G, upper and lower jaws diverge; H, jaws scrape (move back and rotate). Scale bars are 5mm.

Respiratory cycles accelerate during feeding (Fig. 9). In addition, extensive rotation of both lower and upper jaws occurs. During the constriction phase the mandibles not only depress, but also move posteriorly. An additional rotation of the jaws along their axis brings the teeth rows even further posteriorly. At the same time, the premaxillae protrude anteriorly by a rotation around their hinge to the mesethmoid disc (Fig. 10G). During the subsequent expansion phase the mandibles move and rotate so that the tooth row scrapes the glass surface in an anterior direction. An analogous action occurs in the premaxillae : the tooth row scrapes the surface in a posterior direction. At peak expansion the teeth of lower and upper jaws almost touch each other (Fig. 10H). The rough, papillose surface of the large lower lip moves posteriorly and anteriorly together with the lower jaw. Fishes often proceed somewhat anteriorly during each cycle, so that a fresh substrate can be fed upon. Opening of the lateral lip tissue is less pronounced and difficult to observe during feeding. The extensive jaw movements might well allow a sufficient inflow of water. Often (but not always) body and tail movements accompany the feeding sequences.

DISCUSSION

Anatomical observations, aided by the manipulation of muscles in fresh specimens, yielded some remarkable morphological considerations. Together with the limited kinematic data set functional hypotheses could sometimes be formulated. Here we group the morphological structures and kinematic facts in six known functional mechanisms present in loricariids, providing a comprehensive overview of elements, movements, and possible functional-morphological links. The morphological basis of some typical loricariid structures or systems is also discussed and compared to non-loricariids.

The buccal pump system

In *Ancistrus* cf. *triradiatus*, oral expansion has a double function : inflow of water and food, and the maintenance of the suction power when adhering to a substrate. In fishes with a depressed head, the hyoid depression generally has a greater share in the buccal pump system than the suspensorium abduction (Alexander, 1970; Gosline, 1973; Adriaens & Verraes, 1997a). The sternohyoideus

and hyohyoideus inferior muscles, respectively depressing and elevating the hyoid arch in many fishes (e.g., BALLINTIJN & HUGHES, 1965), are among the largest and most vascularized muscles in *A.* cf. *triradiatus*, indicating their importance in this system. The articulation between the hyoid arch and the suspensorium is long and strong, compared to several other teleosts (ANKER, 1974; AERTS, 1991; HUNT VON HERBING et al., 1996). The large suspensorium is rigid (the metapterygoid is connected to the other bones with sutures), and anchored to the skull via the posterior hyomandibular-pterotic suture and the dorsal connection of both the metapterygoid and the hyomandibular to the neurocranium. It is also connected with the tough, armoured skin at the level of the preopercle, even more reducing its mobility. These connections can all be considered reinforcements of the hyoid-suspensorium system [some are characteristic for loricariids or the more apomorphic loricariid taxa (SCHAEFER, 1990; ARMBRUSTER, 2004)], and might be adaptations to the high forces probably generated during suction while adhering to a substrate.

The ossified Baudelot's ligaments, vertical ridges between the basioccipital and the supracleithral part of the compound pterotic, form an ossified posterior wall of the branchial cavity, which ALEXANDER (1965) regarded as an adaptation to withstand the negative pressure caused by the suction, hydrostatically isolating the orobranchial cavity from the intestinal cavity. The relative resilience of the suspensorium and the strengthening of the suspensorial-neurocranial connection could be interpreted in a similar way : a weaker and more movable suspensorium would be susceptible to collapsing when extreme suction is needed.

Figures 6B and 8C clearly show that the masses of the two muscles inserting on the suspensorium are far from equal : the adductor arcus palatini is large, while the levator arcus palatini is almost rudimentary. It can be hypothesized that the relatively large adductor arcus palatini can cause a slight adduction of the suspensorium, and that elevation is mainly a result of the return of the suspensorium to its original position. Our manipulations during dissection and the absence of the levator arcus palatini in some loricariids (ALEXANDER, 1965; HOWES, 1983) corroborate this hypothesis. During dissection, elastic properties of the skin and the ligamentous tissue in the hyomandibular-pterotic suture appeared to be the main cause of such passive elevation, rather than the bending of the bony suspensorium itself, which was suggested by ALEXANDER (1965). This also explains the presence of a relatively robust adductor arcus palatini, needed to overcome the resilience of the suspensorium.

Whether the buccal pump system is able to maintain a negative pressure in the oral cavity has since long been a matter of debate. HORA (1930) believed that the lips could not function as a sucker while respiration continued, since the inflowing water would cause the system to fail. ALEXANDER (1965) demonstrated that respiration and suction can function simultaneously, and that both actions continue when the fish is pulled away from the substrate (a vertical aquarium glass). Our results indicate that inflowing water was limited to a thin stream passing under the sucker immediately posterior to each maxillary barbel, a phenomenon also observed by VANDEWALLE et al. (1986) in *Hypostomus punctatus*.

The branchiostegal membrane

Water leaves the orobranchial cavity through the branchiostegal opening. It does not enter there, as initially supposed by REGAN (1904), but is inhaled via the mouth. The functioning of the muscles associated with the branchiostegal membrane in *Ancistrus* cf. *triradiatus* can not easily be understood. As indicated by manipulation, contraction of the hyohyoidei adductores closes the branchiostegal membrane. SCHAEFER (1997) did not mention this muscle in his myological account on *Otocinclus*, but we found it in *O. vestitus*.

In non-loricariid siluriforms like *Clarias gariepinus* the paired hyohyoideus abductor opens the branchiostegal membrane, inserting on the medialmost branchiostegal ray and originating directly on the hyoid arch, or indirectly, via a medial aponeurosis, thus running more or less rostrocaudally (ADRIAENS & VERRAES, 1997b). Remarkably, in *A.* cf. *triradiatus* the muscle runs completely transversely (Fig. 8A), so that experimental contraction closes the membrane instead of opening it, the muscle having become a functional adductor. The angle between the hyohyoideus abductor and the fibers of the sternohyoideus on which they attach is 90°; hence no interaction of functions is assumed. Also, due to its shortness, the sternohyoideus can't induce a significant change in orientation of the hyohyoideus abductor. Two possible mechanisms might cause the abduction of the membrane in *A.* cf. *triradiatus* : it might have become a passive movement, induced by the high pressure in the branchial cavity caused by the contraction of the hyohyoideus inferior, or it might be initiated by the posterior part of the latter muscle, attaching to the posterior ceratohyal, near the articulation of the first branchiostegal ray. Manipulation suggested that movements of the hyoid have a slight effect on the position of the rays. SCHAEFER (1990) observed that in some loricariids, this part of the hyohyoideus inferior inserts on the branchiostegal rays themselves.

Our anatomical study and observations suggest the movements of the opercle in the respiratory cycle are negligible. It is moved very slightly by the movements of the suspensorium, with which it articulates. In *A.* cf. *triradiatus*, it has a prominent role in the erection of the large cheek spines (HOWES, 1983; GEERINCKX & ADRIAENS, 2006).

Movements of maxillary barbels and lips

Initiation of suction requires a close adhesion of the upper and lower lips to the substrate. In the most plausible theoretical scenario the outer edges of the suction disc, formed by the fused lips, are pushed against the substrate. A subsequent creation of a negative pressure by expansion of the oral cavity (posterior to the valve) enables transferring water from the cavity anterior to the oral valve. A fish adopting a sucking position brings the less mobile upper lip against the substrate by literally swimming against it or pushing the head downward. Pulling both parts of the intermandibularis muscle appears to result in a pursing of the lower lip. Antagonistic movement, i.e. retracting the lower lip toward the body, is most

probably achieved by contraction of the protractor hyoidei pars labialis, as suggested by manipulations (see also The lower jaws).

The maxillae of most loricariids support only small maxillary barbels. It appears that their main function has become to mediate the movements of the lateral lip tissue in which they are embedded. The retractor tentaculi muscle lifts this part of the lips from the substrate, as in many siluriforms (WINTERBOTTOM, 1974), allowing water to enter the oral cavity. Experiments with diluted milk showed that when the fish clings to a vertical substrate water often enters only one side of the mouth, and only this side of the lip is seen moving. This suggests the independent contraction of the left or right retractor tentaculi. VANDEWALLE et al. (1986) hypothesized that the elevation of the lateral lip tissue is mostly caused by lateral movements of the lower jaws. We found, however, no anatomical link connecting both elements. The lateral motion of the lower jaws is negligible when compared to their dorsoventral movements during respiration. Also, when only one of both lip sides is elevated, no visible asymmetry in the movements of the lower jaws is present. The closing of the lip furrow by the action of the levator arcus palatini, suggested by the same authors, is highly improbable, not only because of the absence of an anatomical link, but also because the levator arcus palatini is absent in several loricariids (HOWES, 1983). Our hypothesis, based on the anatomy of *Ancistrus* cf. *triradiatus*, that the extensor tentaculi pars lateralis may close the lip opening, should ideally be tested by electromyographical experiments. The extensor tentaculi [not subdivided in catfishes other than loricariids (SCHAEFER, 1990; DIOGO, 2005)] is responsible for a downward or forward movement of the maxillary barbel in most catfishes (ALEXANDER, 1965; GOSLINE, 1975). The origin of the pars lateralis in the lateral ethmoid-metapterygoid groove is unique among siluriforms, and might have evolved several times within the loricariid family (HOWES, 1983).

The autopalatine-maxillary mechanism of *A.* cf. *triradiatus* and other loricariids is comparable to the situation seen in the African mochokid *Euchilichthys*, also having a sucker-like mouth. Here too, the cartilaginous rostral tip of the autopalatine is situated on top of the premaxilla, the extensor tentaculi muscle is directed completely rostrocaudal, and the line between the lateral ethmoid-autopalatine joint and the muscle insertion on the autopalatine is almost vertical instead of horizontal (GOSLINE, 1975).

The oral valve

The oral valve of loricariids attaches to the anterodorsal mouth roof, and, when closed, separates a small anterior cavity from the larger oral cavity. The muscle we name retractor veli has previously been called muscle d or retractor palatini (HOWES, 1983; SCHAEFER, 1997; DIOGO & VANDEWALLE, 2003). It is, however, not homologous to the retractor palatini of LUBOSCH (1929, in WINTERBOTTOM, 1974) and HOFER (1938), which is a derivative of the adductor arcus palatini in some perciform and tetraodontiform fishes. Contrary to HOWES' (1983) claim, ALEXANDER (1965) did not name it retractor premaxillae, but omitted it. The muscle is unique for loricariids (SCHAEFER, 1990). Its function has at best been vaguely described, based on dissection of preserved specimens. It would insert on a "complexly divided connective tissue sheet" (HOWES, 1983 : 313), attached to the autopalatine, the premaxilla, and the lower jaw, and would pull the autopalatine ventrally in preserved specimens. However, our manipulation of the muscle in fresh specimens of *Ancistrus* cf. *triradiatus* revealed no ventral movement of the autopalatine.

GRADWELL (1971) described a "muscle of oral valve" in several loricariids : *Hypostomus punctatus*, *Hemiancistrus annectens*, *Rineloricaria microlepidogaster*, *Ancistrus occidentalis* and *Otocinclus mariae*. It would insert on the lateral sides of the oral valve, and originate "on the dorsal surface of the skull, anterior to the eyes and lateral to the nares" (GRADWELL, 1971 : 837). His biomechanical analysis proved it to contract at the moment the valve closed. In spite of the questionable description of the muscle origin mentioned in his text, his figures corroborate our assumption that it is the retractor veli. The retractor palatini of HOWES (1983) and the muscle of the oral valve of GRADWELL (1971) are presented as separate muscles in the myological review of DIOGO & VANDEWALLE (2003), but are identical. Apart from *A.* cf. *triradiatus*, we also observed this muscle in *Pterygoplichthys lituratus*, *Farlowella acus* and *Otocinclus vestitus*. In *F. acus* it inserts on the valve via two tendons, while in *O. vestitus* two separate retractor veli divisions are present, only connected posteriorly. The dorsal division inserts more anteriorly on the valve. In breathing specimens of *A.* cf. *triradiatus* it can be observed that the valve is closed at the moment the hyoid elevates (Fig. 9). The exact movements of the valve, both rostrocaudally and dorsoventrally, are difficult to explain, as retractor veli activity, elastic recoil and water pressure and flow probably have a combined effect.

The upper jaws

The high mobility of the premaxillae must be one of the most important evolutionary innovations encountered in the loricarioid lineage, and is a synapomorphy for callichthyids, scoloplacids, astroblepids and loricariids (HOWES, 1983; SCHAEFER, 1990; DIOGO, 2005). SCHAEFER & LAUDER (1986; 1996) listed this release of constraint as one of the decoupling events observed in the evolution leading to the loricariids. In most siluriforms the premaxillae are firmly attached to the lateral cornua of the mesethmoid; structural and functional changes of the premaxilla and maxilla have lead to the inability to perform protrusion (ADRIAENS & VERRAES, 1997a). However, the development of a small dorsal premaxillary process and the disappearance of the mesethmoid cornua have triggered an important shift in the relation between the neurocranium and the upper jaw, enabling a novel protrusion mechanism. Our results suggest this mechanism is most probably mediated by the extensor tentaculi pars medialis and the retractor premaxillae in loricariids. The latter muscle, derived from the adductor mandibulae complex and constituting a key innovation in the loricarioid lineage (SCHAEFER & LAUDER, 1986), has a direct insertion on the premaxilla in astroblepids and scoloplacids as well, and an indirect connection via a connective sheet in callichthyids (SCHAEFER, 1990; SCHAEFER & LAUDER, 1986;

1996), which also have a dorsal premaxillary process (HUYSENTRUYT & ADRIAENS, 2005), and show some degree of premaxilla protrusion (ALEXANDER, 1965).

The vertical ventral mesethmoid disc, present in all loricariids, and, to a lesser degree, astroblepids (SCHAEFER, 1990), is important for the movements of the premaxillae. The mesethmoid-premaxillary cartilage, a meniscus forming a double articulatory cup, keeps the premaxillae in place, aided by the series of ligaments that assist and direct their movements (Fig. 5A). Consequently, contraction of the retractor premaxillae swings the premaxilla teeth row caudally, thus scraping the substrate. The effect of the assumed antagonistic extensor tentaculi pars medialis is more complex : the muscle unambiguously pulls the rostral tip of the autopalatine ventrally. When manipulating the autopalatine in this direction, the autopalatine-maxilla joint pushes against the posterior part of the premaxilla. Both the premaxilla and the maxilla (the part ventral to the joint) are provided with a strong tissue cushion in the region where they touch (Fig. 5A). The ligamentous suspension of the premaxilla makes it rotate about a transverse horizontal axis instead of merely being pushed forward. As a result the premaxilla protrudes, and swings its teeth row rostrally. Dissection and manipulation of this apparatus showed no significant effect on the movement of the tip of the maxilla. Independent motion of each premaxilla seems to be limited by the short interpremaxillary ligament.

The lower jaws

The evolutionary rotation of the lower jaws to a medial position with the teeth pointing rostroventrally, as well as the loss of both the interoperculo-mandibular ligament and the medial connection between both dento-mentomeckelian bones, have laid open new possibilities concerning rotational mobility (SCHAEFER & LAUDER, 1986). During normal respiration in *Ancistrus* cf. *triradiatus* a slight up-and-down motion of the lower jaws and the adjacent lower lip tissue is seen, probably anatomically and functionally coupled to the hyoid movements. Whether this movement is (partly) caused by the adductor mandibulae muscle remains to be verified. Only at feeding the lower jaws are seen rotating and scraping the substrate, more or less synchronously with the upper jaws. In the most probable hypothetical scenario the adductor mandibulae and protractor hyoidei muscles act as antagonists. The scraping movement, in which the teeth are moved rostrally, would be achieved by a contraction of the adductor mandibulae (dissection and manipulation confirmed that the most dorsal part certainly has to be involved). The protractor hyoidei pars dentalis may then perform the antagonistic movement of swinging the lower jaw and its teeth row back caudally. The protractor hyoidei pars labialis can be considered the retractor of the lower lip.

The cartilage plug, attached to the hyoid arch at the midline, and protruding into the space behind and between the lower jaws, is hypothesized to be a supporting device for the dento-mentomeckelian bones, preventing them from being merely pulled caudally. Their caudal motion is restricted, and the effect of contraction of the adductor mandibulae, inserting on the dorsocaudal aspect of the jaws, is partly transformed in a rotation around the longitudinal axis of the jaws. The consequence is that the teeth can scrape a larger surface. Previously, SCHAEFER & LAUDER (1986; 1996) appointed a different function of the cartilage plug : it was suggested to act as a novel anatomical link between the hyoid and the lower jaws, unique to loricariids, allowing the sternohyoideus muscle to retract the lower jaws via the hyoid arch. This is contradicted by the present study on *Ancistrus* cf. *triradiatus*, as the plug attachment to the hyoid arch is relatively strong, while it is not strongly attached to the lower jaws (Figs 5D-E, 8B). The dento-mentomeckelian bones move and roll against the plug, but are not attached to it. Hence, any force exerted by the sternohyoideus retracts the hyoid arch, and, to a lesser extent, the attached cartilage plug, but has no significant effect on the mandibles. This was shown by manipulation of freshly killed and dissected specimens (tissue characteristics are strongly altered in preserved specimens). Another argument is histological : the cartilage plug connected to the hypohyals consists of elastic cartilage, ideal for a supporting, gliding device, but inappropriate for the efficient transmission of pulling forces in the caudal direction, a mechanism that would benefit more from a tendinous ligament. The mandibulohyoid ligament attaches to the lateralmost aspect of the angulo-articular, and its role in retraction of the medially pointed lower jaw seems very slim. The lower lip is moved rostrally and caudally together with the lower jaws. Unicellular keratinized projections or unculi form numerous brushes on thick epidermal papillae that may aid during substrate scraping (Fig. 5 D). These have been observed in other loricariids as well (ONO, 1980; ROBERTS, 1982).

The remarkable habitus of loricariids evoked GREGORY (1933 : 196) to state that "in these heavily armoured forms the siluroid skull attains its highest specialization." Considering the results of the present paper, in addition to the works of ALEXANDER (1965), HOWES (1983), SCHAEFER (1987; 1997), SCHAEFER & LAUDER (1986; 1996) and others, it can be concluded that this has not been an idle statement. On the contrary, the loricariid head is one of the most impressive examples of structural diversification and refinement shaped by evolution.

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ABBREVIATIONS USED IN FIGURES

ap-o-cb-I	anterior process of os ceratobranchiale I
art-f-cl	articulation facet for cleithrum
bb-IV-V	basibranchialia IV-V (posterior copula)
B-l	Baudelot's ligament
br-cav	branchial cavity
br-m	branchiostegal membrane
c-mx	cartilago maxillaris
c-symp	cartilago symplecticum
ch-pl	cheek plates
ch-sp	cheek spines
comp-pt	compound pterotic bone
crt-h-apal	cartilaginous head of autopalatine
crt-p	cartilage plug
crt-p-h-l	cartilage plug-hyoid ligaments
csh-mx	cushion on maxilla
csh-pmx	cushion on premaxilla
d-pl	dermal plate
eb-soc	epiphysial branch of supraorbital canal
fn-sph	fenestra sphenoidea
fr-a-hh	foramen for artery supplying hyohyoideus inferior
fr-r-op-VII	foramen ramus opercularis nervus facialis
fr-tr-hm-VII	foramen truncus hyomandibularis nervus facialis
fr-v-j-inf	foramen vena jugularis inferior
hb-III	hypobranchiale III
intpmx-l	interpremaxillary ligament
ioc	infraorbital canal
ipb-crt-n	infrapharyngobranchial-like cartilage nucleus
l-mes-pmx-l	lateral mesethmoid-premaxillary ligament
llc	lateral line canal
m-add-apal	musculus adductor arcus palatini
m-add-hm	musculus adductor hyomandibulae
m-add-md-e	external part of musculus adductor mandibulae
m-add-md-i	internal part of musculus adductor mandibulae
m-add-op	musculus adductor operculi
m-dil-op	musculus dilatator operculi
m-ext-tt-l	musculus extensor tentaculi pars lateralis
m-ext-tt-m	musculus extensor tentaculi pars medialis
m-hh-abd	musculus hyohyoideus abductor
m-hh-add	musculi hyohyoidei adductores
m-hh-inf	musculus hyohyoideus inferior
m-intm-d	musculus intermandibularis pars dentalis
m-intm-l	musculus intermandibularis pars labialis
m-lev-apal	musculus levator arcus palatini
m-lev-op	musculus levator operculi
m-mes-pmx-l	medial mesethmoid-premaxillary ligament
m-pr-h-d	musculus protractor hyoidei pars dentalis
m-pr-h-l	musculus protractor hyoidei pars labialis
m-rt-pmx	musculus retractor premaxillae
m-rt-tt	musculus retractor tentaculi
m-rt-v	musculus retractor veli
m-stern	musculus sternohyoideus
mes-apal-l	mesethmoid-autopalatine ligament
mes-crt-l	mesethmoid-cartilage ligament
mes-mx-l	mesethmoid-maxillary ligament
mes-pmx-c	mesethmoid-premaxillary cartilage
o-aa	os anguloarticulare
o-apal	os autopalatinum
o-bb-II	os basibranchiale II
o-boc	os basioccipitale
o-cb-V	os ceratobranchiale V
o-ch-a	os ceratohyale anterior
o-ch-p	os ceratohyale posterior
o-cl	os cleithrum
o-den-m	os dento-mentomeckelium
o-eb-IV	os epibranchiale IV
o-epoc	os epioccipitale
o-exoc	os exoccipitale
o-fr	os frontale
o-hb-I	os hypobranchiale I
o-hh	os hypohyale
o-hm	os hyomandibulare
o-io-II/VI	os infraorbitale II/VI
o-ipb-IV	os infrapharyngobranchiale IV
o-leth	os latero-ethmoideum
o-mes	os mesethmoideum
o-mpt	os metapterygoideum
o-mx	os maxillare
o-nas	os nasale
o-op	os operculare
o-osph	os orbitosphenoideum
o-para	os parasphenoideum
o-par-soc	os parieto-supraoccipitale
o-pmx	os praemaxillare
o-pop	os praeoperculare
o-prot	os prooticum
o-psph	os pterosphenoideum
o-puh	os parurohyale
o-pvm	os praevomerale
o-q	os quadratum
o-sph	os sphenoticum
o-spop	os suprapraeoperculare
oc	otic canal
or-cav	oral cavity
or-v	oral valve or velum
pb-poc	pterotic branch of postotic canal
pb-soc	parietal branch of supraorbital canal
pc	preopercular canal
poc	postotic canal
pr-pl	postrostral plate
prfr-pl	prefrontal plate
prn-pl	prenasal platelet
r-br	radius branchiostegus
ses-b	sesamoid bone
soc	supraorbital canal
susp	suspensorium
sut-v-VI	occipital suture with neural spine of sixth vertebra
tr-pr-c-v	transverse process of complex vertebra
u-ph-j	upper pharyngeal jaw
unc	unculi or unicellular keratinized brushes
vc-V	fifth vertebral centrum

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The Cymothoidae (Crustacea, Isopoda), parasites on marine fishes, from Algerian fauna

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RÉSUMÉ. Study of the cymothoid parasites of marine fish from Algeria, particularly in the Béjaïa and Jijel gulfs, allowed us to identify nine species *(Anilocra frontalis, A. physodes, Nerocila bivittata, N. orbignyi, N. maculata, Ceratothoa parallela, C. oestroides, C. oxyrrhynchaena, C. italica*). Eight have been recorded previously, but *Ceratothoa italica* is a new record for the region. New hosts for *Anilocra frontalis, Nerocila maculata* (host until now unidentified in Algeria) and *Nerocila orbignyi* are reported. For each parasite collected, the host fish, geographic distribution, parasitic specificity and prevalence are given. An up-to-date check-list of the fourteen species now reported from Algeria is given.

KEY WORDS : Cymothoidae, Crustacea, parasitic isopods, Algeria.

Les Cymothoidae (Crustacea, Isopoda), parasites de poissons marins, de la faune Algérienne

RÉSUMÉ. Le présent travail constitue une mise au point sur les Cymothoidae ectoparasites de poissons de la côte algérienne. Pour l'instant, neuf espèces ont été inventoriées par les auteurs : *Anilocra frontalis*, *A. physodes*, *Nerocila bivitatta*, *N. orbignyi*, *N. maculata, Ceratothoa paralella*, *C. oestroides*, *C. oxyrrhynchaena*, *C. italica*. Parmi celles-ci, *Ceratothoa italica* est nouvelle pour la faune algérienne. Toutes ces espèces sont signalées pour la première fois dans le golfe de Béjaïa et dans le golfe de Jijel. Des hôtes nouveaux pour *N. orbignyi* et *A. frontalis*, ainsi que le poisson hôte de *N. maculata* jusqu'à présent inconnu en Algérie, ont été identifiés. Pour chacune des espèces, le(s) poisson(s) hôte(s), la distribution géographique, la spécificité parasitaire et les prévalences sont précisés. Une liste récapitulative des quatorze espèces actuellement signalées en Algérie est enfin établie.

MOTS CLES : Cymothoidae, Crustacea, isopodes parasites, Algérie.

INTRODUCTION

Crustacean ectoparasites on marine fish are diverse. Many species of fish are infected by cymothoids (Crustacea, Isopoda, Cymothoidae). They are blood-feeding; several species settle in the buccal cavity of fish, others live in the gill chamber or on the body surface including the fins. Their life cycle involves only one host (Holoxenic cycle).

According to TRILLES (1986), 46 species of Cymothoidae have been reported in Africa (12 Anilocrinae and 34 Cymothoinae). The cymothoid fauna of diverse localities along the Algerian coasts have been incompletely studied, several of them around a century ago : LUCAS, 1849 (Algiers, Annaba : ex-Bône, Oran, Algeria); SCHIOEDTE & MEINERT, 1881 (Annaba); SCHIOEDTE & MEINERT, 1883 (Oran); CARUS, 1885 (Algiers, Annaba, Oran); GOURRET, 1891 (Algeria); MONOD, 1924 a-b (Oran).

More recent studies are those of TRILLES (1972; Algeria, Oran, Bou-Ismail : ex-Castiglione), TRILLES (1975; Algeria, Bou-Ismail, Skikda : ex-Philippeville, Annaba), DOLLFUS & TRILLES (1976; Algeria, Algiers, Bou-Ismail, Bou-Haroun), TRILLES (1977; Algiers), TRILLES (1979; Bou-Ismail) and TRILLES (1986; Algeria).

The cymothoid fauna of the areas that we have now prospected, the gulf of Béjaïa and the gulf of Jijel as well as the Tamelaht lagoon and the Soummam Oued, have not until now been studied.

MATERIALS AND METHODS

Fish samples were obtained from the gulf of Béjaïa and the gulf of Jijel, as well as the Tamelaht lagoon and the Oued Soummam (East Algeria; near Tunisia) (Fig. 1), between April and September 2005. Fish were captured using diverse fishing techniques : at sea by using trawl, trammel, palangre or harpoon, and in the Soummam Oued and the Tamelaht lagoon by carelet or monofilament. Parasites were collected from fish and immediately preserved in 70% alcohol. Data on collecting period, sampling area, name and size of host as well as the location of fish capture, were noted. Prevalence (P) was calculated. The geographical distribution and host species were also specified. Finally, we compiled a check-list of all cymothoids reported to the present time from the Algerian coasts.

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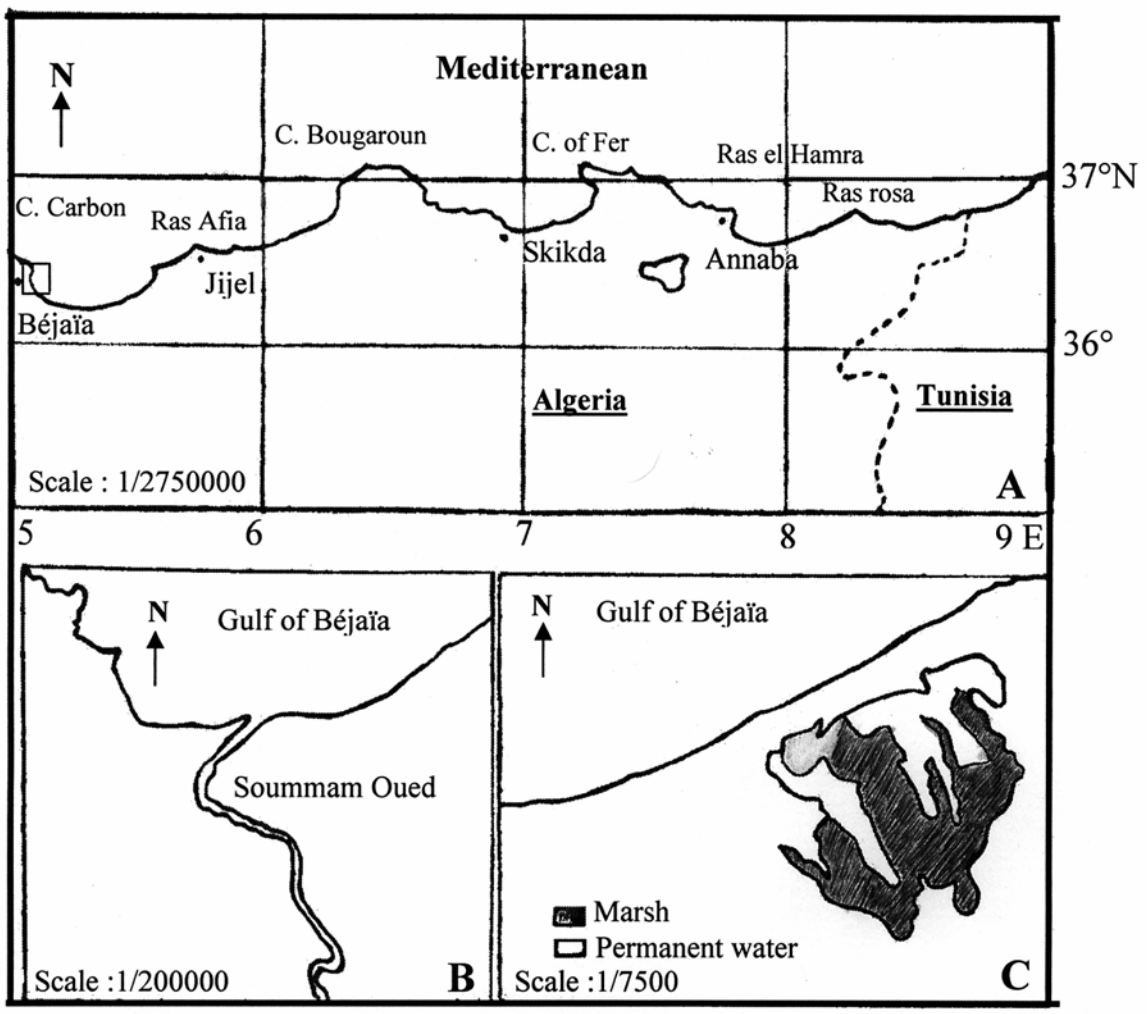

Fig. 1. – Location of the study
A : Situation of the gulf of Béjaïa (between the Cape Carbon and Ras Afia) and the gulf of Jijel (between Ras Afia and the Cape Bourgaroun); **B**, **C** : Situation of the Soummam Oued and the Tamelaht Lagoon.

TABLE 1

Parasitological index of the Cymothoidae collected

Parasites/hosts species	NFE	LS	NFI	P	PC
Anilocra physodes					
Spondiliosoma cantharus	3	[20-26]	1	33.33	GJ
Anilocra frontalis					
Mullus barbatus	82	[10-23]	9	10.98	GB
Umbrina canariensis	4	[19-25]	1	25	GB
Pagellus acarne	44	[14-24]	1	2.27	GB
Diplodus annularis	24	[10-17]	2	8.33	GB

TABLE 1

Parasitological index of the Cymothoidae collected

Parasites/hosts species	NFE	LS	NFI	P	PC
Lithognathus mormyrus	25	[14-22]	1	4	GB
Solea vulgaris	21	[12-21]	1	4.76	GB
Lithognathus mormyrus	7	[14-19]	1	14.28	GJ
Crenilabrus pavo	7	[16-20]	2	28.57	GJ
Nerocila bivittata					
Crenilabrus pavo	9	[16-25]	1	11.11	GB
Nerocila orbignyi					
Mugil cephalus	55	[15-36]	1	1.81	GB (SO)
Crenilabrus pavo	9	[16-25]	2	22.22	GB
Crenilabrus pavo	7	[16-20]	2	28.57	GJ
Trigla lyra	9	[19-26]	1	11.11	GB
Nerocila maculata					
Pagellus acarne	44	[14-24]	2	4.54	GB
Ceratothoa italica					
Diplodus annularis	24	[10-17]	1	4.17	GB
Ceratothoa oestroides					
Boops boops	140	[10-25]	11	7.9	GB
Spicara smaris	25	[11-17]	3	12	GB
Pagellus acarne	44	[14-24]	2	4.54	GB
Trachurus trachurus	109	[11-26]	1	0.92	GB
Ceratothoa oxyrrhynchaena					
Spicara smaris	25	[11-17]	2	8	GB
Boops boops	140	[10-25]	5	3.75	GB
Ceratothoa parallela					
Boops boops	140	[10-25]	12	8.60	GB
Trachurus trachurus	109	[11-26]	3	2.75	GB
Spicara smaris	25	[11-17]	1	4	GB

NFE = Number of fish examined; **LS** = Range of fish size (cm); **NFI** = Number of fish infected; **P** = Prevalence (%); **PC** = Place of collection; **GB** = Gulf of Béjaïa; **GJ** = Gulf of Jijel; **SO** = Soummam Oued.

RESULTS AND DISCUSSION

Cymothoidae now collected by the authors.

ANILOCRINAE

Anilocra Leach, 1814

Anilocra frontalis Milne Edward, 1840

Anilocra frontalis (Table 1) was collected from the body of *Umbrina canariensis* (P = 25%), *Mullus barbatus* (P = 10.98%), *Pagellus acarne* (P = 2.27%), *Diplodus annularis* (P = 8.33%), *Lithognathus mormyrus* (P = 4% in the gulf of Béjaïa; P = 14.28% in the gulf of Jijel), *Solea vulgaris* (P = 4.76%) and *Crenilabrus pavo* (P = 28.57% in the gulf of Jijel). This is the first record of the species in the gulfs of Béjaïa and Jijel.

The distribution of this species is wide : North Sea, Atlantic, Mediterranean and Adriatic (Trilles, 1994), in Tunisia (Trilles & Raibaut, 1971), in Morocco (Trilles, 1975) and more recently in Turkey (Öktener & Trilles, 2004).

In Algeria, *A. frontalis* has been previously recorded (Trilles, 1986, 1994) from Oran (Milne Edward, 1840; Lucas, 1849; Carus, 1885), Mers-el-Kébir (Lucas, 1849; Carus, 1885), Algeria (Trilles, 1975), Bou-Ismail (Trilles, 1975; Trilles, 1979), Algiers (Dollfus & Trilles, 1976).

This species, characterized by a euryxenic or stenoxenic specificity according to the prospected area, was found on several host species, especially Labridae (*Labrus maculatus*, *L. vetula*, *L. bergylta*, *L. merula*, *Crenilabrus melops*, *C. cinereus*, *C. ocellatus*) but sometimes on other fish (*Gadus sp*, *Merlangius pollachius*, *Blennius pholis*, *Cottus bubalis*, *Gobius flavescens*, *G. paganellus*, *G. minutus*, *Onos mustela*, *Spinachia vulgaris*, *Boops boops*, *Boops salpa* and *Spondyliosoma cantharus* (Trilles, 1994). In Algeria, it has been previously collected from *Diplodus annularis*, *Oblada melanura*, *Gobius paganellus* and an unidentified Labridae (Dollfus & Trilles, 1976). Our results are, therefore, in agreement with those from other Mediterranean areas. In Algeria, *Anilocra frontalis* is probably a euryxenic species.

Anilocra physodes (L., 1758)

Anilocra physodes (Table 1) was collected only from the body of *Spondyliosoma cantharus* (P = 33.33%) in the gulf of Jijel. It has been broadly reported from differents part of the Mediterranean, the Adriatic, the Black Sea and the Egean Sea (Trilles, 1975). In the southern and eastern Mediterranean, this species was found in Egypt (Trilles, 1975), Tunisia (Trilles & Raibaut, 1971; 1973; Charfi-Cheikhrouha et al., 2000), Morocco

(Trilles, 1975), Turkey (Öktener & Trilles, 2004) and Lebanon (Bariche & Trilles, 2005).

In Algeria, it was previously reported (Trilles, 1986; 1994) from Algiers (Lucas, 1849; Carus, 1885; Fain-Maurel, 1966; Trilles, 1975; Dollfus & Trilles, 1976), in the gulf of Skikda (ex-Philippeville) and from Bou-Ismail (Trilles, 1975). *A. physodes* is recorded for the first time in the Gulf of Jijel.

This euryxenic species has a wide host range, with, however, a certain preference for the Sparidae and the Maenidae (Trilles, 1975). In Tunisia, it was recently found on *Lithognathus mormyrus*, *Sciaena umbra* and *Uranoscopus scaber* (Charfi-Cheikhrouha et al., 2000). In Lebanon, Bariche & Trilles (2005) collected this species from *Boops boops* and *Lithognathus mormyrus* but also, for the first time, from *Pagrus caeruleostictus* and *Pagellus acarne*. In Algeria, it was previously reported on *Maena vulgaris* (Carus, 1885), *Spicara, Boops, Pagellus* and other Sparidae (Fain-Maurel, 1966), *Diplodus fasciatus*, *Spondyliosoma cantharus*, *Scorpaena porcus*, *Zeus faber* (Trilles, 1975). From Castiglione, Dollfus & Trilles (1976) reported this species on *Smaris chryselis* and *Spondyliosoma cantharus*. At the level of the zone prospected in our study, the specificity of this species may be narrower than in other areas and oïoxenic.

Nerocila Leach, 1818

Nerocila bivittata (Risso, 1816)

Nerocila bivittata (Table 1) was collected from the caudal fin of *Crenilabrus pavo* (P = 11.11%) in the gulf of Béjaïa. In the Mediterranean, it was reported by Trilles (1968; 1977). Along the Tunisian coasts, it has been identified by Trilles & Raibaut (1973) and Charfi-Cheikhrouha et al. (2000). This species was recently found in Turkey (Öktener & Trilles, 2004) and in Lebanon (Bariche & Trilles, 2005).

It has been mentioned from Algeria by several authors (Trilles, 1986; 1994) : Algeria (Gourret, 1891; Dollfus & Trilles, 1976), Annaba (Lucas, 1849; Schioedte & Meinert, 1881; Carus, 1885), Oran (Lucas, 1849; Carus, 1885), Algiers (Carus, 1885; Trilles, 1977) and Bou-Ismail (Trilles, 1975). *N. bivittata* is noted for the first time in the gulf of Béjaïa.

In the Mediterranean, this species is chiefly parasitic on fish belonging to the family Labridae (Trilles, 1994; Charfi-Cheikhrouha et al., 2000), but has sometimes been collected from hosts from other families : Scorpaenidae (*S. scrofa*, *S. porcus*) (Trilles, 1975), Sciaenidae (*Sciaena umbra*), Mullidae (*Mullus surmuletus*), Gobiidae (*Gobius geniporus*), Serranidae (*Serranus scriba*) (Charfi-Cheikhrouha et al., 2000), Sparidae, Gobiidae and Sciaenidae (Öktener & Trilles, 2004), Triglidae and Sparidae (Bariche & Trilles, 2005). *Nerocila bivittata* is a stenoxenic species with a preference for Labribae, but that specificity may change with locality, becoming euryxenic in some areas.

Our results confirm the preferential occurrence of this species on Labridae with perhaps stenoxenic specificity in Algeria.

Nerocila orbignyi (Guérin-Mèneville, 1832)

Nerocila orbignyi was collected (Table 1) from the caudal fin of *Mugil cephalus* (P = 1.81%) in the gulf of Béjaïa and the Soummam Oued, from the head of *Trigla lyra* (male; P = 11.11%) in the Gulf of Béjaïa, and from the caudal fin of *Crenilabrus pavo* in the gulf of Béjaïa (P = 22.22%) and in the gulf of Jijel (P = 28.57%).

This species has already been recorded in the Mediterranean (Trilles, 1977; Trilles, 1994), along the Turkish coasts (Öktener & Trilles, 2004), from Morocco (Dollfus & Trilles, 1976) and Tunisia (Trilles & Raibaut, 1973; Charfi-Cheikhrouha et al., 2000). In Algeria, it was previously reported only from Annaba by Lucas (1849). It is identified for the first time in the gulfs of Béjaïa and Jijel.

This species settles preferentially on the Mugilidae (Trilles, 1994; Öktener & Trilles, 2004). In Africa, particularly in Tunisia, it has been collected from *Mugil cephalus*, *Mugil auratus*, *Mugil capito* and *Mugil labrosus* (Trilles & Raibaut, 1973), *Liza ramada*, *Liza saliens*, *Liza auratus*, *Chelon labrosus* (Charfi-Cheikhrouha et al., 2000). However, this euryxenic species has also been reported from several other fish (Trilles, 1994; Öktener & Trilles, 2004), on *Alosa fallax nilotica* (Trilles & Raibaut, 1973), *Batrachus didactylus*, *Solea senegalensis* (Dollfus & Trilles, 1976), *Dicentrarchus labrax*, *Solea solea*, *Serranus scriba*, and *Diplodus annularis* (Charfi-Cheikhrouha et al., 2000).

Our results are, therefore, in agreement with the previously known characteristics of this euryxenic species. However, the prevalence values observed suggest that in Algeria the preference of *N. orbignyi* for the mugilids is not as marked.

Nerocila maculata (Milne Edward, 1840)

Nerocila maculata (Table 1) was collected from the pelvic fin and from the operculum of *Pagellus acarne* (P = 4.54%) from the gulf of Béjaïa. Trilles (1986; 1994) recorded this species from the Mediterranean to the Atlantic coasts of Southern Europe. It has been identified in Algeria from Annaba by Lucas (1849), Carus (1885) and Trilles (1975). This species is recorded for the first time in the gulf of Béjaïa.

Along the French coasts, it has been collected on *Gadus capelanus* (Trilles, 1968), and from Morocco on *Gadus capelanus* and *Raja alba* (Dollfus & Trilles, 1976).

In Algeria, until now, the identity of the host fish was unknown (Trilles, 1986). Our results are not in agreement with the previous data from other areas; a change in specificity is perhaps possible according to the prospected localities.

CERATOTHOINAE

Ceratothoa Dana, 1852

Ceratothoa italica Schioedte and Meinert, 1883

Ceratothoa italica (Table 1) was found in the buccal cavity of *Diplodus annularis* (P = 4.17%), in the gulf of Béjaïa. This is the first record of *Ceratothoa italica* from the Algerian coast.

This ectoparasite is relatively uncommon; it is known in the Mediterranean and in the Adriatic (Trilles, 1968; Trilles et al., 1989). It has been reported from Tunisia and the north-western Atlantic coasts of Africa, Mauritania and western Morocco (Trilles, 1972; 1986); recently, this species was collected in Turkey (Öktener & Trilles, 2004) and in Lebanon (Bariche & Trilles, 2005).

C. italica has been collected from several species of Sparidae : *Pagellus mormyrus*, *Pagellus erythrinus*, *Oblada melanura*, *Cantharus lineatus*, and *Sargus sp.* (Trilles, 1994; Horton, 2000). In Turkey, Öktener & Trilles (2004) found this species on *Dicentrarchus labrax* and *Spicara maena*, while Bariche & Trilles (2005) found it only on *Dicentrarchus labrax* in Lebanon. Therefore, the specificity of this species varies, being euryxenic, stenoxenic or oïoxenic according to the prospected areas.

Diplodus annularis is a new host for *C. italica* and its specificity may be oïoxenic in the gulf of Béjaïa.

Ceratothoa oestroides (Risso, 1826)

Ceratothoa oestroides (Table 1) was collected from the mouth of *Trachurus trachurus* (P = 0.92%), *Pagellus acarne* (P = 4.54%), *Spicara smaris* (P = 12%) and *Boops boops* (P = 7.9%) from the gulf of Béjaïa.

This species has already been reported from the Mediterranean, the Adriatic and the northwestern Atlantic coasts of Africa (Trilles, 1994; Horton, 2000). It has been collected particularly in Tunisia (Trilles & Raibaut, 1971; Charfi-Cheikhrouha et al., 2000), Morocco and Algeria (Trilles, 1972); recently, it was reported from Turkey (Öktener & Trilles, 2004) and Lebanon (Bariche & Trilles, 2005). Ours is the first record of its collection in the gulf of Béjaïa.

Trilles (1986) notes a preference of this species for the Sparidae and Maenidae. In Tunisia, it has been collected on *Boops boops*, *Diplodus annularis* and *Trachurus trachurus* (Trilles & Raibaut, 1971), in Turkey on *Spicara maena* and *Sardina pilchardus* (Öktener & Trilles, 2004) and from Lebanon on *Sparus aurata* (Bariche & Trilles, 2005). According to Trilles (1994), *C. oestroides* is ubiquitous and euryxenic; it can be found infesting several other fish species : *Physis mediterranea*, *Mullus barbatus*, and *Abudefduf saxatilis*. It has also been collected from cultured *Dicentrarchus labrax* and *Sparus aurata* (Šarušić, 1999). Charfi-Cheikhrouha et al. (2000) report *C. oestroides* from nine species belonging to six families of fish : Sparidae, Carangidae, Clupeidae, Maenidae, Scorpaenidae and Mugilidae.

Therefore, our results are in agreement with previous data. However, *Pagellus acarne* is a new host record for *C. oestroides*.

Ceratothoa oxyrrhynchaena Koelbel, 1878

Ceratothoa oxyrrhynchaena (Table 1) was collected from the buccal cavity of *Spicara smaris* (P = 8%) and *Boops boops* (P = 3.75%) from the gulf of Béjaïa.

The distribution of this parasite is very extended (Trilles, 1986; 1994) : Sea of Japan, Indian Ocean, Mediterranean, Adriatic and Atlantic. In Africa, it has been identified in Tunisia (Trilles & Raibaut, 1971), Mauritania and the Suez gulf (Trilles, 1972). In Algeria, especially from Algiers, it has been reported by Trilles (1972). This species is recorded for the first time in the gulf of Béjaïa.

The distributions of *C. oxyrrhynchaena* and *C. oestroides* are similar, although the first species is distinctly less common (Trilles, 1968; 1994). *Ceratothoa oxyrrhynchaena* has been collected mainly on Maenidae and Sparidae (Trilles, 1968). In Tunisia, this parasite has been reported from the buccal cavity of Maenidae (Trilles & Raibaut, 1971), on *Zeus faber* (Trilles, 1972), *Raja asterias*, *Raja clavata*, *Scyliorhinus stellaris* and *Torpedo marmorata* (Capape & Pantoustier, 1976). In Lebanon, it has been recently collected on *Lithognathus mormyrus* (Bariche & Trilles, 2005). Therefore, our results are in agreement with those of Trilles (1968), Trilles & Raibaut (1971) and Bariche & Trilles (2005).

Ceratothoa parallela (Otto, 1828)

Ceratothoa parallela (Table 1) was collected from the buccal cavity of *Boops boops* (P = 8.60%), of *Spicara smaris* (P = 4%) and of *Trachurus trachurus* (P = 2.75%). It is reported for the first time in the gulf of Béjaïa.

This species has already been collected in the Mediterranean, Adriatic and in the Northeastern Atlantic (Trilles, 1986; 1994). In Africa, it has been collected in Tunisia (Capape & Pantoustier, 1976; Charfi-Cheikhrouha et al., 2000), Algeria (Lucas, 1849; Schioedte & Meinert, 1883; Carus, 1885; Dollfus & Trilles, 1976) and more specifically from Oran and Algiers (Trilles, 1972). It has been recently found in Turkey (Öktener & Trilles, 2004).

Several authors reported this species in the buccal cavity of *Boops boops* (Trilles, 1968; 1994; Charfi-Cheikhrouha et al., 2000; Öktener & Trilles, 2004; respectively from the French, Tunisian and Turkish coasts). *C. parallela* was also occasionally found on other species of fish : *Raja asterias*, *Raja clavata* (Capape & Pantoustier, 1976; Trilles, 1994), *Sparus sp.*, *Dentex vulgaris*, *Boops salpa*, *Esocis belonis*, *Spicara chryselis*, *S. maurii*, *S. alcedo*, *Trigla corax*, *Mullus sp.*, *Gadus capelanus*, *Merlucius merlucius* (Trilles, 1994), cultured *Sparus aurata* (Papapanagiotou & Trilles, 2001) and *Diplodus annularis* (Charfi-Cheikhrouha et al., 2000).

In Algeria, especially in the gulf of Béjaïa, our results are in agreement with the previous data : *Ceratothoa parallela* is a euryxenic species, chiefly parasitic on *Boops boops* and more rarely on *Spicara smaris* and *Trachurus trachurus*.

General list of the Cymothoidae reported to date from Algeria : species, hosts, locations and authors

Cymothoids	Hosts	Locations	Authors
Anilocrinae			
Anilocra physodes (L., 1758)	–	Algiers	Lucas, 1849
	Moena vulgaris	Algiers	Carus, 1885
	Box	Algiers	Fain-Maurel, 1966
	Pagellus		
	Other Sparidae		
	Diplodus fasciatus	Skikda (ex-Phillipeville)	Trilles, 1975
	Spondyliosoma cantharus		
	Scorpaena porcus	Bou-Ismail (ex- Castiglione)	Trilles, 1975
	Zeus faber		
	Spondyliosoma cantharus	Gulf of Jijel	Present study
Anilocra frontalis	–	Oran	Milne Edwards, 1840
Milne Edwards, 1840			Lucas, 1849
			Carus, 1885
			Gerstaeker, 1901
	–	Mers-el-Kébir	Lucas, 1849
	Crenilabrus quinquemaculatus	Bou-Ismail (ex- Castiglione)	Trilles, 1975
	Crenilabrus roissali		
	Labrus viridis	Algeria	Trilles, 1975
	–	Oran	
	Oblada melanura	Algeria	Dollfus & Trilles, 1976
	Labrus bergylta		
	Diplodus annularis		
	Gobius paganellus		
	Crenilabrus sp.		
	Labrus	Bou-Ismail (ex- Castiglione)	Trilles, 1979
	Labrus merula		
	Crenilabrus ocellatus		
	Mullus barbatus	Gulf of Béjaïa	Present study
	Umbrina canariensis		
	Sciaena aquila		
	Pagellus acarne		
	Lithognathus mormyrus		
	Solea vulgaris		
	Diplodus annularis		
	Crenilabrus pavo	Gulf of Jijel	Present study
	Lithognathus mormyrus		
Nerocila orbignyi	–	Annaba (ex Bône)	Lucas, 1849
(Guérin-Méneville, 1832)	*Mugil cephalus*	Gulf of Béjaïa (Soummam Oued)	Present study
	Crenilabrus pavo	Gulf of Béjaïa	Present study
	Crenilabrus pavo	Gulf of Jijel	Present study
	Trygla lyra	Gulf of Béjaïa	Present study
Nerocila maculata	–	Annaba (ex- Bône)	Lucas, 1849
(Milne Edwards, 1840)			Carus, 1885
			Trilles, 1975
	Pagellus acarne	Gulf of Béjaïa	Present study
Nerocila bivittata	–	Algeria	Gourret, 1891
(Risso, 1816)			
	–	Annaba (ex- Bône)	Lucas, 1849
			Schioedte & Meinert, 1881
			Carus, 1885
	–	Oran	Lucas, 1849
			Carus, 1885
	–	Algiers	Carus, 1885
	Crenilabrus melops	Bou-Ismail (ex- Castiglione)	Trilles, 1975
	Crenilabrus pavo		
	Crenilabrus pavo	Gulf of Béjaïa	Present study
Ceratothoinae			
Ceratothoa italica	*Diplodus annularis*	Gulf of Béjaïa	Present study
Schioedte & Meinert, 1883			
Ceratothoa oestroides	Diverse fish species	Annaba (ex- Bône)	Lucas, 1849
(Risso, 1826)		Fort Génois	
	–	Algiers	Carus, 1885
	–	Bou-Ismail (ex- Castiglione)	Trilles (1972; 1979)

General list of the Cymothoidae reported to date from Algeria : species, hosts, locations and authors

Cymothoids	Hosts	Locations	Authors
	–	Bou-Haroun	Dollfus & Trilles, 1976
	Boops boops	Gulf of Béjaïa	Present study
	Spicara smaris		
	Pagellus acarne		
	Trachurus trachurus		
Ceratothoa paralella	–	Algeria	Lucas, 1849
(Otto, 1828)	–	Oran	Lucas, 1849
			Schioedte & Meinert, 1881
			Carus, 1885
			Trilles, 1972
	–	Algiers	Trilles, 1972
	Boops boops	Gulf of Béjaïa	Present study
	Trachurus trachurus		
	Spicara smaris		
Ceratothoa collaris	–	Oran	Lucas, 1849
Schioedte &			Schioedte & Meinert, 1883
Meinert, 1883			Carus, 1885
	Dentex filosus	Oran	Monod, 1924 a-b
	Pagellus sp.		
	Pagellus erythrinus	Oran	Trilles, 1972
	Pagellus acarne		
Ceratothoa oxyrrhynchaena	*Zeus faber*	Algiers	Trilles, 1972
Koelbel, 1878	*Spicara smaris*	Gulf of Béjaïa	Present study
	Boops boops		
Emetha audouini	–	Algeria	Trilles, 1972
(Milne Edwards, 1840)			
Livonecinae			
Idusa dieuzeidei	*Symphurus nigrescens*	Algeria	Dolffus, 1950
Dollfus, 1950			Dollfus & Trilles, 1976
Livoneca pomatomi	*Boops boops*	Algiers	Dollfus & Trilles, 1976
(Gaillat Airoldi, 1940)	*Boops boops*	Bou-Haroun	Dollfus & Trilles, 1976
Livoneca sinuata	*Gobius*	Bou-Haroun	Dollfus & Trilles, 1976
Koelbel, 1878	*Boops boops*	Algiers	Dollfus & Trilles, 1976

Now, 14 Cymothoidae have been reported from Algeria, the majority being widely distributed in the Mediterranean; however, *Ceratothoa collaris* appears to be limited to the Northern and Northwestern coasts of Africa while *C. oxyrrhynchaena* is cosmopolitan. We report nine species, *Anilocra frontalis, A. physodes, Nerocila bivittata, N. orbignyi, N. maculata, Ceratothoa parallela, C. oestroides, C. oxyrrhynchaena, C. italica,* for the first time in the Gulfs of Béjaïa and Jijel. *Ceratothoa italica* is new for the Algerian fauna.

We have identified the host fish for all the cymothoids that we have collected. For some parasites (*A. frontalis*, *N. bivittata*, *C. oestroides*, *C. oxyrrhynchaena* and *C. parallela*), our results are in agreement with the characteristics of specificity known for these species. For others (*A. physodes*, *N. orbignyi*, *N. maculata* and *C. italica*), our data seem to indicate possible variation of the specificity according to the prospected area. It is particularly obvious for *N. maculata;* host of this species, previously unknown in Algeria, is now identified and differs from those previously reported from other Mediterranean zones.

Prevalences are rather weak and correspond to a low general infestation rate, varying, however, according to the host species. Therefore, our results are quite similar to some data about Tunisia. Infestation rates are, moreover, even weaker in the Tamelaht lagoon and the Soummam Oued.

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New occurrence of *Lecane decipiens* (Murray, 1913) and some other alien rotifers in the Schelde estuary (Belgium)

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ABSTRACT. Three alien rotifers were found in the Schelde estuary in spring and summer 2002-2003. *Lecane decipiens* and *Brachionus variabilis*, with a maximum of respectively 6 and 4 ind L^{-1}, were rare and quite localized in the freshwater tidal reach, corresponding to the stage III (non invasive species) in Colautti and Mac Isaac's invasion terminology. This record of *L. decipiens* is the first confirmed one in Europe. *Keratella tropica* was generally present in the freshwater reach but, more tolerant to salinity, it was also found in brackish water. With its maximum abundance of 18 ind L^{-1}, *K. tropica* appeared to be invasive, with a stage IVa (widespread but rare) at the scale of the Schelde estuary. A description with illustrations of these introduced species is given with some information about their ecology.

KEY WORDS : *Lecane decipiens*; *Keratella tropica*; *Brachionus variabilis*; introduced species; Schelde estuary.

INTRODUCTION

As non indigenous species (NIS) entering an ecosystem can induce major changes in community structure and ecosystem functioning, and potentially destroy local species by predation or competition, biological invasions are one of today's concerns in both terrestrial and aquatic systems (Perrings et al., 2002; Kennish, 2002; Lee, 2002; Lee et al., 2003; Tabacchi et al., 2005). For aquatic organisms, shipping activity, with its associated ballast water discharge and hull fouling, is known to be an important mean of transport both overseas and locally (Wasson et al., 2001). Consequently, harbour management strategies take this problem into account in order to prevent biological invasions (Horan et al., 2002; Batabyal & Beladi, 2004; Batabyal et al., 2005).

As the Schelde basin (Belgium and the Netherlands, Fig. 1) is one of the most intensively used shipping ways in Europe, it is a particularly favourable area to the introduction and spreading of non indigenous species. After Rotterdam, the Belgian harbour of Antwerpen, located inland, between km 68 and 89 from the mouth (Vlissingen), is the second European port and the eighth in the world. Navigable for most of its length, the Schelde is connected with a dense canal network giving access to other major ports in the vicinity, as Gent, Brussel, Zeebrugge, Oostend, Liège, and also to other basins, as the Rhine and Meuse basins. The Schelde is also one of the few remaining estuaries in Europe with an extensive freshwater tidal reach (Meire et al., 2005; Van Damme et al., 2005) and as such offers a large variety of environments to the introduced specimens. Not surprisingly, numerous exotic species, both terrestrial and aquatic have been reported from the area. The Chinese mitten crab, *Eriocheir sinensis* (Milne-Edwards, 1854), is perhaps the best known invader in the Schelde estuary (Anger, 1991), and the mollusc *Dreissena polymorpha* (Pallas, 1771) has been reported on sluice doors. As some other examples we cite : the Atlantic croaker, *Micropogonias undulates* (L.) (Stevens et al., 2004); the North-American amphipods *Melita nitida* Smith, 1873 and *Incisocalliope aestuarius* (Watling and Maurer, 1973) reported by Faasse & Van Moorsel (2003).

The zooplankton community is also concerned as a potential invader. The small size of most of these organisms, combined with the production of resting eggs offers them advantages for propagation. *Acartia tonsa* (Dana, 1848), for example, is a North-American native calanoid well established in all European estuaries. *Daphnia ambigua* Scourfield 1947 and *D. parvula* Fordyce, 1901 are alien cladocerans commonly found in Belgium (Maier, 1996) as well as *Moina weissmanni* Ishikawa, 1896 reported in Flanders by De Meester et al. (2002).

But not all introduced specimens can succeed in establishing themselves. The important variety of situations has given rise to an equally diverse vocabulary, using terms as : alien, exotic, imported, colonizing, invasive… Colautti & Mac Isaac (2004) have clarified the confused "invasive" species terminology by suggesting a classification based on the "propagule pressure concept" (Williamson & Fitter, 1996) with different stages in an invading process. Five stages were identified : stage 0 being the potential invading propagules in their donor region; stage I, the propagules are taken into a transport vector (ballast water for example); stage II, the propagules survive the transport and are released in a new environment; stage III, they establish (survive and reproduce) in the recipient region but stay localized and rare; stage IVa, propagules become widespread but rare; stage IVb, propagules are localized but dominant; finally, at stage V they are widespread and dominant. The stages III to V only concern established species in a new environment. Stages IVa and V are considered as "invasive" opposite to stages III and IVb which are "non invasive". This classification was used in the present work.

The aims of this study were (1) to report the presence of some introduced rotifers (*Keratella tropica, Lecane decipiens* and *Brachionus variabilis*) in the Schelde estuary, (2) to present morphological characteristics of these species in the Schelde focussing on their diagnosis, with new illustrations and (3) to discuss their ecology and their present invasive stage in the Schelde.

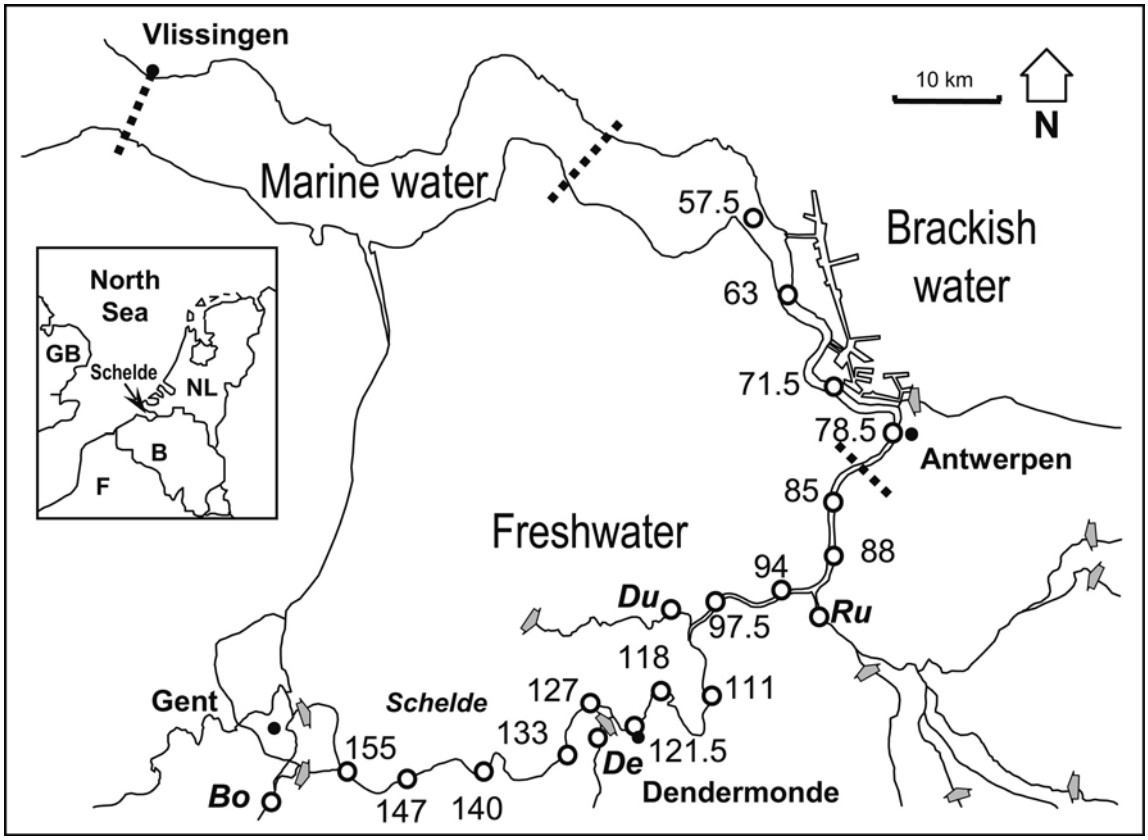

Fig. 1. – Map of the Schelde estuary indicating the marine, brackish and freshwater stretches and the position of the sampling stations (white circles). Stations in the Schelde are denoted according to their distance to the mouth, at Vlissingen. Stations in the tributaries were called "Ru" for Rupel, "Du" for Durme, "De" for Dender and "Bo" for Boven Schelde. Gray arrows indicate position of upper limit of tidal influence.

MATERIALS AND METHODS

The material examined (Table 1) was obtained in 2002-2003 from campaigns in the brackish and freshwater reaches of the Schelde estuary during the multi disciplinary monitoring of the OMES program (Flemish government research program), including investigations about the zooplankton diversity and distribution. 16 stations, identified by their distance (in km) to the mouth of the estuary in Vlissingen and 4 tributaries (Rupel, Durme, Dender and Boven Schelde), were monthly sampled (Fig. 1).

50 litres of the surface water were collected in the middle of the stream with a bucket from a ship and filtered over a 50µm mesh size. Carbonated water was added to the sample in order to narcotize rotifers before fixing them with formalin at a final concentration of 4%. The tributaries were sampled from the shore, at about one km from their mouth.

In 2003, some specimens were also collected from the shore in Antwerpen, Dendermonde and Gent.

Salinity and temperature were measured *in situ* using an YSI 650 MDS multimeter with an YSI 600 R sensor.

TABLE 1

List of material examined, with the number of specimens and sampling station. All stations were in the Schelde estuary except Gent *, in the Boven Schelde. D indicates the distance from the mouth at Vlissingen.

	Number	Station	D (km)	Date
L. decipiens	1	Vlassenbroek	118	05/2002
	2	Dendermonde	121.5	05/2002
	2	Dendermonde	121.5	03/2003
	2	Sint Onolfs	127	06/2002
	2	Wetteren	147	06/2002
	2	Melle	155	06/2002
	4	Gent *	–	06/2002
B. variabilis	2	Temse	97.5	07/2002
	1	Mariekerke	111	06/2002
	3	Mariekerke	111	07/2002
	1	Vlassenbroek	118	07/2002
	3	Wetteren	147	07/2002
K. tropica	2	Kruibeke	85	07/2002
	2	Mariekerke	111	07/2002
	2	Dendermonde	121.5	08/2002
	3	Sint Onolfs	127	08/2002
	2	Appels Veer	133	08/2002
	2	Uitbergen	140	07/2002
	2	Wetteren	147	07/2002

In the laboratory, samples were stained with 4-5 drops of erythrosine, prepared at 0.8mg per 100mL of water, to facilitate detection of the organisms in the detritus rich samples. The samples were screened with a stereomicroscope Leica MZ 9.5 (9x – 90x) and specimens of interest mounted on a slide in glycerine. These were further observed with a microscope Nikon Optiphot-2 (50x – 600x) fit with a DIC process and a camera lucida for drawings and measurements.

RESULTS AND DISCUSSIONS

The following species have been diagnosed as alien species in the Schelde estuary :

Lecane decipiens (Murray, 1913)

Description : *Lecane* with a smooth ovate lorica being about four-fifths as wide as long (Fig. 2). Dorsal plate anteriorly narrower than ventral, without lateral extensions but sharp triangular projections at the antero-lateral corners. Deep lateral sulci between the dorsal and ventral plates. The lateral margins of the dorsal plate do not reach the head aperture margin. This aperture having coincident margins, strongly concave, with sinuses very deep, V-shaped and rounded posteriorly. Toe single and long, parallel sided for half and tapering gradually to point, without claw.

Diagnosis : This species can be mistaken with the common *L. hamata* (Stokes, 1896) and with *L. serrata* (Hauer, 1933). It differs from the first by the lateral margin of the dorsal plate which does not reach the head aperture, and from the second by its lorica without any ornamentation.

Measurements : The toe (length), the dorsal and ventral plates (width and length) were measured on 10 specimens from the Schelde. The mean size with standard deviation are given in Table 2.

TABLE 2

Mean size and standard deviation of *Lecane decipiens* Dorsal plate (DP), ventral plate (VP) and toe. 10 specimens were measured.

	DP length	DP width	VP length	VP width	Toe length
Mean size (µm)	76.5	62.9	80.8	54.8	37.2
Standard deviation	3.7	5.3	3.1	3.2	0.5

Distribution and abundance : *L. decipiens* was found in spring (May and June 2002) in 5 stations of the upper reaches of the estuary and in the Boven-Schelde (Fig. 3). The water temperature measured was between 19.4°C, in May, and 25.1°C, in June. *L. decipiens* also appeared in winter (March 2003) in one station (Dendermonde, km 121.5) at a water temperature of 10.0°C. The area inhabited by this *Lecane* covered around 40km, from Vlassenbroek (km 118) to the Boven-Schelde station, upstream from Gent. The salinity ranged from 0.09 to 0.11g L^{-1}. The maximum abundance was 6.1 ind L^{-1}, in June.

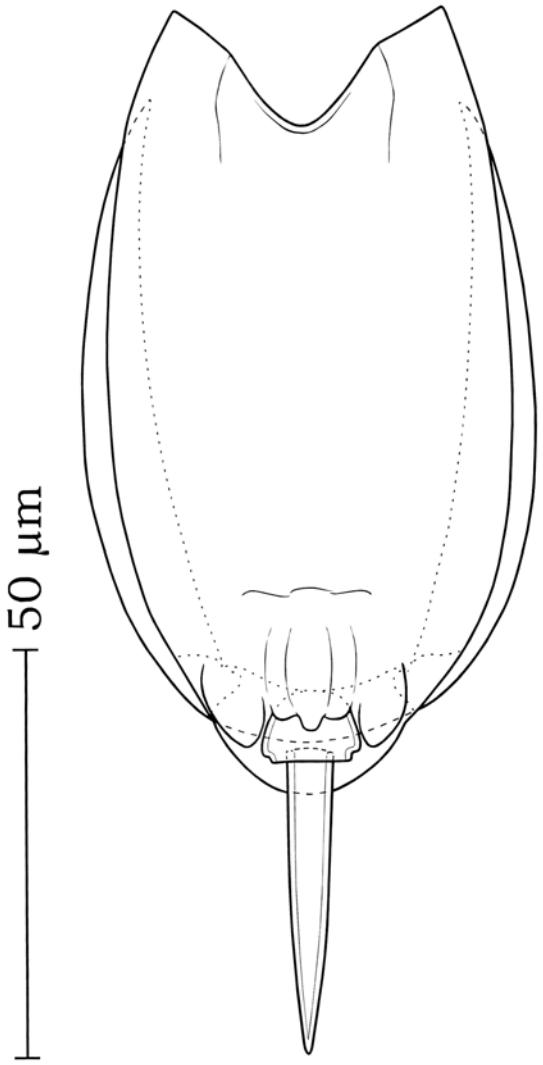

Fig. 2. – Ventral vue of a female of *Lecane decipiens*.

Discussion : The measurements showed that individuals from the Schelde estuary were rather small but in accordance with the data previously reported on the species (Koste, 1978; Sharma, 1980; Segers, 1995).

This *Lecane* originating from South and Central America was cited in Europe although all of these occurrences were uncertain or misidentified *L. hamata* (Pejler, 1962; Brunhes et al., 1982; Segers et al., 1993; Segers, 1995; Segers, 1996). The problem of reliability of the non-illustrated records as well as for some illustrated ones is well known in rotifer studies (Dumont, 1983; Koste & Shiel, 1989; Segers, 1998; Segers, 2001). The presence of *L. decipiens* in the Schelde basin in various locations (6 stations) and time (2002-2003) indicates, despite its quite low abundance in our plankton samples, the possible establishment of a rather abundant population in its typical benthic-periphytic environment. This is the first confirmed occurrence in Europe. Its presence in March 2003 associated with low temperatures indicates a large tolerance to this factor whereas the salinity range in which *L. decipiens* occured was low.

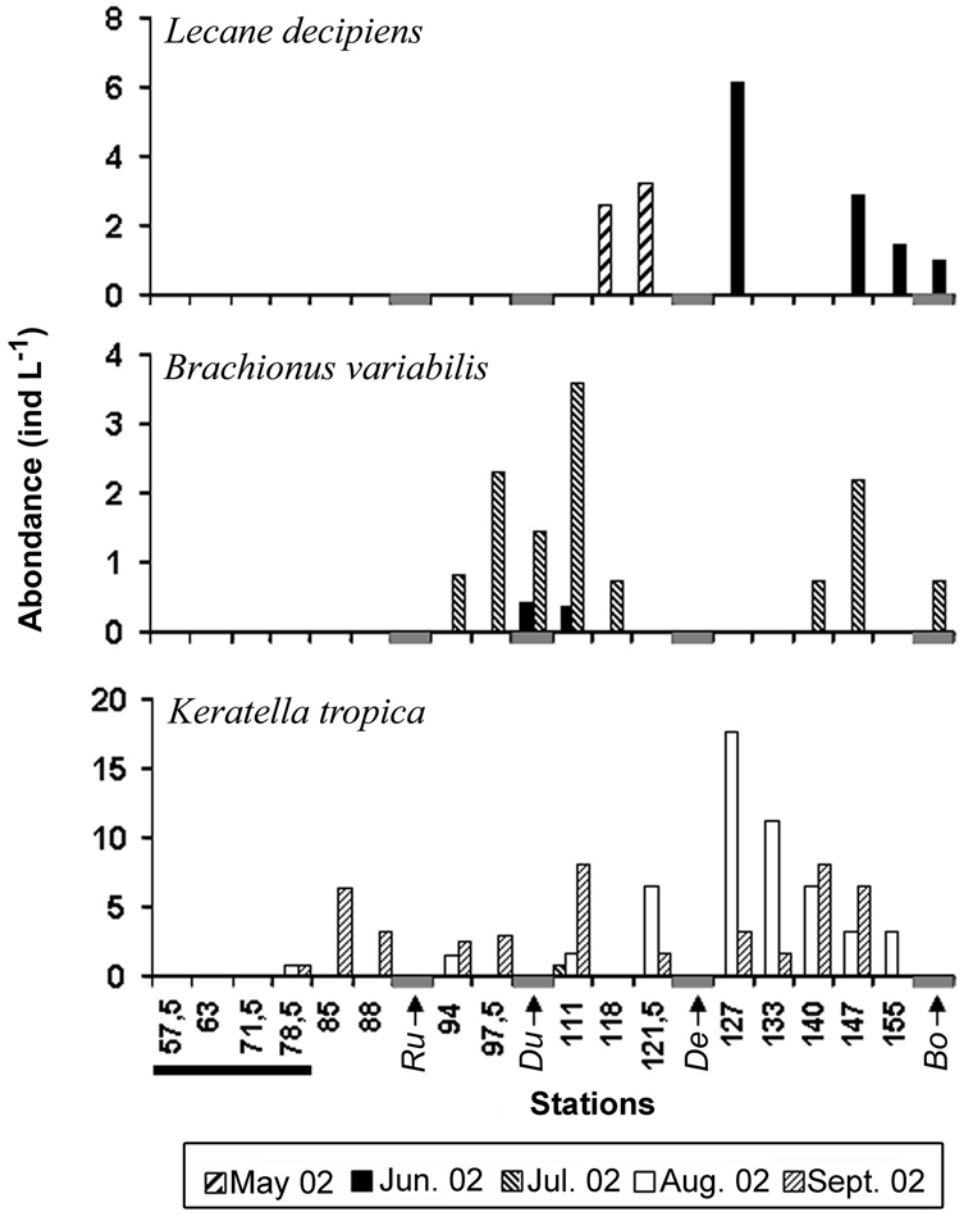

Fig. 3. – Abundance of *L. decipiens*, *B. variabilis* and *K. tropica* in the Schelde estuary in 2002. Stations in the Schelde are denoted according to their distance (in km) to the mouth, at Vlissingen. Stations in the tributaries were called "Ru" for Rupel, "Du" for Durme, "De" for Dender and "Bo" for Boven Schelde. The black line indicates the brackish area of the estuary.

According to the invasive species terminology of Colautti & Mac Isaac, 2004, this species can be considered as "non invasive" with a stage III (established species, localized and numerically rare). In May, the rotifer population was dominated by *B. calyciflorus* Pallas, 1766, and by both *B. calyciflorus* and *B. angularis* Gosse, 1851 in June. During these periods, *L. decipiens* never represented more than 0.7% of the total rotifer abundance (Fig. 4). We can assume that the impact of *L. decipiens* on the functioning of the ecosystem is negligible.

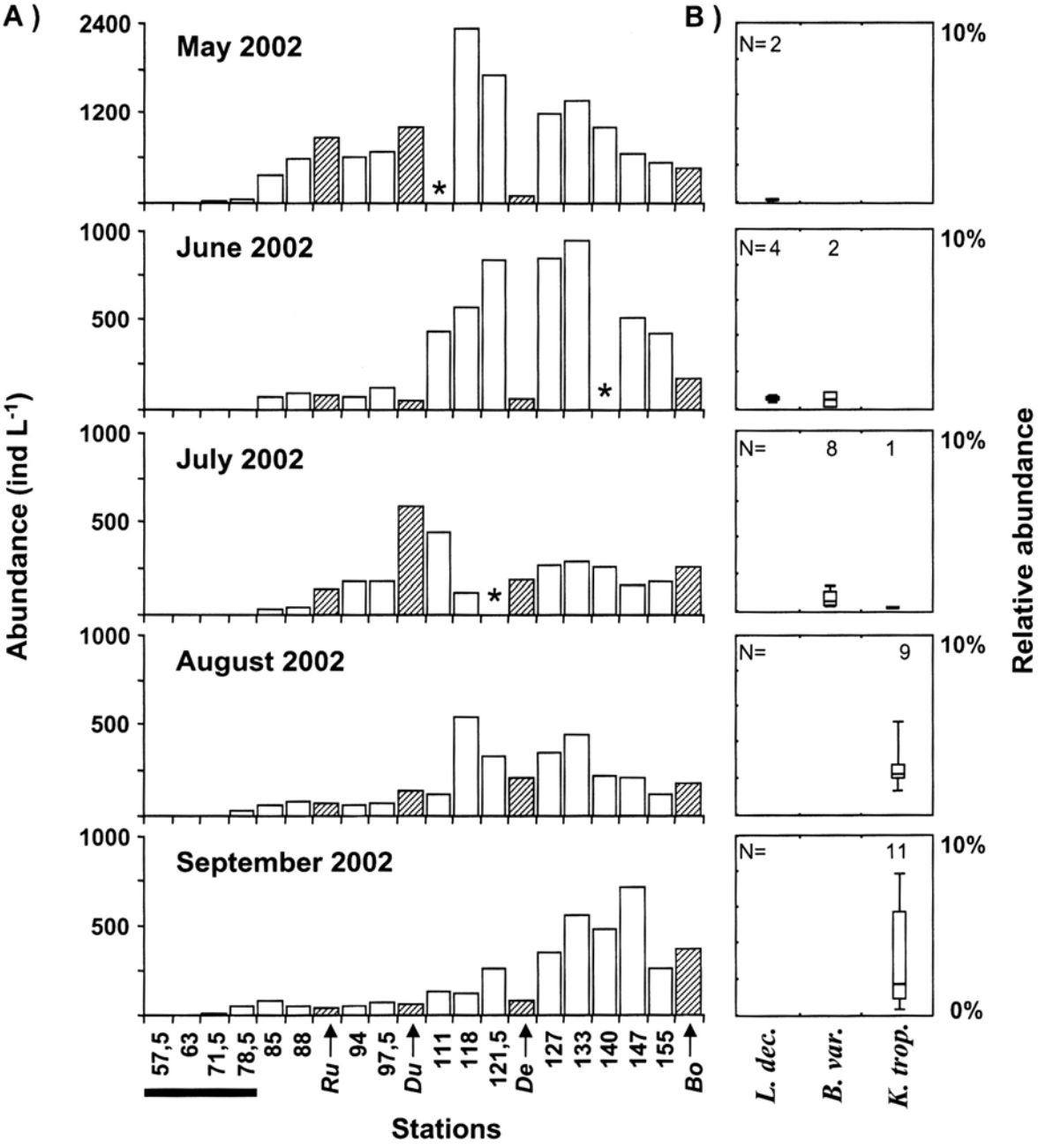

Fig. 4. – Total abundance (ind L^{-1}) of rotifers (A) from may to september 2002 in the Schelde estuary (white bars) and its tributaries (striped bars). Stations are denoted as in Fig. 3. Asterisk indicates an absence of sample.
The relative abundance (B) of the introduced rotifers, *L. decipiens* (*L. dec*.), *B. variabilis* (*B. var.*) and *K. tropica* (*K. trop*.) compared to the total rotifer abundance is represented by box-plots with the mean, the minimum and maximum relative abundance at the stations where they occurred. N indicates the number of these stations.

Brachionus variabilis Hempel, 1896

Description and diagnosis : Lorica flattened dorso-ventrally bearing six spines at the antero-dorsal margin; the median ones being the longest; the intermediate and the lateral barely equal (Fig. 5). Antero-ventral margin with two rounded median projections. Two posterior spines, very variable, can appear or not, and reach a considerable length. All specimens found in the Schelde had long spines. This *Brachionus* presents a dorsal tongue-shaped projection over the foot opening which characterise the species. The similar *B. novae-zelandiae* (Morris, 1913) does not have such projection.

Measurements : The length of the lorica with and without spines was measured on 10 specimens from the Schelde, as well as their width. The mean size with standard deviation is given in Table 3.

Distribution and abundance : *B. variabilis* was only present in the upper reaches of the estuary (Fig. 3) in summer, with quite low abundances. The maximum abundance occurred in July with 4 ind L^{-1}. Compared to the total rotifer population, the relative abundance was quite low, reaching a maximum of 1.4% of the rotifer abundance (Fig. 4). The area inhabited by *B. variabilis* covered around 70km, from Steendorp (km 94) to the Boven-Schelde station, upstream from Gent. The salinity ranged from 0.09 to 0.24 g L^{-1}. Water temperature was 20.2 to 24.1°C.

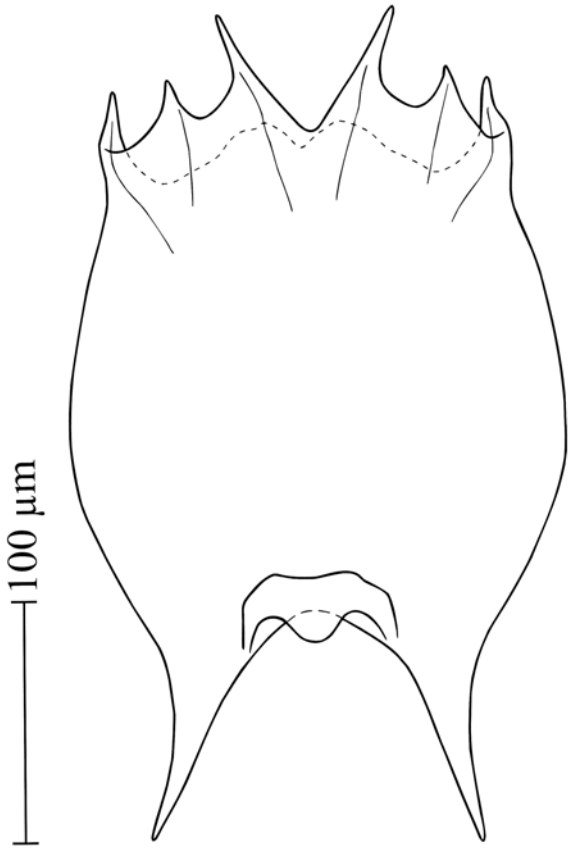

Fig. 5. – Dorsal view of a female of *Brachionus variabilis*.

TABLE 3

Brachionus variabilis length and standard deviation. 10 specimens were measured.

	Total length	Length without spines	Width
Mean size (µm)	348.2	197.5	206.7
Standard deviation	3.9	3.3	4.4

Discussion : The first occurrence of *B. variabilis* in Europe was reported in the boat racing canal in Gent (Coussement et al., 1976). Its sporadic occurrence in Belgium was noted by Dumont (1983) : "(…) *Brachionus variabilis* (…) suddenly appeared "en masse" in Belgium during the hot summer of 1976. After a short re-occurrence in 1977, it died out (?) again". At present, this American species, originally described from Havana, Illinois, and recorded from the Palearctic, Neoarctic, Oriental, Australian and Neotropical regions (De Ridder & Segers, 1997) is observed in the Schelde. All specimens were found in freshwater. In Belgium, this species could develop in high numbers around Antwerpen (e.g. Blokkersdijk) and in the creek region of the North West, (e.g. Sint Jan in Eremo) according to an anonymous referee. Considering its large distribution, the non-indigenous status of *B. variabilis* in Belgium is arguable. As well as *L. decipiens*, *B. variabilis* can be considered as "non invasive", stage III (localized and numerically rare). Further data are necessary to precise the status of the Schelde basin population but an invasive stage II would suppose some recurrent introductions and dispersal along the estuary to explain its large distribution in the estuary. Nevertheless this species is not sufficiently abundant to have a significant impact on the system.

Keratella tropica Apstein, 1907

Description : Lorica flattened dorso-ventrally bearing three median plates; a fourth small posterior plate characterise the species; three pairs of marginal plates (Fig. 6). Six spines at the anterior-dorsal margin, with the median ones, long and curved ventral ward. The lateral ones are usually a little longer than the intermediate. Two unequal posterior spines both variable in length but the right one is always longer than the left one which can be absent. The variations of the posterior spines length are illustrated in Fig. 7 with the different patterns found in the Schelde.

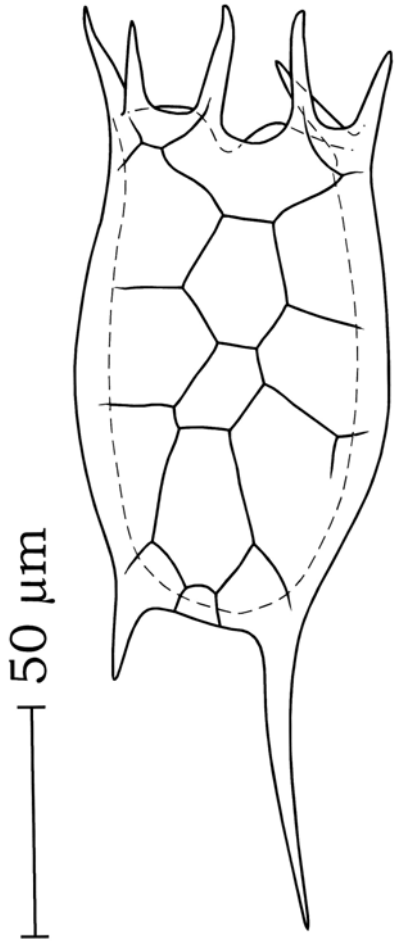

Fig. 6. – Dorsal view of a female of *Keratella tropica*.

Diagnosis : The small hindmost median plate of the lorica easily differentiates *Keratella tropica* from *Keratella valga* (Ehrenberg, 1834).

Measurements : The length of the lorica without spines was measured on 15 specimens from the Schelde, as well as the length of the two posterior spines. The mean size with standard deviation is given in Table 4.

Distribution and abundance : *K. tropica* was mainly present in the upper reaches of the estuary (Fig. 3) in the late summer (July, August and September). Some specimens were also found in Antwerpen (km 78.5) in the brackish water. The salinity ranged from 0.09 to 2.62g L^{-1}. Water temperature ranged between 18.5 and 23.6°C. The maximum abundance, observed in August, was 17.6 ind L^{-1}. The species was also observed the following years (2003-2004) during the same season. The relative abundance was much higher than that of *L. decipiens* and *B. variabilis* : negligible in July 2002 (0.2% of the rotifer population) but between 1.3 and 5.1% in August 2002, and 0.6 to 7.9% in September 2002 (Fig. 4) according to the station where this species occurred.

TABLE 4

Mean size and standard deviation of *Keratella tropica* lorica and posterior spines. 15 specimens were measured.

	Lorica	Left posterior spine	Right posterior spine
Mean size (µm)	109.3	56.2	9.3
Standard deviation	4.3	17.2	8.0

Fig. 7. – Diversity of patterns in posterior spines of *Keratella tropica* in the Schelde estuary.

Discussion : This *Keratella* is widely distributed in tropical freshwaters and also in subtropical and temperate areas in summer. Specimens from the Schelde estuary were rather small in size compared to those from other locations and generally they had a short left posterior spine. The spatio-temporal distribution of the different forms of *K. tropica* did not present any tendency : specimens with long and short posterior left spine were both present during summer in all locations. HYNES (1970) reported the existence of a diminutive form of this species from a river (Batuluoya, Ceylon) and attributes it to an adaptation to running waters. According to GREEN (1980), the spine length was correlated with the presence of calanoid copepods. In the Schelde, calanoids were rare during summer but the cyclopid copepod *Acanthocyclops trajani* Mirabdullayev & Defaye, 2002 (previously identified as *A. robustus)*, was dominant in the freshwater tidal and is a potential predator of rotifers.

As in other estuaries, *K. tropica* was mainly found in the freshwater reaches of the estuary (SHARMA & NAIK, 1996; HOLST et al., 1998; SHIEL, 1986) but it shows a tolerance to salinity, being present in the brackish area as well. It appears to be resistant to pollutions and eutrophic conditions (DUGGAN et al., 2002; MISHRA & SAKSENA, 1998), as encountered in the Schelde estuary. Moreover, it's presence in the entire freshwater area of the estuary lead us to consider it as a stage VIa (widespread but numerically rare) invasive species. In 1980, LEENTVAAR reported its presence in the cooling water of a factory in the Netherlands. Its warm-stenothermy determines its occurrence. *K. tropica* is now a common rotifer in Europe in summer and it is at present impossible to tell whether this is truly non-indigenous (SEGERS, pers. com.). Whatever its invasive status is, with its rather important relative abundance (max 7.9% of the rotifer abundance), *K. tropica* could have a significant role in the trophic web of the estuary.

GENERAL DISCUSSION

The OMES study was the only recent one focusing on rotifer diversity in the Schelde estuary. As such, the three species considered in this paper could have been introduced a long time ago without being observed till 2002. The date of their introduction in the Schelde remains unknown. All have been recorded in Europe before (see DE RIDDER & SEGERS, 1997). Nevertheless, the present report of *L. decipiens* is the first illustrated, hence reliable, record from Europe. All previous illustrated records of the species from Europe are misidentified *L. hamata* (SEGERS, 1996).

Even if the shipping is clearly an important vector of propagation for some species, it cannot be generalized to

all species. Transcontinental transport concentrates in marine and brackish waters so these ships are probably less appropriate for propagation of typically freshwater species as *L. decipiens* and *B. variabilis*. The freshwater reaches of the Schelde however also deal with a lot of more localized transport, which could facilitate dispersion within the adjacent basins of Rhine and Meuse.

For introduced species found in low abundance it is problematic to determine whether they are established (stages III and IVa) or not (stage II) in their new environment. The regular shipping traffic may lead to multiple introductions year by year. Nevertheless, the distribution and frequency of the occurrences are informative to determine the invading stage. *Keratella tropica* is the only rotifer species appearing to be invasive (stage IVa) at the scale of the investigated stretch of the Schelde estuary. *Lecane decipiens* and *Brachionus variabilis* are non invasive (stage III).

In combination with the adaptation capacity of some exotic species, as *K. tropica*, the actual climate change could be favourable to spreading of introduced species. Global change models predict an increase of run-off in North and Western Europe (Arnell, 1999). Muylaert et al. (2001) showed changes in phytoplankton composition of the Schelde following freshets. During the period 1996-2000, the run-off of the Schelde has strongly and continuously increased and Struyf et al. (2004) have shown a possible effect on the modification on the Schelde estuarine nutrient fluxes. In 2002 the discharge of the Schelde was indeed very high (Van Damme et al., 2005). These phenomena have a strong influence on the ecosystem functioning, which increases the difficulty to predict the becoming of the introduced species. Nevertheless, the 3 probably alien rotifers presented here show a development during the warmest periods of the year. Hence, an increase of the water temperature could facilitate their development in this area. However, to our knowledge, no rotifer has up to date been reported to be invasive in any estuary.

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Does reproduction accelerate the growth of eye lens mass in female voles?

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ABSTRACT. Although the eye lens mass method has long been used for determining age in small rodents from natural populations, the effects of breeding on growth rates of lenses are rarely considered. Under laboratory conditions, we examined eye lens mass in two groups of 100- day old females of the common vole (*Microtus arvalis*) : one comprising the females that have already bred, each delivering two litters, and another comprising controls that did not reproduce. The breeding females were heavier than the nonbreeding ones and also had heavier dried eye lenses. However, the effect of breeding on eye lens mass disappeared when the variation in body mass was accounted for in the statistical model. The total number and mass of offspring that the females produced did not affect the lens mass. We conclude that different reproductive histories did affect the growth of eye lens in female common voles through its influence on body size. These results suggest that besides age, the construction of calibration curves for aging voles in natural populations should also include individual body mass as an additional covariate to account for variation in body mass due to differences in reproductive condition.

KEY WORDS : age estimation, eye lens mass, reproduction

INTRODUCTION

Demographic structuring based on age is an important concept in population ecology and biological conservation. Age determination by measuring the mass of eye lens is a method widely used in many mammals (LORD, 1959; BOTHMA et al., 1972; BROEKHUIZEN & MAASKAMP, 1979), including small microtinae rodents (MARTINET, 1966; LELOUARN, 1971; HAGEN et al., 1980, ADAMCZEWSKA-ANDRZEJEWSKA, 1981; MORAVEC, 1985; GLIWICZ, 1994; 1996; TAKAHASHI & SAITOH, 1997). This method is usually considered as one of the most reliable ones among those available for field research (ADAMCZEWSKA-ANDRZEJEWSKA, 1973; GURNELL & KNEE, 1984). At the same time, it is well-recognised that it also has significant limitations in animals with varying biological rates, such as somatic growth rates and developmental rates (MEUNIER & SOLARI, 1972; HANSSON, 1983a). In particular, short-lived rodents, such as voles, which exhibit large cohort variation in body growth and reproductive output over the year, are difficult to age even with this method. Large variation in biological rates, when translating into lens mass growth, may lead to large inaccuracies in estimated ages between individuals.

Typically, female voles from spring cohorts have the fastest somatic growth and sexual development and their life-time reproduction is concentrated very early in life. In contrast, autumn-born females grow slowly and enter reproduction after overwintering at an advanced age, concentrating life-time reproduction late in life (BERGSTEDT, 1965; GLIWICZ et al., 1968; MARTINET & SPITZ, 1971; MYLLYMÄKI, 1977; GLIWICZ, 1994; TKADLEC & ZEJDA, 1998a; b). Photoperiod and food quality are usually thought to be the major triggering factors driving the acceleration of biological rates in spring-born animals (MEUNIER & SOLARI, 1972; MARTINET & SPITZ, 1971; HANSSON, 1983b; BATZLI, 1986). Indeed, the growth of eye lenses has been observed to be faster in spring-born voles than later-born voles (ADAMCZEWSKA-ANDRZEJEWSKA, 1973; MEUNIER & SOLARI, 1972; HLAVÁČ, 1979; MORAVEC, 1985).

It remains an issue whether the acceleration of biological rates early in life may have negative consequences to processes late in life. A related concept of reproductive costs (WILLIAMS, 1966; STEARNS, 1976; KOIVULA et al., 2003) implicitly assumes that reproduction, being definitely among the very demanding life periods coupled with high metabolic rates, contributes significantly to the acceleration of biological rates and is, in various ways, paid for later in life. If so, the breeding females should have heavier lenses and appear older than the nonbreeding females of the same age. However, since most females in spring populations are in reproductive condition, observations from natural populations do not allow discriminating between the effects of reproduction per se and other factors connected with the ongoing season.

The main objective of the present paper is to disentangle the effects of reproduction from the effects of other factors, such as daylight and food, on biological rates by measuring the growth of eye lens mass in breeding and nonbreeding captive female voles. The hypothesis that reproduction accelerates biological rates predicts that breeding females will have heavier lenses than nonbreeding ones of the same age.

MATERIALS AND METHODS

Experiment

The experimental animals originated from F1 and F2 generations produced by wild-caught common voles (*Microtus arvalis*, Pallas 1778) bred in captivity at 14 hours of light, 10 hours of dark, and a temperature of 20 ± 2°C. A special palletised food for voles, based on cereals, dry lucern, dry milk, vitamin and mineral supplement, was provided *ad libitum*. Immediately after weaning at 20 days of age, 54 females were paired with breeding males and kept as monogamous pairs until they gave birth to two litters. Two females died and the six others which did not reproduce were removed. By 100 days of age, reproduction occurred in 46 females, two of them reproduced only once, one female was in advanced pregnancy with a second litter and one other aborted the second litter. Delayed removal of a male caused third pregnancies in three females. The different number of litters and offspring produced were considered in calculating reproductive effort (RE). Another 59 females were kept in cages alone as nonbreeding controls, with 54 of them surviving to the required age. Surviving females from both samples were sacrificed at 100 days of age. Eyeballs were removed and then fixed in 10% formalin for more than 3 weeks. After draining out excessive fluid by filter paper, the preserved lenses were weighed on an analytical balance to the nearest 0.1mg (the formalin lens mass). The lenses were then dried at 55°C for 24 hours and weighed again (the dried lens mass).

Statistical analysis

The experimental and control females were produced by 36 mothers. Consequently, there were 36 sibling groups, within which the data were likely to be correlated, violating the basic assumption of data independence. Therefore, to avoid pseudo-replication when analysing the treatment differences in female body mass, formalin lens mass, and dried lens mass, we fitted generalized linear mixed models assuming normal error distribution and including mother's identity as a random effect, using procedure MIXED from the package SAS 9.1.3 (SAS INSTITUTE INC., 2004; LITTELL et al., 1996). Both the intercept and treatment effect were assumed to have a random component. We used restricted maximum likelihood method to estimate variance components. According to theory, breeding females, by having a higher metabolic rate, were expected to have both higher body and lens masses. We therefore adopted one-tailed significance testing procedure by applying the F-test, with the Kenward-Roger's method for computing the denominator degrees of freedom. The reproductive effort of a female was calculated according to the MILLAR (1977) and slightly modified as $RE = N\ W_w^{\ 0.75}\ (m^{0.75})^{-1}$, where N = total litter size, W_w = the mass of all offspring at weaning divided by N, and m = adult female mass at the time of sacrifice, mostly shortly before or after weaning the second litter. The W_w was computed as the quotient of the total mass of weaned offspring and total litter sizes, because the nestlings which did not survive to weaning should be considered as well.

RESULTS

As expected, the females that bred were, on average, heavier by about 20% than those that did not ($F_{1,26.2} = 10.86$, $p = 0.003$, Table 1). Because there were no differences in body mass at the beginning of the experiment ($F_{1,10} = 0.30$, $p = 0.60$), the higher body mass of breeding females was most likely due to reproduction. Females that were heavier had larger both formalin ($F_{1,89.7} = 4.97$, $p = 0.028$, Fig. 1a) and dried lens masses ($F_{1,37.3} = 6.59$, $p = 0.014$, Fig. 1b). While there was no difference in formalin lens mass between the breeding and nonbreeding females ($F_{1,91.6} = 0.20$, $p = 0.66$), the difference in dried lens mass was significant ($F_{1,20.7} = 2.91$, $p = 0.10$), suggesting that reproduction does have an effect on the growth rate of lenses, enhancing their mass by about 5%. The treatment effect on dried lens mass, however, disappeared completely in a combined statistical model including both treatment and the body mass (treatment : $F_{1,25.6} = 0.94$, $p = 0.34$; body mass : $F_{1,68.2} = 5.94$, $p = 0.017$). As the regression of eye lens mass on body mass could differ between the two groups, we tested for interaction between the treatment and body mass (test of heterogeneity of slopes). We found no difference in slopes for body mass of breeding and nonbreeding females (interaction treatment*body mass : $F_{1,64.7} = 0.35$, $p = 0.56$). This indicates that reproduction influences the growth of lenses through its overall effect on body mass (Fig. 1). In other words, there is an allometric relationship between the dried eye lens mass and body size of voles. The estimated treatment effect for dried lenses in females at 100 days of age was 0.12mg which may correspond to a bias of about 50 days of age and progressively more at later ages because of the non-linearity in the age-lens regression. Neither formalin nor dried lens mass depended on the total mass of all offspring at weaning (formalin : $F_{1,42.5} = 0.35$, $p = 0.55$; dried : $F_{1,24} = 1.30$, $p = 0.27$) or the total number of offspring produced at weaning (formalin : $F_{1,42,8} = 0.12$, $p = 0.73$; dried : $F_{1,26} = 1.21$, $p = 0.28$). Female's body mass at 100 days of age tends to be negatively related to RE ($F_{1,35.8} = 3.96$, $p = 0.054$); however, neither the formalin ($F_{1,42.7} = 0.05$, $p = 0.83$) nor the dried lens mass ($F_{1,39.3} = 0.02$, $p = 0.90$) depended on the total RE.

DISCUSSION

Exploring the differences in body mass and eye lens mass in breeding and nonbreeding female common voles, we found that the breeding females had heavier eye lenses but at the same time, that this effect could be explained fully by the increased body mass of breeding females compared to the nonbreeding ones. The resulting body size in breeding females at 100 days of age seems to be negatively affected by their reproductive effort, perhaps because of the high metabolic requirement during lactation. We conclude that breeding, no matter the size of reproductive allocation, can accelerate the eye lens mass growth rate of mothers and hence, the body mass of individuals at capture should be considered as an additional covariate in calibration equations for aging voles in natural populations.

TABLE 1

The comparison of least square means with 95% confidence intervals for body and lens masses between breeding and nonbreeding female common voles in captive experiments estimated by fitting generalized linear mixed models.

Variable	Breeding		Nonbreeding	
	Mean	95% c.i.	Mean	95% c.i.
Body mass at weaning (g)	14.4	13.4 – 15.3	14.0	13.2 – 14.9
Body mass at 100 days (g)	30.2	27.8 – 32.6	24.7	22.4 – 27.1
Formalin lens mass (mg)	10.43	10.10 – 10.76	10.34	10.1 – 10.68
Dried lens mass (mg)	5.62	5.38 – 5.85	5.35	5.12 – 5.59

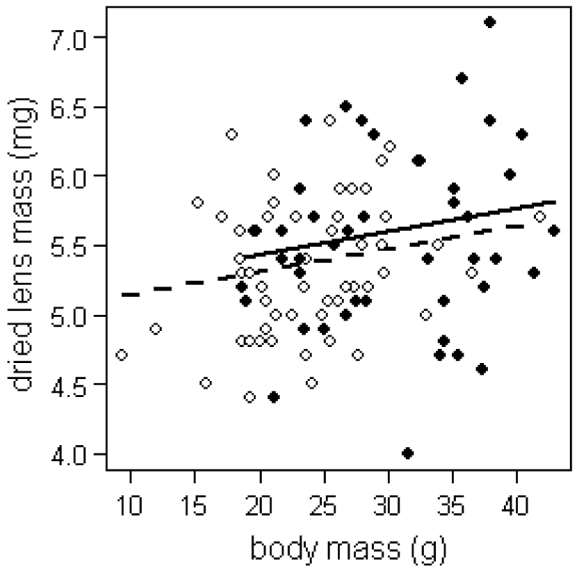

Fig. 1. – Relationship between the dried lens mass and body mass estimated by fitting generalized linear mixed model for breeding (solid line) and nonbreeding female common voles (dashed line) at 100 days of age. The circles are the observed data for breeding (closed circles) and nonbreeding females (open circles).

In voles, the sexual maturity of individuals and their entering into reproduction is connected with the increased body size (ZEJDA, 1971; 1992; MARKOWSKI, 1980). The rapid recruitment of early maturing individuals into the population and their fast body growth is usual in the spring (GLIWICZ et al., 1968; MARTINET & SPITZ, 1971; MYLLYMÄKI, 1977; GLIWICZ, 1994; TKADLEC & ZEJDA, 1998a; b). An accelerated growth of the eye lenses occurs in the same part of the year (MEUNIER & SOLARI, 1972; HLAVÁČ, 1979; MORAVEC, 1985) and results in higher lens mass in mature as compared to immature animals (HANSON, 1983b). We found that these two processes operated together in laboratory-bred animals as well, even though only the dried lens mass turned out to be sensitive enough to respond to breeding. This emphasizes again that aging of voles should indeed be based on the dried lens mass rather than the wet (formalin) one (e.g., MALLORY et al., 1981, JÁNOVÁ et al., 2003) if the higher precision of estimates is required. The differences in eye lens mass between breeding and nonbreeding are likely to increase progressively with age and individuals born towards the end of breeding season. In late summer and autumn, body growth rates begin to slow down in preparation for overwintering and the age of sexual maturation is delayed until the next year. Age at first reproduction and sexual maturation is also under a strong influence of population density which fluctuates greatly in voles (MALLORY et al., 1981; BOONSTRA, 1989; TKADLEC & ZEJDA, 1995; TKADLEC & ZEJDA, 1998a).

Body mass tended to be negatively related to RE, most likely because of higher energetic demands on a female's body when weaning a larger litter (MCINROY et al., 2000). However, the effect of variation in RE on body mass appeared to be rather weak, with no effect on the eye lens mass at all, even though body and lens masses were observed to be correlated. Perhaps, the variation in RE imposed by the experimental design was too small in order to observe any response beyond that of the body size.

The eye lens method of aging is widely used in small mammals. We demonstrated that the different reproductive histories among female voles do impose a bias in estimates of age through their effect on body size. Because of the allometric relationship between the mass of eye lenses and body size, larger voles, usually those that breed, appear to be older than smaller, usually nonreproducing voles. Hence, reproduction does accelerate the growth of eye lens mass in female voles. Assuming that the similar allometric relationship exists for males as well, which is very likely, we suggest that the construction of calibration curves for aging voles in natural populations should also include individual body mass as an additional covariate to account for variation in body mass due to differences in reproductive condition.

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On the presence of the osteoglossid fish genus *Scleropages* (Teleostei, Osteoglossiformes) in the continental Paleocene of Hainin (Mons Basin, Belgium)

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ABSTRACT. Some bony remains, otoliths and squamules belonging to the osteoglossid genus *Scleropages* are described from the continental Paleocene of Hainin (Mons Basin, Belgium). The hypotheses to explain the occurrence of such a freshwater fish in Europe at that time are discussed.

KEY WORDS : Teleostei, Osteoglossidae, *Scleropages*, fossil remains, continental Paleocene, Belgium.

RÉSUMÉ. Quelques restes osseux, des otolithes et des squamules appartenant à l'ostéoglossidé *Scleropages* sont décrits dans le Paléocène continental de Hainin (Bassin de Mons, Belgique). Les hypothèses expliquant la présence de ce poisson dulçaquicole en Europe à cette époque sont discutées.

MOTS-CLÉS : Teleostei, Osteoglossidae, *Scleropages*, restes fossiles, Paléocène continental, Belgique.

INTRODUCTION

Continental deposits of Paleocene age, overlying the marine Calcaire de Mons (Danian), were described by RUTOT (1886) from the railway section at Hainin, about 13km West of Mons. In 1970, a test hole (HA 1) was drilled (at x = 107.250, y = 124.890) by the Geological Laboratory of the "Faculté Polytechnique de Mons" at the municipal football field of Hainin, about 600m North of the railway section, with the aim to realize a complete section through the lacustrine continental deposits (observed between 8.5m and 38.4m depth). Another test hole, HA 2 (at x = 107.045, y = 124.990) was drilled, some 250m North West of HA 1, with the intention to explore the underlying marine Danian deposits (GODFRIAUX & ROBASZYNSKI, 1974).

According to STEURBAUT (1998 : 144-145), the concerned "continental Montian" deposits, now called Hainin Formation, represents the lowstand systems tract of sequence TP 1.4, which corresponds to the oldest Seelandian of Europe (about 60 million years old).

Sediments from the HA 1 hole provided the oldest mammal teeth from the European Cenozoic (GODFRIAUX & THALER, 1972; VIANEY-LIAUD, 1979; SUDRE & RUSSEL, 1982; CROCHET & SIGÉ, 1983; SIGÉ & MARANDAT, 1997). This fauna has been chosen as reference-level MP1-5 of the mammalian biochronological scale for the European Paleogene (SCHMIDT-KITTLER, 1987; AGUILAR et al., 1997). The "Montian" fauna of Hainin also contains fossil amphibians (GROESSENS-VAN DYCK, 1981), turtles (GROESSENS-VAN DYCK, 1984; SCHLEICH et al., 1988), crocodiles (GROESSENS-VAN DYCK, 1986), lizards (FOLIE et al., 2005) and many fish remains. Additional fossils were obtained in 1974, from a hole with a diameter of 1m, known as "Puits Hainin", located at 25m North of the mentioned exploration drilling. This hole was burrowed till a depth of 25m and provided the bulk of the now available Hainin fossils.

In 1994, MAGIONCALDA published additional data on three new boreholes made at Hainin in 1986 and located in the area immediately East of the HA 1 hole. He mentioned otoliths of osmerids (?), *Thaumaturus* Reuss, 1844, percoids, umbrids and esocids, based on otolith identification by Nolf. The list of Magioncalda does not mention osteoglossids, because his work did not considered the "Puits Hainin", from where a small sample examined by Nolf about 20 years ago yielded osteoglossid otoliths, identified in the collection as *Scleropages* Günther, 1864 but never published until now.

Osteoglossiformes are one order of the primitive teleostean super-order Osteoglossomorpha, commonly known as the "bony tongue" fishes by reason of their peculiar bite between parasphenoid, pterygoid bones, dermobasihyal and dermobasibranchials. Osteoglossiformes are heavily ossified teleosts, with a medio-parietal skull, very large posterior infraorbitals, a more or less prognathous lower jaw, and generally large acute teeth on both jaws and a reduced or lost subopercle (TAVERNE, 1977; 1978). Their scales frequently offer a reticular pattern formed by small bony plates, the squamules, separated from each other by very narrow grooves and resting on the basal cal-

cified collagenous layer of the scale (GAYET & MEUNIER, 1983; MEUNIER, 1984).

Osteoglossiformes appear in the fossil record as soon as the Early Cretaceous with species such *Laelichthys ancestralis* Silva-Santos, 1985 from Brazil, *Chanopsis lombardi* Casier, 1961 from the Democratic Republic of Congo and *Nieerkunia liae* Su, 1992 and *Xixiaichthys tongxinensis* Zhang, 2004 both from the South East of China. Most fossil and all Recent Osteoglossiformes are freshwater fishes but there are also a few fossil marine species in the Paleocene and Eocene of Europe, North America, Central Asia and Africa. "Genus Osteoglossidarum" *tavernei* Nolf & Stringer, 1996 from the Maastrichtian of Mississipi is the first known marine occurrence for the order. Recent freshwater Osteoglossiformes generally tolerate brackish waters and sometimes enter in marine waters near the estuaries.

The fossil record of the suborder Osteoglossoidei shows a worldwide distribution, essentially because of the wide expansion of marine species during the Lower Paleogene. Recent species offer a remarkable example of Gondwanan distribution with *Osteoglossum* Cuvier, 1829 and *Arapaima* Müller, 1843 in tropical South America, *Heterotis* Rüppel, 1829 and *Pantodon* Peters, 1877 in Africa and *Scleropages* in Australia, New Guinea and Southeastern Asia. Such a Gondwanan distribution already occurs for the oldest osteoglossoid species in the continental Lower Cretaceous deposits.

Recent Osteoglossoidei are moderately large fishes with the exception of the small *Pantodon* reaching only a few centimetres. *Arapaima*, the giant of the group, grows to about 3 or 4 metres in length. Those Recent genera are grouped in three families, Osteoglossidae with *Osteoglossum* and *Scleropages*, Arapaimidae (= Heterotidae) with *Heterotis* and *Arapaima* and Pantodontidae for the sole *Pantodon* (Taverne, 1979). Some consider Arapaimidae as a simple subfamily of Osteoglossidae and also include in this family the fossil subfamily Phareodontinae (NELSON, 2006 : 104-106).

In the present paper, we describe fossil fish remains belonging to *Scleropages* from the continental Paleocene of Hainin in the Mons Basin (Belgium) and we discuss the occurrence of such an osteoglossid genus in those geological layers of that Belgian locality.

The present day distribution of *Scleropages* is restricted to Southeastern Asia, Australia and New Guinea, with a total of six species : *Scleropages formosus* (Müller & Schlegel, 1844) or Asian arowana (Fig. 1) from Indonesia, Malaysia, Thailand and Cambodia, *Scleropages leichardti* Günther, 1864 or spotted barramundi from Eastern Australia, *Scleropages jardinii* (Saville-Kent, 1892) or northern barramundi from Southern New Guinea and Northern Australia, *Scleropages aureus* Pouyaud et al., 2003 from the Siak river in Sumatra, *Scleropages legendrei* Pouyaud et al., 2003 from West Borneo and *Scleropages macrocephalus* Pouyaud et al., 2003 from Borneo too (POUYAUD et al., 2003). *Scleropages* is known as a fossil in the Neogene of Queensland, Australia (HILLS, 1943) and in the Eocene of Sumatra where it is contemporaneous with *Musperia radiata* (Heer, 1874), another fossil osteoglossid from Sumatra (SANDERS, 1934; TAVERNE, 1979). "Genus Osteoglossidarum" *deccanensis* Rana, 1988 and "genus Osteoglossidarum" *intertraptus* Rana, 1988, both from the Maastrichtian "Deccan Intertrappean Beds" of India (RANA, 1988), can also confidently be attributed to *Scleropages* or to a closely related genus and provides the oldest fossil record of the taxon. KUMAR et al. (2005) further mention the presence of an osteoglossid skull, *Taverneichthys bikanericus* Kumar et al., 2005, that seems close to *Osteoglossum* and *Scleropages,* in the continental Paleocene of Rajasthan, India.

Recent Osteoglossiformes no more exist in Europe today but *Scleropages* is not the unique fossil osteoglossiform fish on that continent. However, most other European fossil Osteoglossiformes are marine teleosts. They include *Brychaetus muelleri* Woodward, 1901, "genus Osteoglossidarum" *rhomboidalis* (Stinton, 1977) and "genus Arapaimidarum" *acutangulus* (Stinton, 1977), all three from the Lower or Middle Eocene of the southern North Sea Basin, *Monopteros gigas* Volta, 1796, *Thrissopterus catullii* Heckel, 1856 and *Foreyichthys bolcensis*, Taverne 1979, all three from the lowermost Lutetian of Monte Bolca, and four still unpublished new osteoglossoid genera in the Eocene of Denmark (BONDE, 1966, 1987, in press; TAVERNE, 1998).

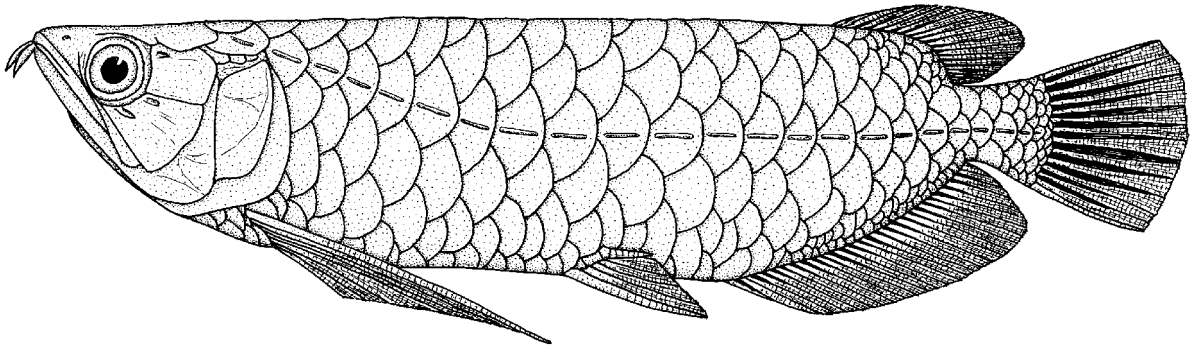

Fig. 1. – *Scleropages formosus* (Müller & Schlegel, 1844) from Southeast Asia (modified from TAVERNE, 1977 : fig. 70). This species can grow to about 90cm in length.

MATERIALS AND METHODS

IRSNB P 8238 : A right premaxilla, Hainin, at 17-17.7m depth (level R1).

IRSNB P 8239 : An incomplete right maxilla, Hainin, at 17-17.7m depth (level R1).

IRSNB P 8240 : A fragment of a right entopterygoid, Hainin, at 13.7-14m depth (level N2).

IRSNB P 8241 : A fragment of a left palato-ectopterygoid, Hainin, at 13.3-13.7m depth (level N1).

IRSNB P 8242 : An abdominal vertebra, Hainin, at 14-14.6m depth (level O1).

IRSNB P 8243 : A left saccular otolith, Hainin, precise depth unknown, and a fragmentary saccular otolith, Hainin, at 17-17.7m depth (level R1).

IRSNB P 8244-8251 : Squamules, Hainin, at 13.3-13. m (level N1), 13.7-14m (level N2), 16-16.6m (level Q1), 16.6-17m (level Q2), 17-17.7m (level R1) and 17.7-18m depth (level R2).

Levels N1, N2 and O1 are grey clays with calcareous concretions broken by peaty ruptures, while levels Q1, Q2, R1 and R2 are rough tufaceous grey marls (GODFRIAUX & ROBASZYNSKI, 1974).

The material has been studied with a Wild M5 stereomicroscope supplied with a camera lucida used for the drawings.

PALEONTOLOGICAL STUDY

Division **TELEOSTEI** Müller, 1846

Super-order **OSTEOGLOSSOMORPHA** Greenwood et al., 1966

Order **OSTEOGLOSSIFORMES** Regan, 1909

Suborder **OSTEOGLOSSOIDEI** Regan, 1909

Family **Osteoglossidae** Bonaparte, 1832

Genus **Scleropages** Günther, 1864

Species **Scleropages** sp.

Bony remains (Figs 2 ; 3)

The preserved right premaxilla (Fig. 2, B1-3) is complete. It is a robust bone, triangular in shape. The oral border bears ten teeth most of which visible only by their roots. Those teeth are large, laterally compressed and closely arranged side by side except the first one which seems to be the larger of the series and which is slightly separated from the other teeth. The second tooth stands in a more inner position than the other teeth. The base of the two partially preserved teeth is covered with bone. The symphyseal ascending process is reduced to a small bony knob. A small hollow is located on the symphyseal border of the bone, just before and below the ascending process. A deep longitudinal groove for the articulation with the maxilla is visible in a median position on the inner side of the bone.

Only the middle portion of a right maxilla (Fig. 2, A1-4) is preserved. The oral border carries the roots of ten teeth. The dorsal rim of the bone forms a small crest.

A fragment of a right entopterygoid (Fig. 3, A1, 2), representing its postero-dorsal part, is preserved. A short but deep vertical groove is visible at the raised top of the external face of the bone. On the internal face, at a short distance from the dorsal margin of the bone, there is a longitudinally disposed single row of large, conical, pointed teeth of whose the most posterior are the largest. Below that row of large teeth, the surface of the bone is covered with much smaller teeth irregularly ranged.

A middle portion of a left palato-ectopterygoid (Fig. 3, C1, 2) is also preserved. The external face of the bone bears an osseous horizontal crest close to its ventral border. The internal face is covered by numerous very small teeth, irregularly disposed. The teeth of the ventral margin are conical and a little larger than the others.

Only one abdominal vertebra (Fig. 2, C1, 2) is known. It bears on its left side a long, large and obliquely oriented haemapophysis (= parapophysis). The right haemapophysis is lost. Only the base of the broad neural arch is preserved. Both the lateral faces of the centrum and the haemapophysis are deeply sculptured by crests and holes.

The saccular otolith is large and robust, with a strongly prominent rostrum and a well marked angle in the central portion of the ventral rim. Although the dorsal rim is somewhat corroded, the lacking part does not substantially alter the shape of the otolith. The anterior part of this rim is somewhat concave. The outer face is very slightly convex, nearly flat. Its surface is smooth, but shows some fine concentric growth lines, especially near the ventral rim. The greatest thickness of the otolith is located in its posterior portion. The inner face is clearly convex, especially in the dorso-ventral direction. The sulcus, entirely located in the upper half of the outer face, is not divided in an ostial and a caudal part, and is completely filled with colliculum which shows a rough surface, which strongly contrasts with the very smooth surface of the ventral area. The ventral rim shows a sharp profile, which becomes gradually smoother towards the posterior rim. This rim shows an angulous profile at the junction with the inner face, but a smooth transition to the outer face.

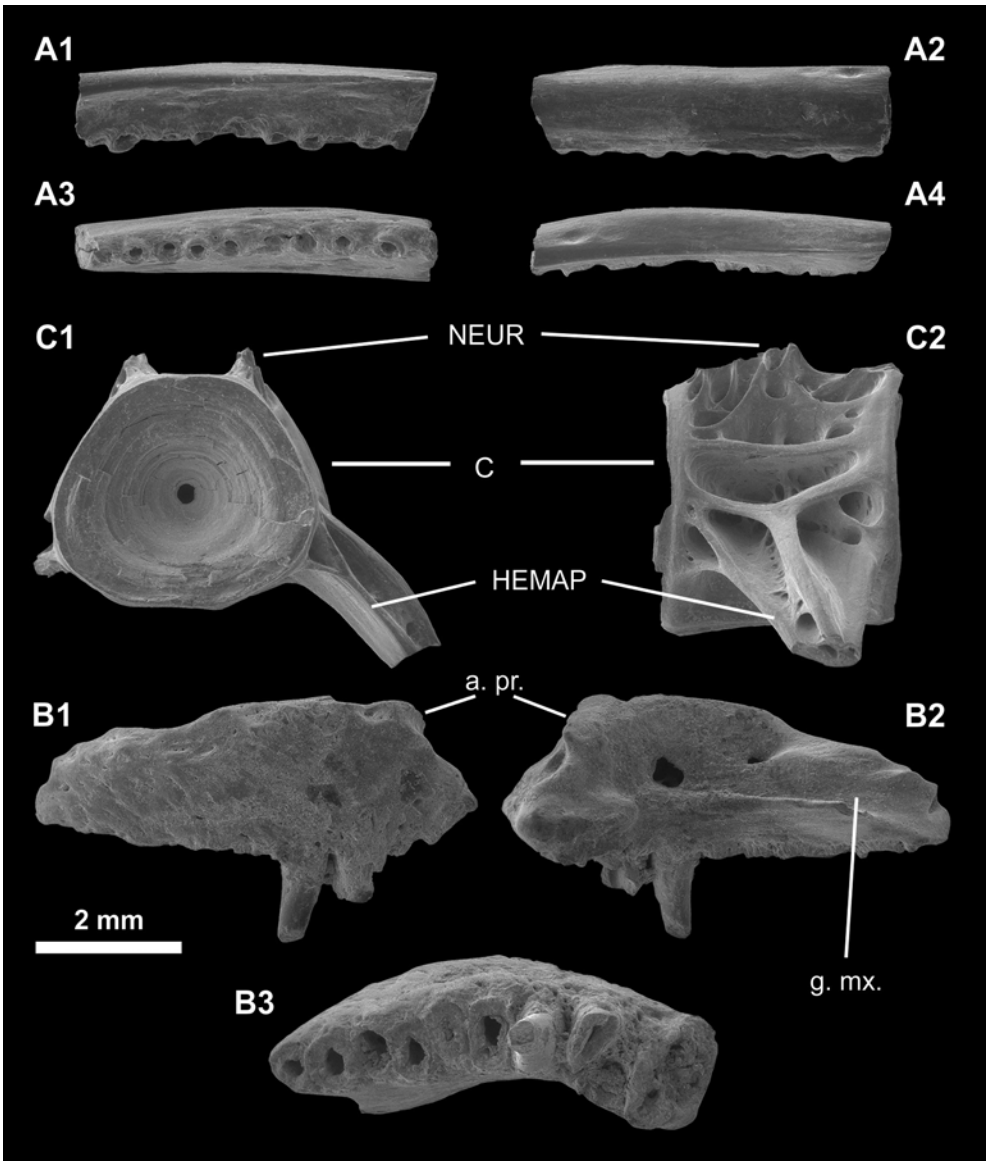

Fig. 2. – *Scleropages* sp. from the Paleocene of Hainin. (A) Fragment of a right maxilla (IRSNB P 8239) in internal (A1), external (A2), ventral (A3) and dorsal view (A4). (B) Right premaxilla (IRSNB P 8238) in external (B1), internal (B2) and oral view (B3). (C) Abdominal vertebra (IRSNB P 8242) in anterior (C1) and left lateral view (C2). Legend : **C** : vertebral centrum; **HEMAP** : haemapophysis (= parapophysis); **NEUR** : neural arch; **a. pr.** : symphyseal ascending process of the premaxilla; **g. mx.** : groove on the inner face of the premaxilla for the articulation of the maxilla.

Scales (Fig. 5)

Numerous small quadrangular, rhombic and polygonal squamules have been found at Hainin. The lower face of each squamule is concave, with the margin raised in the form of a ridge, and thus is looking like an opened box. The internal face generally is smooth but in some squamules a few large widely spaced tubercules are present. The apex of those internal tubercules sometimes is perforated by a minute hole. The external face of the squamules is flat and always ornamented with granular tubercules. Those tubercules are very small and more or less arranged in irregular concentric rows on the squamules from the anterior field of the scale. The tubercules are much larger and irregularly ranged on the squamules from the posterior field of the scale. Circuli and radii do not exist.

DISCUSSION

The discovery of squamules ornate with granulations in the Hainin continental Paleocene deposits attests the presence there of a fossil osteoglossoid fish. The African osteoglossomorph family Mormyridae also possesses scales with squamules but their ornamentation is formed by normal circuli and not by granulations as in Osteoglossoidei.

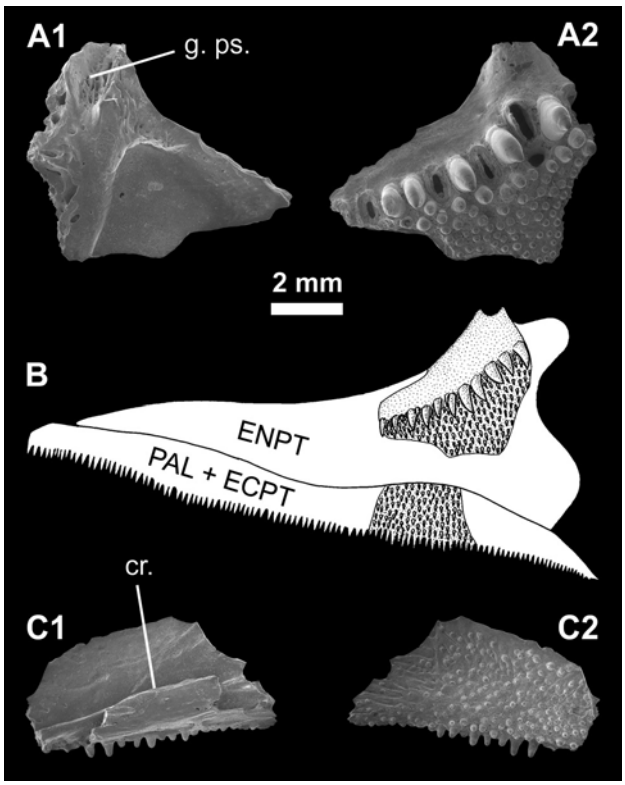

Fig. 3. – *Scleropages* sp. from the Paleocene of Hainin. (A) Fragment of a right entopterygoid (IRSNB P 8240) in external (A1) and internal view (A2). (B) Schematic drawing of the right entopterygoid and palato-ectopterygoid of *Scleropages* in internal view to show the location of the Hainin pterygoid fragments (IRSNB P 8241). (C) Fragment of a left ectopterygoid in external (C1) and internal view (C2). Legend : **cr.** : crest on the external face of the ectopterygoid; **g. ps.** : groove on the external face of the entopterygoid to receive the basipterygoid process of the parasphenoid.

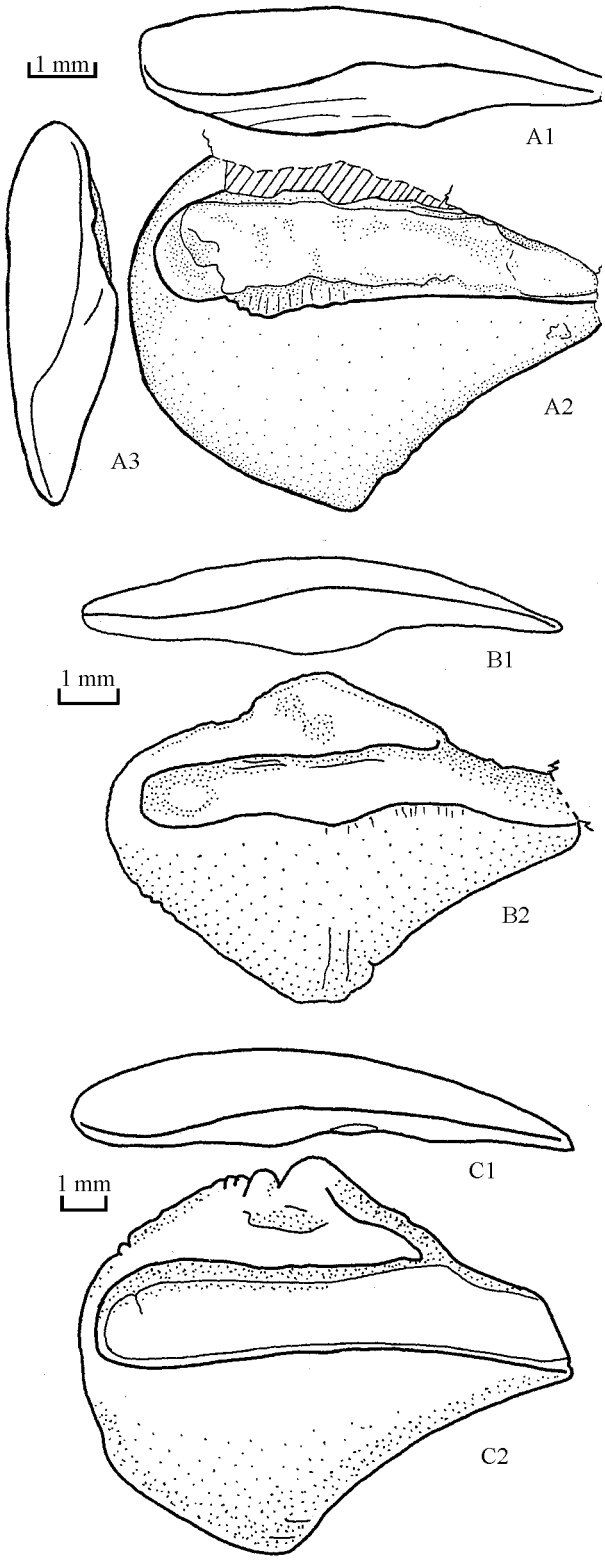

Fig. 4. – (A) Left sagitta (IRSNB P 8243) of the *Scleropages* sp. from the Paleocene of Hainin, in dorsal (A1), profile (A2) and external view (A3). (B) Left sagitta of *Scleropages formosus* (Müller & Schlegel, 1844) in external view (modified from TAVERNE, 1977 : fig. 81). (C) Left sagitta of *Scleropages leichardti* Günther, 1864 in external view (modified from NOLF, 1993 : Fig. 4A).

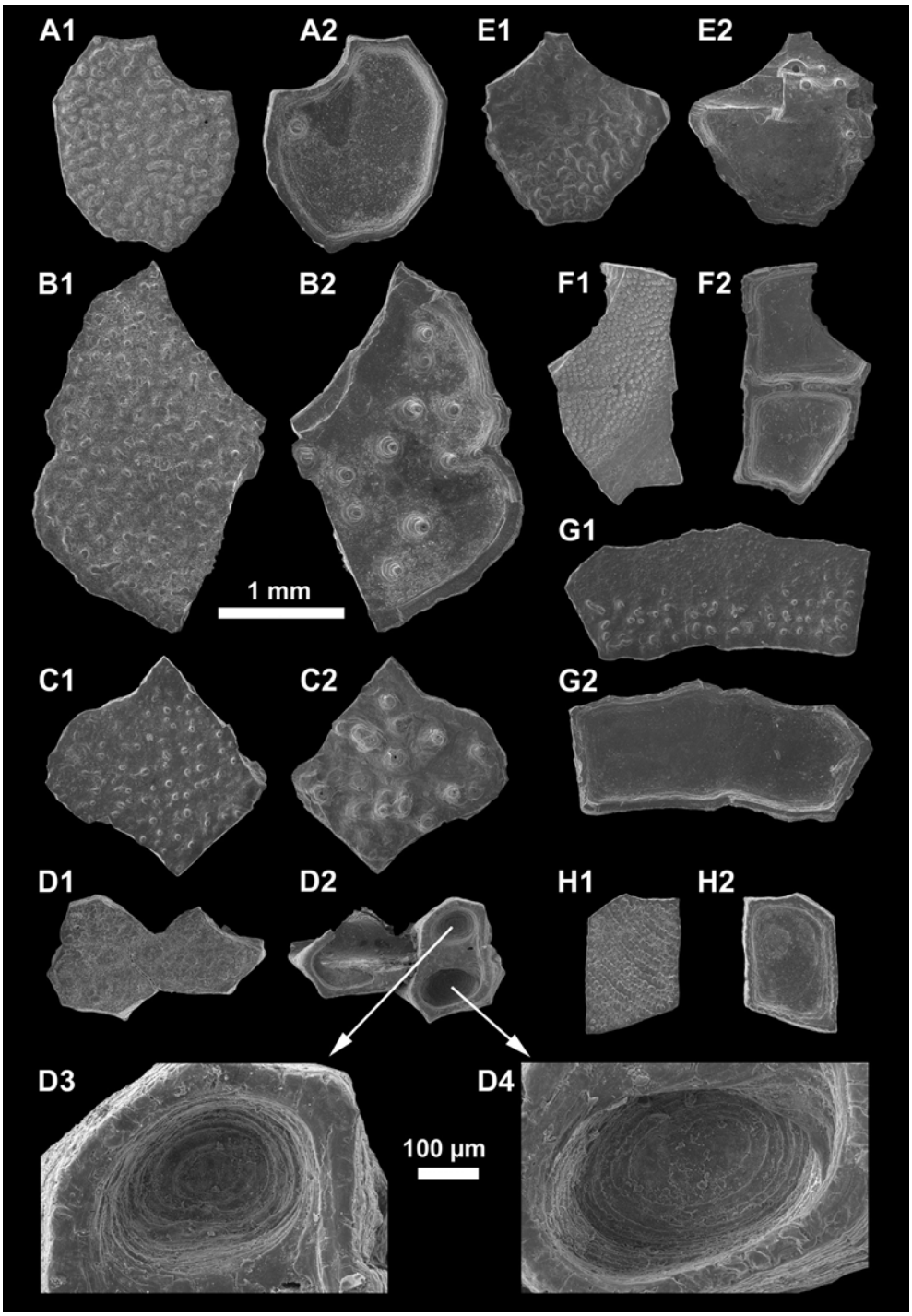

Fig. 5. – *Scleropages* sp. from the Paleocene of Hainin. Squamules (IRSNB P 8244-8251) of the posterior field of the scale in external (A1, B1, C1, E1, G1) and internal view (A2, B2, C2, E2, G2). Three united squamules from the lateral field in external (D1) and internal view (D2), with enlargement of (D2) in (D3) and (D4). Two united squamules of the anterior field of the scale in external (F1) and internal view (F2). A squamule of the anterior field of the scale in external (H1) and internal view.

A deep groove is present at the top of the external face of the entopterygoid in the Hainin fossil fish. Such a groove is a unique autapomorphy of the osteoglossoid families Osteoglossidae, Arapaimidae and Pantodontidae (Taverne, 1977 : Figs 53, 54, 82, 83, 107, 134; 1978 : Figs 40, 41). No other teleost offers such a specialized structure. That groove receives the paired basipterygoid process of the parasphenoid (Bridge, 1895 : pl. 22, fig. 4) which is firmly fixed there by an elongated anterior process of the hyomandibula, except in *Pantodon* where that hyomandibular process is missing. That very peculiar structure allows a supplementary articulation between the neurocranium and the hyopalatine arch that strengthens the palato-lingual bite in those fishes.

The pattern of the dentition on the inner face of the entopterygoid, with one dorsal row of large teeth and a ventral area with much smaller and irregularly arranged teeth, agrees with a belonging to Osteoglossidae (TAVERNE, 1977 : Figs 54, 83) but not to Arapaimidae and Pantodontinae. Indeed, there are only some large teeth on the upper part of the entopterygoid in *Heterotis* and *Pantodon* (TAVERNE, 1977 : Fig. 107, 1978 : Fig. 41) and very small denticles on practically the whole inner surface of the bone in *Arapaima* (TAVERNE, 1977 : Fig. 134).

Fossil and Recent Osteoglossidae offer premaxillae with a well developed symphyseal ascending process (TAVERNE, 1977 : Figs 42, 47, 49, 1978 : Figs 2, 7, 20; GAYET, 1991 : Fig. 7a; LI, 1996 : Fig. 3; LI et al., 1997 : Figs 3, 8). In *Scleropages* however, this symphyseal process is very short (TAVERNE, 1977 : Fig. 71). The premaxilla of the Hainin osteoglossid, with its very short symphyseal process, completely looks like the corresponding bone of *Scleropages* and more particularly to the one of the Recent Asian *Scleropages formosus* which also possesses ten or eleven premaxillar teeth. In the Recent *Scleropages leichardti* from Australia, there are only three to five teeth on the premaxilla.

The identification of the Hainin fossil fish with the genus *Scleropages* is further confirmed by its saccular otoliths that look very much like those of *Scleropages leichardti* (NOLF, 1993 : fig. 4A). It differs only from them by a wider sulcus and a thicker posterior portion. Otoliths of *Sleropages formosus* (Fig. 4B2) are more elongate and have a narrower sulcus.

The *Scleropages* from Hainin is neither the first mentioned, nor the oldest Osteoglossidae present in the European continental deposits. A fragment of a toothed jaw bone and some squamules of an undetermined osteoglossid have already been described in the continental Campanian (Late Cretaceous) of Champ-Garimond, in southern France (SIGÉ et al., 1997). This French fossil may also belong to the genus *Scleropages*, but those remains are too fragmentary for decisive conclusions.

The occurrence of *Scleropages*, a freshwater genus actually living in Southeast Asia and Oceania, in the continental Paleocene of Belgium and perhaps already in the continental Campanian of France, can be explained by two different ways.

In the first hypothesis, the osteoglossoid distribution is considered as worldwide since the origin of the suborder during the Cretaceous thanks to the tolerance of those fishes for brackish and even marine waters. The present day distribution (see map in BERRA, 2001 : 53), which suggests an ancient "Gondwana pattern", is then viewed as a fallacious picture. In that case, the European fossil *Scleropages* simply is a relic of that old cosmopolite distribution.

Another interpretation of the osteoglossoid geographic distribution is possible. Indeed, the Lower Cretaceous osteoglossoids only are known in Gondwanan regions. It is during the Upper Cretaceous that a few Osteoglossoidei appear for the first time in continental deposits of the northern continents. At that time, only one species is known in Europe, the undetermined Campanien osteoglossid of Champ-Garimont, and two species are reported in the western part of North America, the osteoglossid *Cretophareodus alberticus* Li, 1996 from the Campanian of Alberta, and the osteoglossoid incertae sedis *Chandlerichthys strickeri* Grande, 1986 from the Cenomanian of Alaska. A real worldwide distribution of the Osteoglossoidei only occurs during the Lower Tertiary and is principally due to the numerous marine species then present in the oceans. The disappearance of all those marine Paleogene osteoglossoids, of *Scleropages* in Europe, and of *Phareodus acutus* Leidy, 1873 and *Phareodus testis* (Cope, 1877) in North America after the Eocene has brought back the dispersal of the suborder to Gondwanan frontiers as in the Lower Cretaceous.

That second hypothesis puts a question about the European fossil *Scleropages*. How this freshwater genus has succeeded to reach Europe?

An Indian origin is questionable. During the Upper Cretaceous and the Lower Tertiary, India still was isolated in the ocean and separated from Western Europe by all the length of the Mesogea, a too long marine distance to pass over for a freshwater fish.

A connection between North America and Europe is possible via Greenland and Britain during practically all the Cretaceous times. The marine expanses between those areas during that geological period are not very wide and we know that Osteoglossidae are able to tolerate brackish and even marine waters. However, the two Upper Cretaceous osteoglossoid species are located westward to the large Cretaceous epicontinental seaway then bisecting North America into two land masses. To go over such a wide marine expanse is practically impossible for freshwater teleosts and moreover no freshwater osteoglossoid has ever been found located eastward to that sea in North America during the Cretaceous. Thus, a North American origin for the European *Scleropages* also is improbable.

A connection between North Africa and Europe during the Cretaceous is possible via the Iberian and/or the Apulian platforms and the surrounding islands. The marine expanses between those emerged territories at that time were not very wide. We know also that the occurrence of Osteoglossidae in Africa is attested as soon as the Early Cretaceous with *Chanopsis lombardi* from the Valanginian-Barremian (CAHEN et al., 1959), a geological period during which South-America and Africa were still connected. Other fossil Osteoglossiformes are known in Africa during the Cretaceous but they are younger : the arapaimid *Paradercetis kipalaensis* Casier, 1965 and the kipalaichtyid *Kipalaichthys sekirksyi* Casier, 1965, both from the Cenomanian of the Democratic Republic of Congo (TAVERNE, 1976). In addition to that it is obvious that the Recent South-American *Osteoglossum* and the Southeast Asian and Oceanian *Scleropages* are very close relatives, sharing an almost similar osteology and differing only by a few details (TAVERNE, 1977). Even if no fossil remains of *Sleropages* were found until now in Africa, the actual Gondwanan distribution of those two freshwater genera implicates that an *Osteoglossum-Scleropages*-like osteoglossid was present in Africa in the earliest period of the Cretaceous, before a too important break up of Gondwana. An African origin for the European fossil *Scleropages* is thus the most probable hypothesis.

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Survey of the anguilliform Clariidae (Teleostei, Siluriformes) of Gabon and Republic of Congo, with description of two new species and key to the African clariid genera

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ABSTRACT. A survey of anguilliform African clariids from the Lower Guinea indicated the existence of four species, of which two are new to science. Thirty morphometric features as well as several meristic and descriptive characters have been studied on 174 specimens. *Channallabes ogooensis* n. sp. is recognized by the combination of distinct serrations on the posterior edge of the pectoral spine, 84–97 vertebrae, 100–113 and 85–102 dorsal and anal fin rays, a pale spot on the skull roof between the anterior and posterior fontanel, absence of an epiotic, skull moderately reduced in size, sphenotic and pterotic showing plate-like outgrowths and the posterior border of mesethmoid indented, making the anterior part of the anterior fontanel situated within the mesethmoid. *Channallabes teugelsi* n. sp. is recognized by the combination of serrations only on the anterior edge of the pectoral spine, 70–82 vertebrae, 99–109 and 90–100 dorsal and anal fin rays and a clear, pale spot on the skull roof between the anterior and posterior fontanel. The three anguilliform genera *Channallabes*, *Gymnallabes* and *Clariallabes* are diagnosed and two anguilliform species are reassigned. A revised key to all African clariid genera is provided.

KEY WORDS : Clariidae, systematics, *Channallabes alvarezi, C. longicaudatus, C. ogooensis, C. teugelsi*, new species

INTRODUCTION

A clear taxonomy/systematics and a relatively simple key to identify animals correctly, based on externally visible morphological characters is crucial for field sampling, museums, etc… and thus indispensable for all further biological research.

The anguilliform species of the catfish family Clariidae are of great biological interest, *e.g.* discovery of terrestrial feeding and its importance for understanding evolutionary transitions in the history of vertebrates (VAN WASSENBERGH et al., 2006) and have recently been subject of several studies (DE SCHEPPER et al., 2004; DEVAERE et al., 2005; 2006).

However, anguilliform clariid taxonomy is not well supported. The only key incorporating the anguilliform clariids is that of POLL (1977), who relied on the limited number of specimens used in the original descriptions of the species. However, we find this key fails to account for intraspecific variation; overlap among species in the ranges of characters used in this key, such as presence of paired fins and number of ribs and vertebrae, limits its utility.

We examined the anguilliform clariid material, including type-specimens, museum specimens and newly collected specimens from the region of interest. Anguilliform clariids of the Lower Guinea are poorly represented in museums worldwide. Only *Gymnallabes typus*, *G. alvarezi* and *Channallabes apus* are frequently found in museums. Most of this material, however, appears to be incorrectly identified. Because correct identifications of the species are difficult, we conducted a systematic survey and morphological analysis.

MATERIALS AND METHODS

We examined 174 anguilliform clariids from the Lower Guinea. Institutional abbreviations are listed in LEVITON et al. (1985), except for Instituto de Biologia Aplicada, Barcelona, Spain (IBAB). For this study, we used museum material, but most specimens were collected from two sampling campaigns in Gabon (1999, 2000). Three large regions have been sampled : northern Gabon in the Woleu River system, close to the border with Equatorial Guinea and Cameroon; the Ivindo River system around Makokou; the Ogowe River system in the vicinity of Franceville. All sampling sites were characterized by shallow, muddy, still water. Most specimens were caught using fyke nets and fish hooks by local fishermen.

For all specimens, 36 point-to-point measurements were taken using digital callipers to 0.1mm (digital ruler, Mauser), following DEVAERE et al. (2004) : total length (TL); standard length (SL); preanal length (PaL); prepelvic length (PPvL); prepectoral length (PPcL); predorsal length (PdL); distance between the occipital process and the dorsal fin (SPDFL); pelvic fin length (PvFL), pectoral fin length (PcFL); pectoral spine length (PcSL); caudal peduncle depth (CPD); body depth at anus (ABD); maxillary barbel length (MxB); external mandibular barbel length (EmnB); internal mandibular barbel length (ImnB); nasal barbel length (NB); interpelvic distance (IpvD); interpectoral distance (IpcD); head length (HL); preorbital length (PoL); skull width (SkW); supraoccipital process length (SpL); supraoccipital process width (SpW); interorbital distance (IoD); anterior nostril interdistance (ANID); posterior nostril interdistance (PNID); rostral skull width (RSkW); orbital skull width (OSkW); skull height (SkH); eye diameter (ED); snout height (SnH); prehyoid length (PhL); internal mandibular interdistance

(ImnID); external mandibular interdistance (EmnID); mouth width (MW) and skull roof width (SkR). Measurements of bilaterally paired structures were taken on the left side. The following counts were made using radiographs : number of ribs (RB), number of non-rib bearing precaudal vertebrae (NRPCV), precaudal vertebrae (PCV), caudal vertebrae (CV), total number of vertebrae, including those comprised in the vertebral complex of the Weberian apparatus (TV), dorsal- and anal-fin ray counts (DFR, AFR). Due to the high level of decalcification of some museum specimens not all meristics could be counted on the radiographs. Seven specimens were cleared and stained following TAYLOR & VAN DYKE (1985).

A standard Discriminant Function Analysis (DFA) was performed on the metric dataset using statistica 6.0 (Statsoft, Inc.). The analysis involved all specimens, categorized in four a priori defined groups (based on geographic distribution and meristic data) as follows : (1) all specimens of the Woleu River system, including the type material of *Gymnallabes alvarezi* (N = 85), (2) specimens of the Ivindo River system, including type material of *Clariallabes longicaudatus* (N = 67), (3) specimens of the Middle Ogowe River system (N = 6) and (4) specimens from the following locations; uppermost Ogowe, near Magogo, Lésala and Ndengué, Republic of Congo and Ivindo, Makokou, Gabon (N = 17). Group diagnoses are further explained in the Results section below. Qualitative and absence/presence characteristics were not included in the analyses but help to further identify and discriminate the species.

RESULTS

Figure one shows that the specimens in group I can be separated from the other groups based on the high number of dorsal fin rays (110–160), as well as a high number of vertebrae (96–105). Group I includes all the specimens originating from the Woleu River system as well as the holotype of *G. alvarezi* (Fig. 2). The discriminant function analysis (Fig. 3) shows that group I differs significantly ($P < 10^{-6}$) from all other groups (Table 1). Group I is clearly separated along root 1, which is highly correlated with the pelvic fins being absent (Table 2). The three specimens from group I isolated along root 3 are three aberrant specimens with one pelvic fin (Table 3)

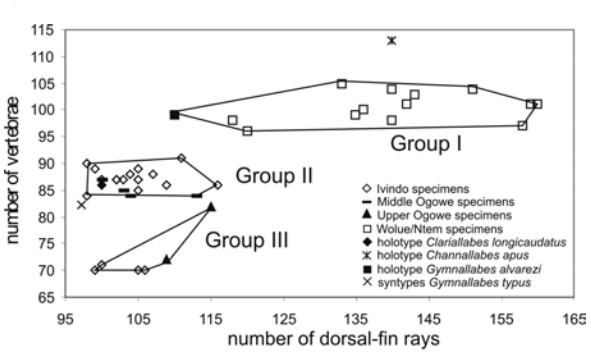

Fig. 1. – Scatterplot of dorsal fin rays counts against total number of vertebrae.

Secondly, group II includes most specimens from the Ivindo and Ogowe River System (Fig. 2) and can be separated by a lower number of both dorsal and anal fin rays (96–116 and 75–105 respectively) and vertebrae (82–91) compared to group I but still a higher number of vertebrae than the specimens in group III. The specimens from the Ivindo and the Middle Ogowe do overlap (Fig.1). However, the osteology of group II specimens allows distinguishing between both populations. The Ivindo specimens have an epiotic bone, which is absent in the Middle Ogowe specimens. Specimens from both populations have well developed serrations on the posterior edge of the pectoral spine, whereas the anterior edge of the pectoral spine is clearly serrated only in the Ivindo population. In the Middle Ogowe population serrations on the anterior edge are never as numerous and well–defined as in the Ivindo group (Fig. 4). The reduction of some of the skull bones is less extensive in the Middle Ogowe specimens. On the other hand, plate–like outgrowths of the sphenotic and pterotic of these specimens are more elaborate compared to those in the Ivindo specimens. Furthermore, in the Middle Ogowe specimens the posterior border of the mesethmoid is indented, bordering the anterior part of the anterior fontanel. This is not the case in the Ivindo specimens, with the fontanel being completely enclosed in the frontals. The discriminant function analysis shows not only that group II differs significantly ($P < 10^{-6}$) from each other group (Table 1), but also that the Ivindo specimens (Group IIa) are discriminated from the Middle Ogowe specimens (Group IIb) along root 3 (Fig. 3), based on the absence of pelvic fins of the Middle Ogowe specimens. Group II is separated from the other groups along root 1 (Table 2).

TABLE 1

VALUES OF *P*–LEVELS, F–VALUES (UPPER RIGHT) AND SQUARED MACHALANOBIS DISTANCES (D^2, LOWER LEFT) OF ALL FOUR GROUPS, reflecting the significant discrimination between the groups.

	Group I (*C. alvarezi*)	**Group II (*C. longicaudatus*)**	**Group II (*C. ogooensis*)**	**Group III (*C. teugelsi*)**
Group I (*C. alvarezi*)		86.2*	12.9*	25.1*
Group IIa (*C. longicaudatus*)	86.8		6.5*	20.7*
Group IIb (*C. ogooensis*)	102.5	52.3		9.6*
Group III (*C. teugelsi*)	70.0	60.2	94.6	

* : $P < 0.000001$

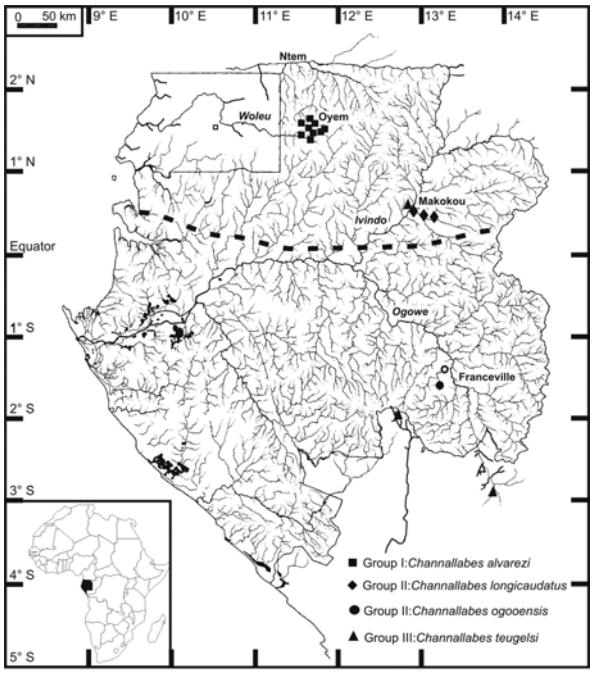

Fig. 2. – Geographic distribution of *C. alvarezi, C. longicaudatus, C. ogooensis* and *C. teugelsi* based on the localities of the examined specimens. Dotted line indicates the dividing line between the central and southern west coastal equatorial freshwater ecoregions. Open symbols are type localities. Holotype of *C. longicaudatus* not shown due to the ambiquity of the location.

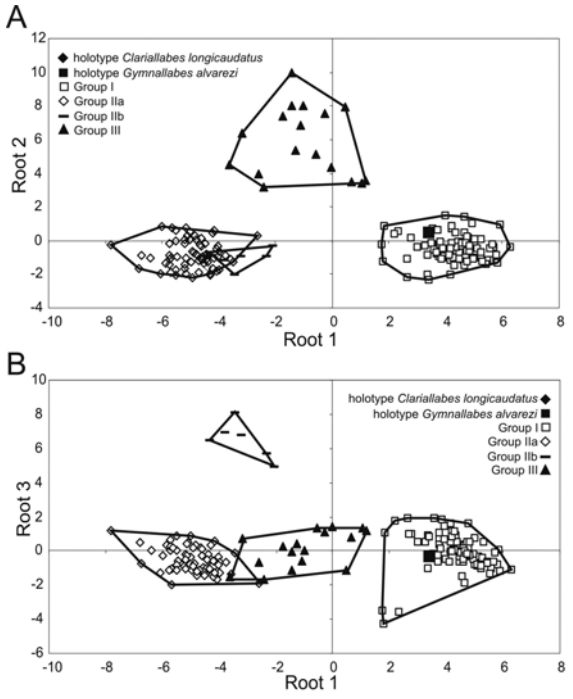

Fig. 3. – Discriminant Function Analysis (DFA) of four groups along (A) root 1 *vs*. root 2 and (B) root 1 *vs.* root 3. Group I : all specimens of the Woleu River system, including the type material of *Gymnallabes alvarezi* (N = 85), Group IIa : specimens of the Ivindo River system, including type material of *Clariallabes longicaudatus* (N = 67), Group IIb : specimens of the Middle Ogowe River system (N = 6) and Group III : specimens from the following locations; uppermost Ogowe, near Magogo, Lésala and Ndengué, Republic of Congo and Ivindo, Makokou, Gabon, with low number of vertebrae (N = 17).

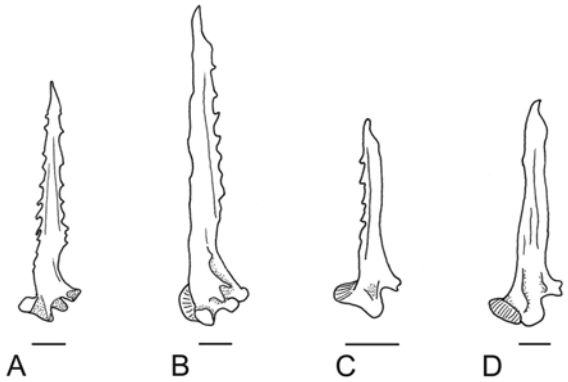

Fig. 4. – Illustrations of pectoral spine serrations (right pectoral fin). (A) both edges of the spine show strong serrations, pattern found in *C. longicaudatus*; (B) only the posterior edge of the spine shows strong serrations, anterior edge may show few, reduced serrations, pattern found in *C. ogooensis*; (C) only the anterior edge of the spine shows strong serrations, pattern found in *C. teugelsi*; (D) no serration found, pattern found in *C. alvarezi*.

TABLE 2

FACTOR LOADINGS FOR THE DISCRIMINATION FUNCTION ANALYSIS. The most important loadings of each root are in bold. Abbreviations are defined in the text.

	Root 1	**Root 2**	**Root 3**
% of variance	78,3%	15,3%	6,4%
PAL	0,169408	**-0,681881**	-0,089365
PDL	0,162934	**-0,632736**	-0,108065
SPDFL	0,182599	**-0,617988**	-0,121439
PvFL	**-0,503113**	-0,142215	**-0,614812**
PcFL	-0,035329	**-0,571193**	0,054308
PcSL	-0,092248	**-0,642360**	0,045391
CPD	0,096979	-0,467579	-0,058974
ABD	0,104527	-0,506432	-0,036495
MxB	0,013148	-0,432189	0,072631
EMnB	-0,020167	-0,479219	0,047128
IMnB	0,019274	-0,389841	0,002671
NB	-0,004556	-0,371535	0,008710
SKL	0,111548	-0,511756	-0,056860
POL	0,127276	-0,459005	-0,071790
SPL	0,047255	-0,217631	-0,124983
SKW	0,057597	**-0,581935**	-0,047982
SPW	0,133996	-0,139753	-0,085663
IOD	0,043390	**-0,560181**	-0,065722
AIND	0,028406	-0,346476	0,057940
PIND	0,055437	-0,444574	-0,035375
RSKW	0,077946	-0,444793	-0,008590
OSKW	0,072724	-0,532620	-0,056747
SKH	0,130920	-0,409255	-0,148632
OD	0,060164	-0,387114	-0,097085
SnH	0,099646	**-0,598212**	-0,053530
OSKH	0,127567	-0,537991	-0,081838
PHL	0,095121	-0,486623	0,024198
IMnID	0,038383	-0,502322	-0,035450
EMnID	0,073970	-0,525558	-0,028242
MW	0,038949	**-0,576369**	0,021140
SKR	-0,059704	-0,479303	0,058735

Finally, Group III includes specimens from three locations in the uppermost Ogowe drainage and from one location in the Ivindo River basin (Fig. 2). The specimens of group III show an even lower number of vertebrae (70–82) than the two other groups (Fig. 1). The discriminant function analysis shows again the significant difference ($P < 10^{-6}$) with all other groups (Table 1, Fig. 3). Group III is mainly separated from the other groups along root 2 (Table 2).

Group I, includes all the specimens from the Woleu system as well as the holotype of *G. alvarezi*, and thus represents a homogenous group of specimens that should be referred to this species. The data presented here shows that group III is sufficiently distinct and consequently should be considered as representing a separate species which we describe as *C. teugelsi* n. sp. below. Group II assembles most Ivindo specimens, as well as the holotype of *Clariallabes longicaudatus,* a junior synonym of *G. typus*. As the syntype of *G. typus* does not cluster with this group (Fig. 1), we consider *Clariallabes longicaudatus* as a valid species and assign the group II Ivindo specimens to it (Fig. 1). Finally, the osteological traits of the Middle Ogowe specimens of group II differ considerably from congeners and support the hypothesis that these specimens represent an undescribed species, which we describe as *C. ogooensis* n. sp. below.

Consequently, the following species are recognized : *C. alvarezi, C. longicaudatus, C. ogooensis* n. sp. and *C. teugelsi* n. sp., *Gymnallabes alvarezi* and *Clariallabes longicauda* are rediagnosed, redescribed, and their new generic assignments discussed.

Channallabes alvarezi (Roman, 1970)

Gymnallabes alvarezi Roman, 1970 : 5, Fig. 1 (type locality : Rio Kie, cerca de Ebebiyin, Rio Muni)

Holotype : IBAB, 317mm SL; Equatorial Guinea : Rio Kie, close to Ebebiyin, Rio Muni, Roman, 1970.

Nontype material : MRAC A4-31-P-1-13, A4-31-P-14-18, A4-31-P-55-60, A4-31-P-76-77, A4-31-P-78-89, 38, 193–383mm, Gabon, Aben Lang, Metui, 1° 29'N-11° 36'E, D. Adriaens, June 2000. MRAC A4-31-P-19, MRAC A4-31-P-21, A4-31-P-24, A4-31-P-26, A4-31-P-30, A4-31-P-31, A4-31-P-32, A4-31-P-67-72, A4-31-P-90-93,17, 202–375mm, Gabon, Ebeigne, Woleu River, 1° 28'N-11° 36'E, S. Devaere, D. Adriaens, A. Herrel, September 2000. MRAC A4-31-P-20, A4-31-P-25, A4-31-P-27-28, A4-31-P-29, 5, 225-342mm, Gabon, Assok Ngomo, Woleu River, 1° 41'N-11° 39'E, S. Devaere, D. Adriaens, A. Herrel, September 2000. MRAC A4-31-P-22-23, 2, 301–412mm, Gabon, Okoallissis, Otolo, Otagna, Woleu River, 1° 31'N-11° 31'E, S. Devaere, D. Adriaens, A. Herrel, September 2000. MRAC A4-31-P-47-54, A4-31-P-73, 9, 160–398mm, Gabon, Zogongone, close to Oyem, 1° 34'N-11° 31'E, S. Devaere, D. Adriaens, A. Herrel, September 2000. MRAC A4-31-P-61-63, A4-31-P-74-75,5, 221–345mm, Gabon, Mbenga, close to Oyem, 1° 37'N-11° 41'E, S. Devaere, D. Adriaens, A. Herrel, September 2000. MRAC A4-31-P-64-65, A4-31-P-66, A4-31-P-94, A4-31-P-1-95-96, A4-31-P-97, A4-31-P-98, 8, 238–413mm, Gabon, Oyem, 1°36'N-11°34'E, S. Devaere, D. Adriaens, A. Herrel, September 2000.

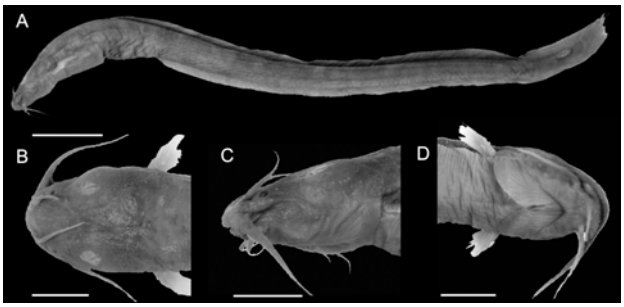

Fig. 5. – Holotype of *Channallabes alvarezi* (317mm SL), IBAB, (A) lateral view (scale = 35mm), (B) dorsal view of the head, (C) lateral view of the head and (D) ventral view of head. (scale = 10mm); (Photographs : Instituto de Biologia aplicada, Barcelona).

Diagnosis : *Channallabes alvarezi* can be recognized by the combination of following characters : a high number of vertebrae (92–105), no serrations on the pectoral spine (Fig. 4) and the body lateral line system is clearly visible as a white dotted line along the flank. It differs from all other *Channallabes,* except *C. apus*, in lacking a pale spot on the skull roof, and in a high number of dorsal and anal fin rays (110–160 *vs.* 98–116 and 101–155 *vs.* 75–105 respectively). It can be distinguished from *C. apus*, by the absence of an interdigitating joint between the entopterygoid and the quadrate and the presence of a large, well-pronounced supraorbital process on the fourth infraorbital, reaching the rostral border of the eye (Fig. 6A).

Description : Measurements and meristics for holotype and additional specimens given in Table 3. *Channallabes alvarezi* has a very elongated body (ABD 3.2–7.2% SL), preanal length 17.7–38.8% SL (Fig. 5), very short skull length, 5.8–13.8% SL. Skull width 55.6–90.7% of skull length. Very narrow skull roof, width 12.0–31.1% of skull length, remains clearly visible between bulging jaw muscles. Eyes small, but visible. Tube-like anterior nostrils small. Upper lip extends slightly beyond lower lip.

Fleshy, unpaired fins continuous. Pectorals fins always present, length 2.5–7.2% SL, with a small, unserrated pectoral spine, length of 1.3–5.4% SL. Seven branched pectoral fin rays. Pelvic fins present in only three specimens; in other specimens (n = 82) no evidence of pelvic fins. Vertebrae 92–105 (mode = 102). Ribs 12–14 (mode = 14). Branchiostegal rays 8. Dorsal fin rays 110–160. Anal fin rays 101–155.

Reduced lateral plate of frontal, only reaches up to level of orbitosphenoid (Fig. 7A). Narrow skull roof. Comparably reduced plate-like outgrowth present on posttemporo-supracleithral bone. Epiotic always absent. One or two small suprapreopercular bones present, with small plate-like outgrowth on proximal one. On prevomer, one or two posterior processes present. Entopterygoid contacting metapterygoid only on rostro-dorsal side of latter. Posteriorly on hyomandibula, three processes caudally increasing in length are present for interdigitation with neurocranium (Devaere et al., 2001; Fig. 6). Oral teeth present on dentary, premaxilla and prevomer.

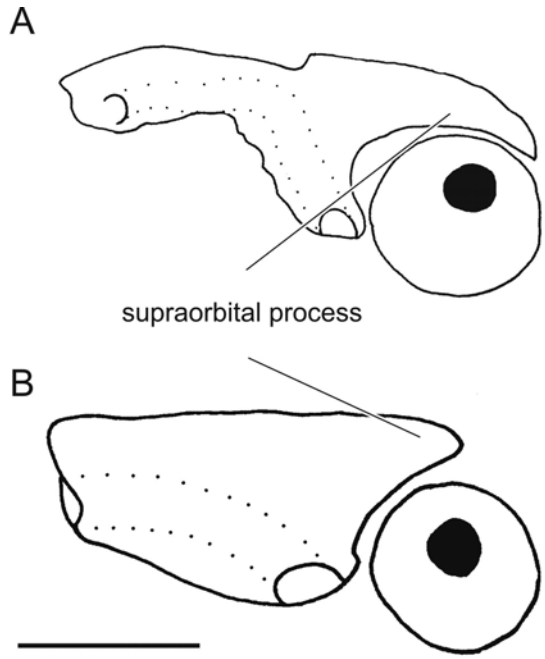

Fig. 6. – Illustration of the extent of the supraorbital process of infraorbital IV (right lateral view). (A) supraorbital process reaching the rostral border of the eye, pattern found in *C. longicaudatus*, *C. ogooensis*, *C. teugelsi* and *C. alvarezi*; (B) supraorbital process not reaching the rostral border of the eye, pattern found in *C. apus* (scale = 1mm).

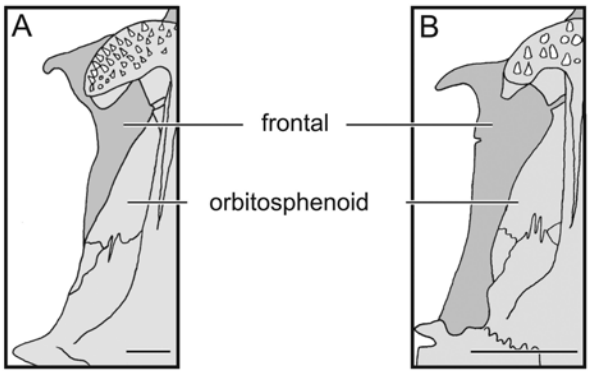

Fig. 7. – Illustration of the extent of the lateral plate of the frontal in relation to the orbitosphenoid (ventral view). (A) lateral plate reaches up to the lateral margin of the orbitosphenoid (scale = 1mm); (B) lateral plate extends more laterally than the margin of the orbitosphenoid (scale = 5mm).

Colour : Alcohol preserved specimens gradually fade from dark brown on dorsal side to whitish brown on ventral side. Both sides separated by white dotted line of lateral line. Skin on jaw muscles has paler brownish colour than surrounding skin of head. Barbels have darkish-brown coloration. *Channallabes alvarezi* shows no distinct pale region on skull roof.

Distribution : Currently known from the Woleu River system in the region of Oyem, Gabon, type locality is Equatorial Guinea (Fig. 2).

Channallabes longicaudatus (Pappenheim, 1911)

Clariallabes longicaudatus Pappenheim, 1911 : 519, Fig. 3 (type locality : in der Mabelle, Süd–Kamerun)

Holotype : ZMB 18401, Zoologisches Museum, Humboldt Universitat (ZMHU), Berlin, 220mm SL; in der Mabelle, South Cameroon, Pappenheim, 1911.

Nontype material : MRAC A4-31-P-99-105, A4-31-P-137-151, A4-31-P-152-157, A4-31-P-159-162, A4-31-P-163-164, 34, Gabon, Makokou, River Ivindo, 0° 33'N-12° 51'E, S. Devaere, D. Adriaens, A. Herrel, September 2000. MRAC A4-31-P-106-131, A4-31-P132-136, 31, Gabon, Etakaniabe, River Liboumba, 0° 31'N-12° 59'E, S. Devaere, D. Adriaens, A. Herrel, September 2000. MRAC A4-31-P-158, 1, Gabon, Iyoko, Makokou, 0°32'N-12° 54'E, S. Devaere, D. Adriaens, A. Herrel, September 2000.

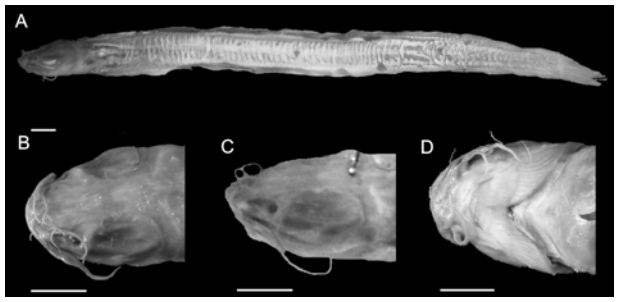

Fig. 8. – Holotype of *Channallabes longicaudatus* (220mm SL), ZMB 18401, (A) lateral view, (B) dorsal view of the head, (C) lateral view of the head and (D) ventral view of head. (scale = 10mm); (Photographs : Museum für Naturkunde der Humboldt-Universitaet, Berlin).

Diagnosis : *Channallabes longicaudatus* differs from *C. alvarezi*, *C. ogooensis* and *C. teugelsi* in having clear serrations on both sides of the pectoral spine (Fig. 4). *Channallabes longicaudatus* can be distinguished from *C. alvarezi* in the low number of dorsal and anal fin rays (98–116 *vs.* 110–160 and 75–105 *vs.* 101–155 respectively) and the presence of a pale spot on the skull roof. Further, it differs from *C. apus* in the presence of an interdigitation between entopterygoid and quadrate and in a large, well-pronounced supraorbital process on the fourth infraorbital, reaching the rostral border of the eye (Fig. 6A). Additionally, *C. longicaudatus* can be differentiated from *C. ogooensis* by the presence of an epiotic and a straight posterior edge of the mesethmoid, leaving the anterior fontanel completely enclosed by the frontal bones.

Description : Measurements and meristics for holotype and additional specimens given in Table 3. *Channallabes longicaudatus* has an elongated body (ABD 4.0–9.8% SL), preanal length 27.3–43.0% SL (Fig. 8). Very short skull length to SL ratio (8.7–15.9%). Skull width 64.4–79.6% of skull length. Skull roof always exposed, width 13.3–42.0% skull length. Eyes always visible. Whitish tube-like anterior nostrils strikingly visible. Lower lip clearly shorter than upper lip.

Unpaired fins continuous. Pectorals always present, length 5.0–9.7% SL, with a firm pectoral spine, length

2.9–6.8% SL. Both edges of pectoral spine serrated. Nine branched pectoral fin rays. Pelvic fins mostly present except in one specimen (intraspecific variation at this level already described by ADRIAENS et al. (2002)). Vertebrae 84–91 (mode = 88). Ribs 12–14 (mode = 13). Branchiostegal rays 8–9. Dorsal fin rays 98–116. Anal fin rays 75–105.

Lateral plate of frontal, broader than orbitosphenoid (Fig. 7B). Narrow skull roof. No other plate-like outgrowths present. Epiotic present. Only one suprapreopercular bone present. Entopterygoid contacting metapterygoid completely on rostro-dorsal side and partially on ventral side. Three processes present on hyomandibular–neurocranium connection, increasing in size caudally. Posterior border of mesethmoid forms a straight line. Teeth present on dentary, premaxilla and prevomer.

Colour : Alcohol preserved specimens light brown colour, gradually becoming more pale ventrally. Undefined paler median line present. *Channallabes longicaudatus* shows well-defined pale spot on skull roof, due to lighter thinning of neurocranium in that area.

Distribution : Due to the ambiguity of the type locality, no complete distribution can be given. Currently known from the Ivindo River system. This species mainly occurs in the Makokou region, Gabon (Fig. 2).

Channallabes ogooensis, new species

Holotype : MRAC A4-31-P-170, 150mm SL, Moanda, Gabon (1° 33'S-13° 16'E), S. Devaere, D. Adriaens and A. Herrel, September 2000.

Paratypes : MRAC A4-31-P-165-169, 5, 109–244mm, Gabon, Malima, River Kahjaka Kanjaka, 1° 40'N-13° 20'E, S. Devaere, D. Adriaens, A. Herrel, September 2000.

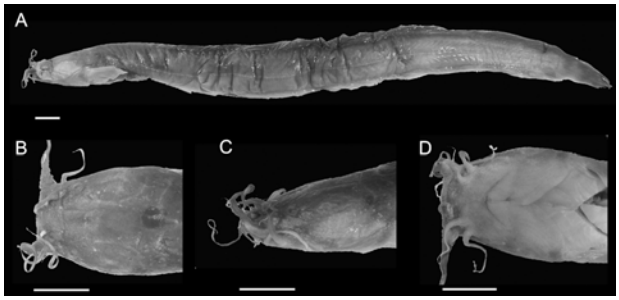

Fig. 9. – Holotype of *Channallabes ogooensis* (150mm SL), MRAC A4-31-P-170, (A) lateral view (scale = 65mm), (B) dorsal view of the head, (C) lateral view of the head and (D) ventral view of head. (scale = 10mm); (Photographs : S. Devaere).

Diagnosis : *Channallabes ogooensis* differs from *C. longicaudatus* and *C. teugelsi* by numerous and distinct serrations on posterior edge of the pectoral spine only (anterior edge shows a more irregular serration pattern) (Fig. 4). Additionally, *Channallabes ogooensis* differs from *Channallabes longicaudatus* by the absence of an epiotic, several skull bones show plate-like outgrowths (sphenotic and pterotic) giving the skull an overall less reduced appearance and the posterior border of the mesethmoid is indented, such that the anterior part of the anterior fontanel is bordered by the mesethmoid. *Channallabes ogooensis* can be distinguished from *Channallabes alvarezi* in the low number of dorsal and anal fin rays (100–113 *vs.* 110–160 and 85–102 *vs.* 101–155 respectively) and the presence of a pale spot on the skull roof. Further, it differs from *C. apus*, in the presence of an interdigitating joint between entopterygoid and quadrate, as well as the presence of a large, well-pronounced supraorbital process on the fourth infraorbital, reaching the rostral border of the eye (Fig. 6A).

Description : Measurements and meristics for holotype and additional specimens given in Table 3. *Channallabes ogooensis* has elongated body (ABD up to 5.7–7.5% SL), preanal length 28.4–36.2% SL (Fig. 9). Short skull length, 12.0–13.8% SL. Skull width 68.4–73.4% of the skull length. Skull roof always clearly visible, width 16.1–32.9% of skull length. Eyes always visible. Whitish tube-like anterior nostrils clearly visible. Lower lip clearly shorter than upper lip.

Unpaired fins continuous. Pectorals always present, length 6.4–11.1% SL, with a firm pectoral spine, length 4.4–7.1% SL. Both edges of pectoral spine serrated, with anterior edge more irregularly serrated. Eight branched pectoral fin rays. Pelvic fins always absent. Vertebrae 84–87 (mode = 84). Ribs 12–13 (mode = 12). Branchiostegal rays 8–9. Dorsal fin rays 100–113. Anal fin rays 85–102.

Less extensive reduction of the neurocranium (compared to *C. alvarezi, C. longicaudatus* and *C. teugelsi)* evident by presence of lateral plate on frontal, wider than the orbitosphenoid (Fig. 7B) and presence of limited plate-like outgrowth on posttemporo-supracleithrum, pterotic and sphenotic. Narrow skull roof. Epiotic absent. Only one suprapreopercular bone present. Posterior border of mesethmoid shows clear indentation, bordering anterior fontanel. Entopterygoid contacting metapterygoid completely rostro-dorsal side and also partially on ventral side. Hyomandibular–neurocranium connection comprising of two processes. Teeth present on dentary, premaxilla, and prevomer

Colour : Alcohol preserved specimens are brown, gradually becoming lighter ventrally. Indefinite paler median line present, which connects different pores of lateral line system. *Channallabes ogooensis* shows well-defined pale spot on skull roof, due to lighter pigmentation of neurocranium in that area.

Distribution : Currently known from the Ogowe River system. The specimens appear in the Franceville region, Gabon (Fig. 2).

Etymology : Named for the Ogowe River where the species is found.

Channallabes teugelsi, new species

Holotype : MRAC 78-22-P-1046, 80mm SL, Magogo, 1km from Lékoli, Komono-Sibiti road, Rep. Congo (2° 36'S-13° 38'E), W. Wachters, July 1978.

Paratypes : MRAC 78-22-P-1047-050, 4, 87–144.6mm, Rep. Congo, Zanaga, Lésala, River Ogowe, 2° 46'S-13° 50'E, W. Wachters, July 1978. MRAC 78-22-P-1051, 1.51mm, Rep. Congo, Ndengué, Moungoundou-Ndziba-Ndziba road, 2° 50'S-12° 41'E, W. Wachters, July 1978. MRAC 75-24-P-683-693, 11, 31–97mm, Gabon, Loa Loa, M'Passa, Makokou, 0° 34'N-12° 45'E, A. Heymer, November 1974.

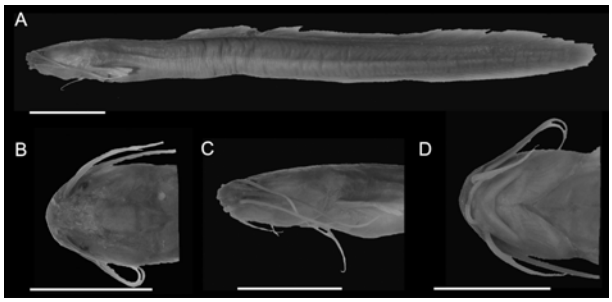

Fig. 10. – Holotype of *Channallabes teugelsi* (80mm SL), MRAC 78-22-P-1046, (A) lateral view, (B) dorsal view of the head, (C) lateral view of the head and (D) ventral view of head. (scale = 10mm); (Photographs : S. Devaere).

Diagnosis : *Channallabes teugelsi* differs from *C. longicaudatus* and *C. ogooensis* by serrations on the anterior edge of the pectoral spine only (Fig. 4). *Channallabes teugelsi* can be distinguished from *C. alvarezi* in the low number of dorsal and anal fin rays (99–109 *vs.* 110–160 and 90–100 *vs.* 101–155 respectively) and the presence of a pale spot on the skull roof. Further, it differs from *C. apus* in the presence of an interdigitation between entopterygoid and quadrate and in a large, well-pronounced supraorbital process on the fourth infraorbital, reaching the rostral border of the eye (Fig. 6A). *Channallabes teugelsi* can be diagnosed by the combination of following characters : a number of vertebrae that ranges between 70–82 and a lower lip that reaches almost equally far as the upper lip.

Description : Measurements and meristics for holotype and additional specimens given in Table 3. Body elongate, but not as extreme as other three described species, ABD 6.0–11.6% SL. Preanal length 29.8–40.6% SL (Fig. 10), indicating smaller tail section than *C. alvarezi, C. longicaudatus* and *C. ogooensis*. Skull length 12.2–27.0% SL. Skull width 45.4–71.5% of skull length. Narrow skull roof, width 14.9–57.8% of skull length, clearly visible. Eyes small, remain visible. Whitish tube-like anterior nostrils clearly present. Lower lip almost reaches upper lip.

Unpaired fins continuous. Pectoral fin length 4.5 – 11.3% SL, small pectoral spine, length 2.9–4.6% SL, sometimes present. Pelvic fin length 3.8–7.0% SL. No evidence of pelvic fins in four specimens (MRAC 8-22-P-1047-050). Only anterior edge of pectoral spine serrated. Nine branched pectoral fin rays. Vertebrae 70–82 (mode = 72). Ribs 10–12. Branchiostegal rays 8. Dorsal fin rays 99–109. Anal fin rays 90–100.

Lateral plate on frontal wider than orbitosphenoid (Fig. 7B). Distinct plate-like outgrowth on posttemporo-supracleithrum present. Epiotic always present. Two suprapreopercular bones always present, proximal one with plate-like process. Two posterior processes present on prevomer. Entopterygoid contacting metapterygoid on rostro-dorsal side and partially on ventral side of latter. Two extended processes present on hyomandibula, for interdigitating with pterotic. Teeth present on dentary, premaxilla and prevomer.

Colour : Alcohol preserved specimens equally light brown along the whole body. Clear whitish spot on the skull roof, between the anterior and posterior fontanel, due to lighter thinning of the neurocranium in that area.

Distribution : Currently known from the Ivindo River system in the region of Makokou, Gabon and the Ogowe River system in the region of Zanaga, Ndengué and Magogo, Rep. Congo (Fig. 2).

Etymology : Named after the late Guy Teugels, as a tribute to his career and his efforts on African catfish taxonomy, especially Clariidae.

TABLE 3

MEASUREMENTS AND MERISTIC DATA FOR THE FOUR *Channallabes* SPECIES FROM GABON AND REPUBLIC OF CONGO. Abbreviations are defined in the text. *N*–values (in parentheses) vary because measurements and meristics were excluded from specimens with damage or unusual preservation artefacts.

	Channallabes alvarezi **(Roman, 1970)** **(*N* = 85)**			***Channallabes longicaudatus*** **(Pappenheim, 1911)** **(*N* = 67)**			***Channallabes ogooensis*** **sp. n.** **(*N* = 6)**			***Channallabes teugelsi*** **sp. n.** **(*N* = 16)**		
	Holo-type	**Range**	**Mean ± SD**	**Holo-type**	**Range**	**Mean ± SD**	**Holo-type**	**Range**	**Mean ± SD**	**Holo-type**	**Range**	**Mean ± SD**
TL (mm)	329	169–445		241	102–323		169	120–223		85	36–155	
SL (mm)	318	150–413		220	95–295		150	109–200		80	31–145	
Measurements in % standard length												
PaL	24.1	17.7–38.8	26.0 ± 2.5	30.7	27.3–43.0	32.7 ± 2.3	35.6	28.4–36.2	34.0 ± 3.0	33.9	29.8–40.6	35.6 ± 3.3
PPvL		23.4–28.3 (3)	26.2 ± 2.5	27.8	21.7–43.0	30.5 ± 2.6				32.1	32.0–37.8 (12)	34.7 ± 2.3
PPcL	8.2	5.5–13.3	8.6 ± 1.0	12.0	10.2–17.2	12.3 ± 1.2	13.3	11.3–13.4	12.5 ± 0.9	14.1	11.6–20.8	16.0 ± 2.8
PdL	16.9	15.2–27.5	18.9 ± 1.7	22.3	20.9–33.8	24.3 ± 2.4	27.5	21.4–27.5	24.4 ± 2.1	25.4	24.5–36.0	29.0 ± 3.7
SPDFL	10.1	6.6–13.1	10.2 ± 1.1	10.6	9.1–17.6	11.6 ± 1.7	13.1	9.3–13.1	11.2 ± 1.3	11.2	8.3–15.8	12.0 ± 1.7
PvFL		2.1–2.2 (3)	2.2 ± 0.1	4.3	1.9–5.6	3.8 ± 0.7				4.0	3.8–7.0 (12)	5.5 ± 1.0
PcFL	3.6	2.5–7.2	3.4 ± 0.6	5.4	5.0–9.7	6.7 ± 1.2	7.2	6.4–11.1	7.9 ± 1.8	7.5	4.5–11.3	5.3 ± 2.2
PcSL	1.6	1.3–5.4	2.0 ± 0.5	2.9	2.9–6.8	4.6 ± 0.7	5.4	4.4–7.1	5.5 ± 1.1	4.4	2.9–4.6 (9)	3.8 ± 0.7
CPD	1.8	1.4–3.6	2.1 ± 0.3	2.1	2.0–8.2	3.1 ± 0.8	3.6	2.6–3.9	3.2 ± 0.5	3.4	2.9–5.9	4.2 ± 0.9
ABD	4.8	3.2–7.2	4.4 ± 0.6	6.2	4.0–9.8	6.3 ± 0.9	7.1	5.7–7.5	6.9 ± 0.7	6.4	6.0–11.6	8.6 ± 1.7
IpvD		1.1 (3)	1.1 ± 0.01	2.3	1.5–5.3	2.5 ± 0.6				2.3	2.2–4.9 (12)	3.3 ± 0.8
IpcD	5.8	3.2–9.3	5.2 ± 0.8	7.7	6.3–10.6	8.4 ± 0.8	9.3	8.1–9.6	9.1 ± 0.7	8.5	7.5–15.7	11.7 ± 2.9
SkL	7.6	5.8–13.8	8.5 ± 1.0	11.8	8.7–15.9	12.3 ± 1.1	13.8	12.0–13.8	13.0 ± 0.6	14.6	12.2–27.0	19.0 ± 5.0

TABLE 3

MEASUREMENTS AND MERISTIC DATA FOR THE FOUR *Channallabes* SPECIES FROM GABON AND REPUBLIC OF CONGO. Abbreviations are defined in the text. *N*–values (in parentheses) vary because measurements and meristics were excluded from specimens with damage or unusual preservation artefacts.

	Channallabes alvarezi **(Roman, 1970)** **(*N* = 85)**			***Channallabes longicaudatus*** **(Pappenheim, 1911)** **(*N* = 67)**			***Channallabes ogooensis*** **sp. n.** **(*N* = 6)**			***Channallabes teugelsi*** **sp. n.** **(*N* = 16)**		
	Holo-type	**Range**	**Mean ± SD**	**Holo-type**	**Range**	**Mean ± SD**	**Holo-type**	**Range**	**Mean ± SD**	**Holo-type**	**Range**	**Mean ± SD**
Measurements in % head length												
PoL	76.9	62.9–97.2	71.0 ± 4.3	69.0	62.5–92.0	68.4 ± 3.6	68.5	64.3–68.7	67.1 ± 1.7	72.2	62.5–87.0	74.4 ± 6.9
SpL	21.4	8.3–24.3	14.0 ± 3.4	16.0	7.8–24.0	15.0 ± 2.8	16.5	6.4–16.5	12.8 ± 2.6	18.6	13.0–28.6	20.0 ± 3.2
SkW	88.5	55.6–90.7	64.6 ± 5.3	69.0	64.4–79.6	71.5 ± 4.8	68.4	68.4–73.4	71.1 ± 2.0	63.4	45.4–71.5	57.8 ± 8.0
SpW	21.8	11.9–39.7	21.1 ± 6.5	16.2	10.8–28.8	16.7 ± 3.5	22.7	11.3–22.7	15.4 ± 3.9	29.2	18.6–33.6	27.1 ± 4.1
IoD	41.8	23.7–43.6	31.6 ± 3.2	38.4	30.0–50.0	36.0 ± 3.1	36.9	30.0–37.8	34.6 ± 2.9	35.2	24.9–35.2	30.2 ± 3.0
ANID	15.5	6.9–20.6	13.1 ± 2.5	12.0	8.3–25.0	15.0 ± 2.7	19.6	15.2–19.8	17.1 ± 2.2	17.3	12.1–20.7	16.4 ± 2.0
PNID	39.6	19.8–39.6	24.8 ± 2.7	28.1	20.9–36.3	27.4 ± 3.1	27.5	26.2–29.5	27.4 ± 1.2	34.4	23.3–34.4	28.0 ± 2.9
RSkW	46.0	27.9–46.2	35.2 ± 3.6	35.7	14.5–49.1	36.6 ± 4.2	36.7	35.2–44.3	38.6 ± 3.6	42.7	25.6–42.7	37.7 ± 4.7
OSkW	59.0	36.1–65.2	48.5 ± 4.1	49.2	25.5–73.0	52.1 ± 5.2	51.8	48.5–56.3	51.2 ± 2.7	49.9	40.0–52.4	46.8 ± 3.6
SkH	54.4	35.1–64.8	47.8 ± 7.6	46.1	32.9–65.6	43.1 ± 5.5	39.1	20.1–41.0	36.4 ± 8.0	41.5	33.1–52.0	41.8 ± 5.9
ED	11.3	5.0–11.3	7.1 ± 1.1	7.2	5.9–13.3	7.8 ± 1.4	6.6	5.3–9.4	7.2 ± 1.5	6.7	6.7–11.8	8.7 ± 1.3
SnH	26.0	13.3–32.0	18.2 ± 2.9	17.6	12.3–24.5	18.0 ± 2.5	19.7	14.8–19.7	18.0 ± 1.7	17.5	7.7–17.5	13.4 ± 2.6
OSkH	35.6	23.5–44.2	32.8 ± 4.7	29.6	23.0–55.5	30.1 ± 4.9	28.4	25.3–31.9	28.7 ± 2.2	32.4	17.1–32.3	24.4 ± 4.4
PhL	48.1	20.1–39.7	28.9 ± 4.9	22.9	20.2–35.0	28.5 ± 3.7	33.8	27.6–34.6	32.2 ± 2.6	25.8	19.1–30.3	24.9 ± 3.2
IMnID	33.1	16.3–33.1	22.2 ± 2.6	35.1	17.7–40.6	25.4 ± 3.5	24.6	23.7–27.7	25.2 ± 1.4	22.7	13.2–25.3	20.8 ± 3.5
EMnID	44.1	29.1–47.4	35.1 ± 3.2	37.1	25.6–53.3	37.4 ± 3.7	35.8	35.8–42.9	38.4 ± 2.7	40.0	25.0–40.0	33.4 ± 4.7
MW	36.4	19.4–40.9	29.1 ± 4.5	32.5	23.8–46.6	33.1 ± 3.8	33.3	33.7–38.8	36.4 ± 2.0	33.7	19.0–33.7	27.4 ± 5.0
SkR	11.5	12.0–31.1	18.3 ± 4.1	23.9	13.3–42.0	24.2 ± 6.1	22.6	16.1–32.9	26.0 ± 6.1	21.4	14.9–57.8	26.7 ± 11.1
Meristics		**Mode**			**Mode**			**Mode**			**Mode**	
RB	14	12–14 (76)	14	12	12–14 (52)	13	12	12–13	12	12	10–12 (3)	
TV	99	92–105 (76)	102	86	84–91 (52)	88	84	84–87	84	72	70–82 (6)	72
DFR	110	118–160 (16)	135	>100	98–116 (15)	105	104	100–113 (4)			99–109 (5)	
AFR	101	105–155 (16)	120	>100	75–105 (15)	98	88	85–102 (4)			90–100 (5)	100

Key to the species of *Channallabes*

1a Large, well-pronounced supraorbital process present on infraorbital IV (Fig. 6A), reaching rostral border of eye; fenestra between scapulo-coracoid and cleithrum present; no contact between entopterygoid and quadrate, in Gabon and Republic of Congo 2

1b Small supraorbital process on infraorbital IV (Fig. 6B), not reaching rostral border of eye; fenestra between scapulo–coracoid and cleithrum absent; interdigitation between entopterygoid and quadrate . . . . . *C. apus*

2a Spot present on skull roof between anterior and posterior fontanel, low number of dorsal (98–116) and anal (75–105) fin rays. 3

2b No spot present on skull roof, high number of dorsal (110–160) and anal (101–155) fin rays. . . . *C. alvarezi*

3a Serrations only on the posterior edge of the pectoral spine (Fig. 2B) . . . . . *C. ogooensis* n. sp.

3b Serrations only on the anterior edge of the pectoral spine (Fig. 2C) . . . . . *C. teugelsi* n. sp.

3c Serrations on both edges of the pectoral spine (Fig. 2A) . . . . . *C. longicaudatus*

DISCUSSION

Generic diagnoses

The four species recognized in this study are all assigned to the genus *Channallabes*, as well as the ones formerly assigned to *Gymnallabes* (*G. alvarezi*) and *Clariallabes* (*C. longicaudatus*). This generic transfer relies especially on osteological similarities between the species of *Channallabes* on the one hand and differences with other *Gymnallabes* and *Clariallabes* species on the other hand. Those diagnostic characters are discussed below and listed in Table 4.

Gymnallabes : we diagnose *Gymnallabes* by the combination of the following characters. Lateral skull bones are extremely reduced. The infraorbital series consists of small, tubular bones, lacking any plate-like processes. Consequently, infraorbital IV lacks a supraorbital process. The suprapreopercular bones similarly lack any plate-like outgrowth. Plate-like extensions of the frontal, sphenotic and pterotic bones are absent thus exposing the dorsal side of the adductor mandibulae complex completely (Fig. 11A–C).

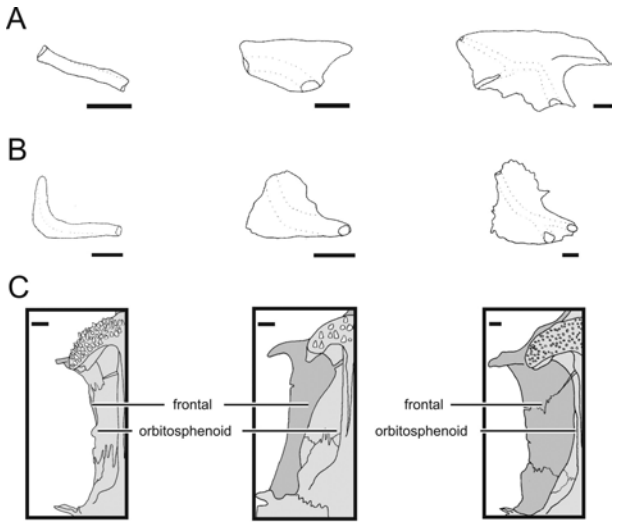

Fig. 11. – Illustration of the most important generic diagnostic characters of *Gymnallabes*, *Channallabes* and *Clariallabes* (left to right). (A) size of the plate–like extensions on the fourth infraorbital, (B) size of the plate–like extensions on the proximal suprapreopercular bone, (C) ventral view of the lateral plates of the frontals (scale = 1mm).

Clariallabes : the complex genus *Clariallabes* is characterized by an elongated body, that is somewhat intermediate between that of most species of *Clarias* and the extremely anguilliform genera (postanal length in *Clariallabes* between 50–65% SL). *Clariallabes* can be diagnosed by the combination of the following characters. The lateral skull bones (infraorbitals and suprapreopercular) are quite large in *Clariallabes*, all bearing a clear plate-like outgrowth (Fig. 11A, B). A small gap is present between the fourth infraorbital and suprapreopercular bone. The lateral plates of the frontals (Fig. 11C), sphenotics and pterotics are large, covering the adductor mandibulae complex partially. The first dorsal fin pterygiophore is situated anterior to the sixth post-Weberian vertebrae. The dorsal and anal fins are not or only partially confluent with the caudal fin, with the different fins clearly distinguishable (TEUGELS et al., unpubl.). It has to be noted that little is known about the osteology of *Clariallabes* species, or of interspecific variation within the genus. The diagnosis given above is based on the current knowledge of species like *C. melas, C. longicauda* and *C. mutsindoensis*.

Channallabes : the following combination of characters helps to discriminate *Channallabes*; the infraorbitals are reduced but the third and fourth infraorbitals still bear a small plate-like outgrowth (Fig. 11A). On the fourth infraorbital a supraorbital process is present, partially or completely bordering the eye dorsally (Fig. 6). The suprapreopercular bones are reduced but a small plate-like extension is present on the proximal one (Fig. 11B). The lateral plates of the frontals are reduced, reaching only slightly more lateral than the orbitosphenoid (Fig. 7, 11C). Finally, the first dorsal fin pterygiophore is situated posterior to the sixth post-Weberian vertebrae.

TABLE 4

COMPARISON AMONG *Channallabes, Gymnallabes* AND *Clariallabes*

	Channallabes	***Gymnallabes***	***Clariallabes***
Size of the infraorbital bones	bears small plate-like outgrowths (especially posterior most ones)	tubular	bears large plate-like outgrowths
Size of the suprapreopercular bones	bears small plate-like outgrowths (especially proximal one)	tubular	bears large plate-like outgrowths
Extent of lateral plates on frontals	equals or slightly exceeds the boundaries of the orbitosphenoid	absent	largely exceeds the boundaries of the orbitosphenoid
Lateral plates on sphenotic and pterotic	absent	absent	present
Supraorbital process on infraobital IV	present	absent	present
Position of first dorsal fin pterygiophore	posterior to sixth post-Weberian vertebrae	anterior to sixth post-Weberian vertebrae	anterior to sixth post-Weberian vertebrae

As for the other (monotypic) genera of anguilliform clariids, the unique combination of characters presented here, clearly separates them from *Channallabes* and *Gymnallabes. Platyallabes* has a very small distance between the origin of the dorsal fin and the supraoccipital process (2.2–6.6% SL), two separate tooth plates on the prevomer and no suprabranchial organ (DEVAERE et al., 2005). *Platyclarias* is recognized by an extremely flattened skull (22.9% – 37.1% of SkL) and the presence of an extra muscle (musculus adductor mandibulae A_3'' pars levator tendinis) in the adductor mandibulae complex. (DEVAERE et al., 2006). *Dolichallabes* is characterized by the presence of a single fontanel on the skull roof and the antorbital and infraorbital IV as the only circumorbital bones present (DEVAERE et al., 2004).

Species belonging to *Channallabes*

The current analysis of anguilliform clariids of the Lower Guinea enables us to formulate three conclusions with respect to species of the genus *Channallabes* : (1) *Gymnallabes alvarezi* needs to be transferred to *Channallabes*; (2) the nominal species *Clariallabes longicaudatus* needs to be resurrected but transferred to

Channallabes; and (3) two new species belonging to *Channallabes* can be recognized. This adds the total number of valid species of *Channallabes* in the Lower Guinea ichthyological province to four species, with another two species known from the Congo basin (*C. apus* and a new species under description).

Differences between *C. apus* of the Congo basin and the Lower Guinea species are apparent both genetically (JANSEN et al., 2006) and osteologically. For example, *C. apus* differs in having an articulation process on the second infraorbital that never contacts with the lateral ethmoid (an obvious contact is found in the Lower Guinea taxa). Also, *C. apus* is distinct in bearing an interdigitating joint between the entopterygoid and the quadrate (DEVAERE et al., 2001) and in the absence of a fenestra between the scapulo-coracoid and the cleithrum.

The distinction between *C. alvarezi* and the other new species is clear and based on several characteristics, as noted by DE SCHEPPER et al. (2004). It is the only Lower Guinea species lacking a pale spot on the head and is characterized by a high number of dorsal and anal fin rays.

Channallabes teugelsi can be distinguished from the other Lower Guinea species mainly based on meristic characters, especially the low number of vertebrae, with a maximum of 82 (Fig. 1). Even though these specimens are small, size-related allometry could not explain the low vertebral count. Moreover, an independence of the total number of vertebrae from the length of the fish has been reported for fishes in general as well (LANDRUM & DARK, 1968).

Channallabes longicaudatus and *C. ogooensis* show the greatest overall similarity, and as a group can easily be distinguished from the rest (Fig. 1). Several qualitative, osteological features are useful in their discrimination, such as serrations on both edges of the pectoral spine in *C. longicaudatus* (instead of only on the posterior edge) and the presence of the epiotic in *C. longicaudatus* (absent in *C. ogooensis*). In addition, the indented mesethmoid distinguishes *C. ogooensis* from *C. longicaudatus*.

The geographic distribution shows that the four species mainly occur in the following large river systems : *C. alvarezi* occurs in the Woleu and the other species originate mainly from the Ivindo/Ogowe. While *C. teugelsi* can be found both in the Ogowe and Ivindo, *C. longicaudatus* is situated mainly in the Ivindo River (the exact type locality is unclear), and *C. ogooensis* only in the Ogowe River (more specifically upstream from the confluence of the two large rivers). According to THIEME et al. (2004), these locations are located in two separate freshwater ecoregions : the central west coastal equatorial and the southern west coastal equatorial, respectively. The northern tributaries of the Ogowe River (Abanga, Okano and Ivindo) are included in the central west coastal equatorial freshwater ecoregion because of faunal affinities with the other rivers of that ecoregion, and show less affinity with the mainstem Ogowe (THIEME et al., 2004).

Because of biased sampling, *i.e.* swamps generally being less sampled than rivers, the occupation of that swampy niche by the anguilliform clariids could explain the possible non-continuous distribution of *C. teugelsi* in the Ogowe and Ivindo. Additional targeted sampling would be required to verify this.

As a consequence of the reassignment of several species in different genera, as well as the description of new species with a more elaborate analysis of intraspecific and interspecific biometric ranges presented in this paper, an updated key based on that of POLL (1977) to the genera of the African Clariidae is presented here.

Key to the genera of the African Clariidae

1a Adipose fin absent or very short (less than 25% SL); more than 50 dorsal fin rays 2

1b Adipose fin large (24–33% SL); less than 50 dorsal fin rays . . . . . *Heterobranchus*

2a Unpaired fins separate from or only partially fused with caudal 3

2b Unpaired and caudal fins form continuous fin fold . 10

3a Eyes present, sometimes small; skull roof exposed between jaw muscles 4

3b Eyes absent; skull roof invisible . . . . . *Uegitglanis*

4a Eyes lateral, adjoining lateral border of skull; dorsal and caudal fins clearly separate (gap 10% SL); lateral dermal skull bones large and separate 5

4b Eyes laterodorsal or dorsal, not adjoining lateral border of skull; dorsal and caudal fins fused or slightly separated (gap max 5% SL); lateral dermal skull bones continuous, closely adjoining or are reduced in size 6

5a Dorsal fin rays 54 or less; elongated neural spines 9–12; suprapreopercular and posttemporo-supracleithrum in contact; no serrations on pectoral spin . . . . . *Dinotopterus*

5b Dorsal fin rays 59 or more; elongated neural spines 5–8; suprapreopercular and posttemporo-supracleithrum with distinct gap; pectoral spine serrated anteriorly . . . . . *Bathyclarias*

6a Suprabranchial organ with developed arborescent structures 7

6b Suprabranchial organ absent or vestigial and incomplete 8

7a Head short (11–26% SL); lateral head bones separate; distance between anus and caudal fin base 50–65% SL . . . . . *Clariallabes*

7b Head long (20–34% SL); lateral head bones in contact (often fused in larger specimens); distance between anus and caudal fin base 50% SL or less . . . . . *Clarias*

8a Skull length 14–19% SL; skull roof width 15–36.5% of skull width; infraorbital IV and suprapreopercular reduced and distinctly separate; vertebrae 59–71 . . . 9

8b Skull length 20–21% SL; skull roof width more than 50% skull width; large infraorbital IV and suprapreopercular in close proximity; vertebrae 51–52 . . . . . *Xenoclarias*

9a Extremely dorsoventrally flattened skull (skull height 22.9–37.1% of skull length); abdominal depth 4.1–6.5% SL; skull roof width 26.0–36.5% of skull width; vertebrae 65–71; ribs 9–11 . . . . . *Platyclarias*

9b No dorsoventrally flattened skull (skull height 50% of skull length); abdominal depth 16% SL; skull roof width 15% of skull width; vertebrae 59; ribs 8 . . . . . *Tanganikallabes*

10a Suprabranchial organ with reduced arborescent organ; distance between the occipital process and the dorsal fin large (5.2–17.1% SL). 11

10b Suprabranchial organ without arborescent organs; distance between the occipital process and the dorsal fin small (2.2–6.6% SL) . . . . . *Platyallabes*

11a Infraorbital series consisting of tubular bones; pale spot on skull roof always absent 12

11b Infraorbital series with plate-like extensions; pale spot on skull roof sometimes present. . *Channallabes*

12a One fontanel on neurocranium; vertebrae 95–116; skull length 6–10% SL . . . . . *Dolichallabes*

12b Two fontanel on neurocranium; vertebrae 62–86; skull length 11–18% SL. . . . . *Gymnallabes*

COMPARATIVE MATERIAL EXAMINED

Gymnallabes typus : BMNH 1866.12.4.1–2, 2, 139–150mm, Nigeria, probably Old Calabar, West Africa (Syntypes).

Channallabes apus : BMNH 1873.7.28 : 16, 1, 135mm, Angola, interior of Ambriz, 7° 50'Z-13° 06'E (Holotype).

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SHORT NOTES

First recordings of the soprano pipistrelle *Pipistrellus pygmaeus* (Leach, 1825) in Belgium

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Microchiropteran bats species can be discriminated according to morphometrical, behavioural and genetical features (1) (2). In addition, as bat species often emit distinct echolocation calls, the analysis of signal characteristics is a good identification tool and can even lead researchers to the discrimination of sibling species. In 1993, JONES & VAN PARIJS (3) showed a bimodal distribution in the echolocation calls of the common pipistrelle *Pipistrellus pipistrellus* (Schreber, 1774) with individuals emitting calls with maximum energy peak around 45kHz and others around 55kHz. Subsequent works have shown that the two phonic types also showed differences in diet, habitat use (4) and social calls (5) (6). Genetic analysis finally separated the two phonic types into different cryptic species *P. pipistrellus* and *P. pygmaeus* (Leach, 1825), the common name given to the latter species being soprano pipistrelle (7). The distribution of *P. pygmaeus* is poorly known because of the recent distinction between the two phonic types. To date, it seems that soprano pipistrelles occur in Portugal, Sweden (8), Norway (9) and Denmark (7) (9) (10), whereas its sibling species, *P. pipistrellus,* does not. In other countries : Greece (11), Great Britain (3), Switzerland (12) (13), Northern Ireland (14), Germany (15) (16), France (17), Italy (18), and Spain (19) (16), the two species (*P. pipistrellus* and *P. pygmaeus*) are sympatric. Despite its presence in bordering countries, *P. pygmaeus* has never been identified in the Benelux so far. Two species of pipistrelles are known to occur in Belgium (20) : *P. pipistrellus* is widely distributed in Europe whereas *P. nathusii* (Keyserling & Blasius, 1839) is much less frequent and usually found around forest edges and riparian habitats (21). Here, we present the first acoustic records of the soprano pipistrelle in Belgium.

Echolocation calls were recorded by means of time expansion bat detectors (D-240(x) and D-980, Pettersson Elektronik AB, Sweden) and stored on a Mini-disc recorder (Sony) or a DAT (Sony). They were then analysed with Bat Sound software (Pettersson Elektronik AB, Sweden). The shape of the signal and maximum energy frequency were used to identify the species. Pipistrelles all use FM-qCF echolocation calls (22), a Frequency Modulated signal that ends up in a quasi Constant Frequency. However, as illustrated (Fig. 1), the different species can be discriminated according to the ending frequency of the qCF : *P. nathusii* around 35kHz, *P. pipistrellus* around 45kHz and *P. pygmaeus* around 55kHz (8) (23).

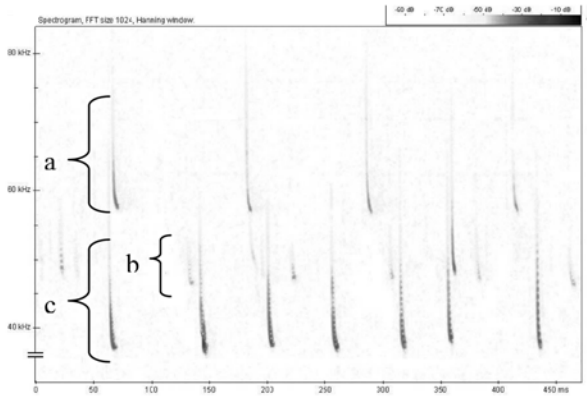

Fig. 1. – Sonagram of three *Pipistrellus* species recorded simultaneously at the Silex pond : a/ *P.pygmaeus*, b/ *P.pipistrellus*, c/ *P.nathusii*.

The first records of the soprano pipistrelle in Belgium came from different locations in the **Flemish Region** : Ieper (May 1998), Moen (June 1999) and Beernem (July 2000) in the Province of West-Flanders, in Zoersel (February 2002 and May 2003) and Merksem (May 2004) in the Province of Antwerp and, in Bree (September 2003) in the Province of Limburg. In April and June 2002 the species was first recorded in the **Brussels Region** respectively in the Silex domain (natural reserve in Boitsfort) and the Rouge Cloître domain (Auderghem). In the **Walloon Region** no confirmed observations were made until now (F. Forget, pers. comm.). Results are presented Table 1. In the Silex Domain (Brussels), soprano pipistrelles were recorded by chance in 2002 on two occasions. In 2003 a sustained monitoring experiment was performed during 50 nights, from April to September. The species was noted on 38 nights throughout the activity season. In summer 2004 and 2005 additional recordings confirmed that the species was still present on the site.

In the Flemish Region, despite an increasing sampling effort, in places where signals of *P. pygmaeus* had been recorded, the species has never been identified again. So far, all our recordings referred to single bats, most of which were recorded during spring (Table 1). This might suggest a temporally presence of the species in the Flanders, as a result of migration or accidental transportation.

TABLE 1

First records of the soprano pipistrelle in Belgium

Date	Location	Recorded (R) Observed (O)	Terminal QCF frequency (kHz)	Peak frequency (kHz)	N recorded signals	Habitat description
May 17, 1998	Ieper	R	54.3	55.2	15	Bank of a moat
June 06, 1999	Zwevegem (Moen)	R/O	57.9	58.7	10	Near a bridge, over a canal
July 02, 2000	Beernem	R/O	55.8	56.4	9	Ruins of a castle in a private woodland
February, 2002	Zoersel forest	R	56.7	59.6	7	Forest path
May, 2003	Zoersel forest	R	55.0	59.4	17	Forest path
September 3-4, 2003	Zuid-Willemsvaart Beek (Bree)	R	59,2	60,6	5	Near a bridge, over a canal
May 6 & 9, 2004	Fort Merksem	R	55,7	56,7	18	Bank of a moat
April 4, 2002	Boitsfort (Brussels)	R/O	56,0	57,8	16	Bank of a pond
June 20, 2002	Auderghem (Brussels)	R/O	56,9	57,6	7	Bank of a forest pond

Terminal QCF frequency = terminal frequency of the signal derived from spectrogram analysis (BatSound) (average calculated from N recorded signals)
Peak frequency = frequency of the highest peak derived from power spectrum (BatSound) of the selected signal (average calculated from N recorded signals)

A recent study has shown that *P. pipistrellus* and *P. pygmaeus* are sometimes mis-identified because of intraspecific variation, with some individuals using frequencies above or below the mean value (13). These authors found that false identification occurred in 50%, based on echolocation calls only. Is it possible that *P. pygmaeus* does not occur in Belgium and that we misidentify the species? It seems not likely. First of all, it is generally admitted that the frequency at maximum intensity of the search phase calls is the parameter showing the lowest inter-specific overlap and that best discriminates those two sibling species (1) (3) (4) (11) (18) (23). Second, Wicht et al. (2003) (13) only compared genetic and acoustical data of four soprano pipistrelles, which is a rather small sample to make any valuable interpretation. Third, they recorded their signals from hand-released animals, which could influence the emission of sounds (24). Finally, the distribution range of *P. pygmaeus* is particularly large in comparison with that of other European bat species ranging from Scandinavia to the Mediterranean area (1). The species is present in France, Germany and England and it would be surprising if the species was absent in the Benelux.

Our recordings suggest that *P. pygmaeus* is not widely distributed in Belgium but present. However, many areas have not been intensively surveyed. It is also likely that this species has been overlooked in the past years, because bat researchers did not expect and thus did not look for pipistrelles with maximum peak frequencies over 50kHz.

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Antrobathynella stammeri (Jakobi, 1954) : the first record of Bathynellacea (Crustacea : Syncarida) in Belgium

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KEY WORDS : Bathynellacea, first record, morphology, continental freshwater fauna, Belgium

The suborder Bathynellacea Chappuis, 1954 is a small crustacean group (up to 200 species known) of extant Syncarida Packard, 1886 living nearly exclusively in the interstitia of subterraneous freshwater habitats (1). Ever since their discovery at the end of the 19-th century they have been considered as rare animals although they show a world wide distribution. Because of their interstitial way of living and their small size (mostly less than 1.5mm) they probably have often been overlooked (2).

The study of crustaceans in Belgium has a long history, but Bathynellacea have never been reported until 2003 when *Antrobathynella stammeri* (Jakobi, 1954) was mentioned in PEETERS et al. (3). The information, based upon unpublished data, was not accompanied with details about sample localities and morphology.

The samples in which *A. stammeri* was detected are :

Stat. WAL 470. Hyporheos of the Crupet rivulet near Pierauchamps (Assesse, Province Namur). Coordinates (Lambert) : 191.000, 115.200; altitude : 170m. 10L water sample with the Bou-Rouch pump, about 30cm deep. Filtered through a hand net with a 100µm mesh size. Leg. G. Michel & Th. Kuyken, 06-08-2002. *A. stammeri* : 2 males, 1 female and 1 juvenile. Co-occurring crustaceans : Copepoda : *Diacyclops belgicus* Kiefer, 1936, *Attheyella (A.) crassa* (Sars, 1863), *Bryocamptus (B.) minutus* (Claus, 1863).

Stat WAL 438. Trou Balza (Cave). Small cavern – partially natural, partially anthropogenic modified – near Ivoi (Assesse, Province Namur) giving access to the saturated karst (Visean limestone). Pool at the end of the gallery 6m long, 2m wide, 30cm deep. Coordinates (Lambert) : 191.31, 117.320; altitude : 225m. Approximately 5L of water collected with a hand pump between the debris at the bottom of the pool. Filtered through a hand net with a 90ìm mesh size. Leg. P.- A. Duchesne & F. Fiers, 08-11-2002. *A. stammeri* : 1 female. Co-occurring crustaceans : Copepoda : *Diacyclops belgicus* Kiefer, 1936; Ostracoda : *Pseudocandona* spec.; Amphipoda : *Niphargus schellenbergi* Karaman, 1932; Isopoda : *Proassellus cavaticus* (Leydig, 1871).

Samples were fixed with buffered formaldehyde up to a concentration of roughly 5%. Observations were performed on temporary slides with a Leitz Diaplan light microscope. One 1 male (from stat. WAL 470) was partially dissected (uropods, furca, thoracopod (= pereiopod) VIII and pleiopods, mounted on 3 slides), partially preserved in alcohol. All other specimens were preserved in alcohol. The material is deposited in the crustacean collection of the Royal Belgium Institute of Natural Sciences (Invent. Number : 29.687)

A complete description of all appendages can be consulted in HUSMANN (4) while NOTENBOOM & DE BOOM (5) and SERBAN & GLEDHILL (6) provided specific details about Dutch and English specimens, respectively. The morphology of the Belgian specimens generally coincides with that of the German, English and Dutch populations but deviate in some details. The following differences were observed : (a) the male thoracopod VIII exopodite bears 5 setae (1 inner, 2 apical and 2 outer : Fig. 1A) as in the German and English specimens while the same structure has only 4 setae (1 inner, 2 apical and 1 outer) in the Dutch populations; (b) the 4 spines on the sympodite of the uropod (Fig. 1D) are ornamented with spinules along the distal 3/4 of the stem as in the German and English specimens while only a few spinules are present in the Dutch specimens; (c) the exopodite of the uropod bears 5 setae (6 in the Dutch and 4 in the German and English specimens); (d) the pleiopods (Fig. 1B) have only 4 elements instead of 6 or 7 as seen in the German and English specimens (situation unknown for the specimens from the Netherlands); and (e) as in the Dutch specimens the Belgian males lack a sexual dimorphic element on the mandibular palp (modified in the German and English specimens).

In addition, the Belgian specimens possess a large hyaline structure on the outer margin of the furca (Fig. 1C) which can be considered as homologous with the "Furkalorgan" often observed in representatives of the Parabathynellacae (2). Because of its transparency, this structure is hardly visible and probably has been overlooked in previous descriptions.

Although, aquatic animals from various subterraneous habitats in Belgium have been studied from the early thirties of the former century on (7, 8) bathynellids have never been mentioned. However, the presence of these syncarids in Belgium was suspected and more particularly in the watershed of the river Meuse since NOTENBOOM & DE BOOM (5) found several specimens of *A. stammeri* in the alluvial deposits of that river in its Dutch part. Moreover, *A. stammeri* among other bathynellids have been frequently found in the alluvia of the river Rhine and its tributaries (4) of which the watershed of the river Meuse formed part until the end of the Mindel Glaciation (0.4mYr).

Bathynellids are in most cases found together with representatives of the harpacticoid copepod families Parastenocarididae Chappuis, 1933 and Chappuisiidae Chappuis, 1940. This mesopsammal community has been referred to as the *Bathynella-Parastenocaris*-biocenosis

(9). No representatives of these taxa were present in belgian localities. The sample from Trou Balza (WAL 438) contained exclusively subterraneous animals (viz. Cyclopoida, Amphipoda and Isopoda) while the sample of the Crupet rivulet (WAL 470) clearly is a mix of both subterraneous (*D. belgicus*) and exogenous animals (*Attheyella* (*A.) crassa* and *Bryocamptus* (*B.*) *minutus*).

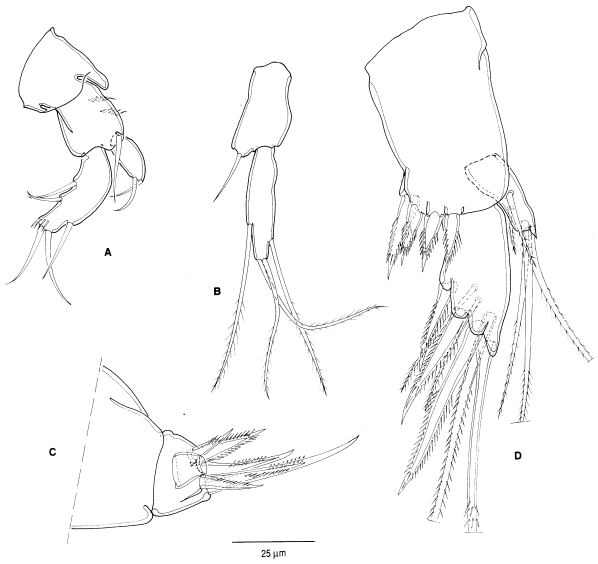

Fig. 1. – *Antrobatthynella stammeri* (Jakobi, 1954), male. A. Thoracopod VIII (basipodid omitted); B. Left pleiopod; C. Furca (outer lateral view); D. Right uropod.

Rivulet Crupet receives its water mainly from the drainage of tertairy and quaternary deposits and partially from the overflow of a natural seep (water catchment) at the southern border of a limestone massive (Visiaan age). Trou Balza, situated north of the catchment is an outcrop of this limestone massive.

The presence of *A. stammeri* in the saturated zone of the Trou Balza indicates that this animal thrives in the vast domain of the limestone massive and that the specimens found in the rivulet Crupet probably represent strays washed from the main population living in the limestone massive.

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Analysis of the inland cladocerans of Flanders (Belgium) – Inferring changes over the past 70 years

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KEY WORDS : Cladocera, faunistics, new record, non-indigenous species, Red List

The four crustacean orders of the cladocerans represent, together with copepods and rotifers, the most common zooplankton taxa in all types of lentic freshwater bodies (1). They exhibit a parthenogenetic (clonal) reproduction mode during periods of favourable environmental conditions, and produce sexual dormant eggs (ephippia) when conditions deteriorate (2). As such they are capable of remaining dormant in the habitat for decades (3). Because of their capacity for rapid population growth, some pelagic members of the group (especially the large-bodied *Daphnia* and *Diaphanosoma*) are able of keeping water bodies in a clear water state by grazing down the phytoplankton (4). Most species feed on bacteria, protists, periphyton, and detritus (many chydorids and macrothricids), some are parasitic (e.g. *Anchistropus* on the polyp *Hydra*) or predacious on small-sized zooplankton (e.g. *Leptodora* and *Polyphemus*) (5; 6). Cladocerans themselves are a main food source for fish, macro-invertebrates, and amphibians (7).

So far, several authors have provided species lists and updates on the occurrence of cladocerans in Belgium (summarized in 8; 9; 10; 11). However, there are virtually no published data on the geographic distribution and the frequency of occurrence of these species in Flanders. A notable exception is the monograph of Luyten (12), dating from the first half of the 20th century, and reporting on the occurrence of 56 cladocerans in 35 sites, spread over Flanders. Furthermore, data on the current status of cladocerans from regions in Europe are almost nonexistent (apart from [13] who provided a Red List of Cladocera from Carinthia).

In this paper, we present contemporary data on the frequency of occurrence and geographic distribution of inland cladocerans in Flanders (Belgium), and compare our results with the observations of (12). We try to identify major trends in the occurrence of species, and indicate hot spots of rare species. Finally, we also report the occurrence of two cladocerans new to the Belgian fauna.

During the period 2000-2005, we collected zooplankton samples from 64 different sites that are evenly spread over Flanders (Fig. 1, App. 1). In each site (defined as an area of ca 28km²) we sampled multiple types of water bodies (ditches, temporary pools, ponds, lakes, and canals) once in summertime. During the entire survey, 605 different water bodies were sampled, with an average of 9.5 (SE 0.9) water bodies per site.

Cladoceran samples were obtained with a tube sampler (diameter 75mm, length 2m), taking an integrated sample of the water column at random sites in the water body. The collected water was filtered through a plankton net (mesh size 64µm) and preserved in formaldehyde (4%) saturated with sucrose. When water bodies were too shallow (e.g. temporary pools with a water depth of less than 30cm), samples were taken by a plankton dip net (mesh size 64µm). In 13% (80 out of 605) of the water bodies, samples were taken from both the active and dormant cladoceran community. The dormant egg bank was sampled using a hand corer (diameter 52mm, length 1m). Eggs were isolated from the surficial 3cm of the sediment applying the sugar flotation technique, and hatched under simulated summer conditions (see [14] for protocol details). Cladocerans in the samples were identified to species level following the key of (6), with the exception of the genera *Chydorus* and *Bosmina*, which were identified to the genus level.

For each taxon, we calculated the frequency of occurrence (% of the sites) to obtain an idea of the representation of each species in Flanders. We distinguished between six categories using the ACFOR scale : Abundant (>75%), Common (75%-51%), Frequent (50%-26%), Occasional (25%-6%), Rare (5%-1%), and Not observed (not detected in any of the investigated sites; the species may be extinct). The same categorization was performed on the dataset of (12).

SPECIES LIST AND FREQUENCY OF OCCURRENCE

In total, 88 different cladocerans, belonging to seven families, have up till now been recorded for Belgian freshwater bodies (Table 1). The majority of the 69 cladocerans observed in our study display no distinct geographic distribution across Flanders (Fig. 2). Only a limited number of species seem to be restricted to certain ecoregions (e.g. *Daphnia atkinsoni* and *Macrothrix hirsuticornis* are confined to the Polders region and the sphagnophile *Acantholeberis curvirostris* to the Campine region). On average, the number of species detected at a site was 19 (SE 1), and this species number was not significantly different among sites located in separate ecoregions. Only 6% of the species were found to be abundant (i.e. *Bosmina* s.l. [mostly *Bosmina longirostris*], *Ceriodaphnia pulchella*, *Chydorus* s.l. [mostly *Chydorus*

sphaericus], *Scapholeberis mucronata*, and *Simocephalus vetulus*) (Fig. 3). Overall, 63% of the cladocerans were not widespread in Flanders : occasional (30%), rare (16%), or not observed (potentially extinct, 17%).

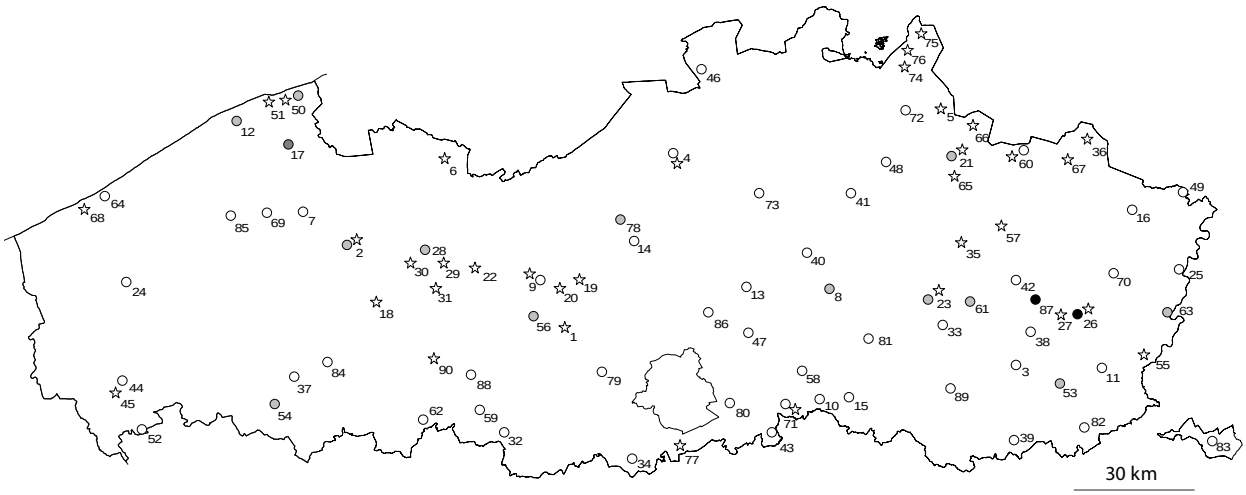

Fig. 1. – Geographic location of the different investigated sites in Flanders (Belgium). Each site is indicated by a circle, and the number of rare cladocerans is shown by the filling (white : 0 rare species; light gray : 1 rare species; dark gray : 2 rare species; black : 3 rare species). Sites that were sampled by (12) are indicated with stars. Numbers accord to the site names listed in App. 1.

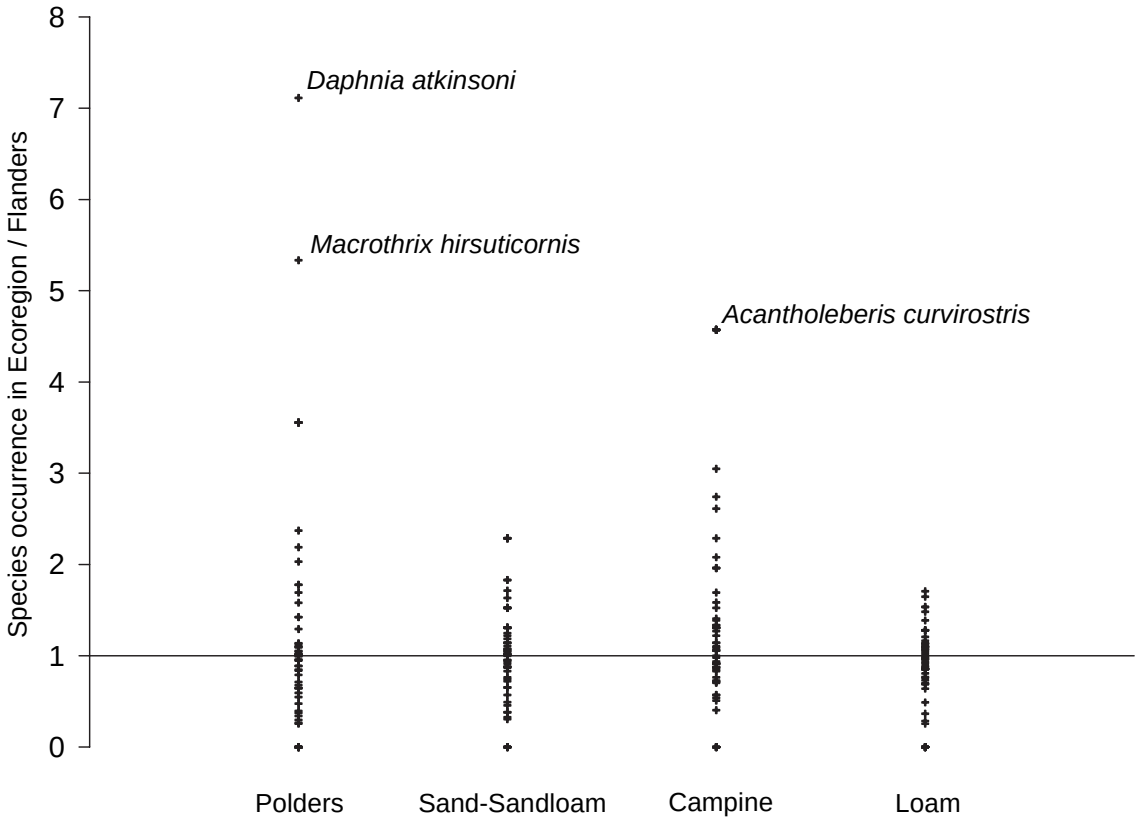

Fig. 2. – The proportion of the frequency of occurrence of each species in the four main ecoregions (spatial entities which are homogenous with respect to abiotic characteristics) of Flanders on its frequency of occurrence in Flanders (the Coastal dunes and Meuse ecoregions were omitted because less than three sites were sampled). Three species which are clearly linked to a certain ecoregion are indicated.

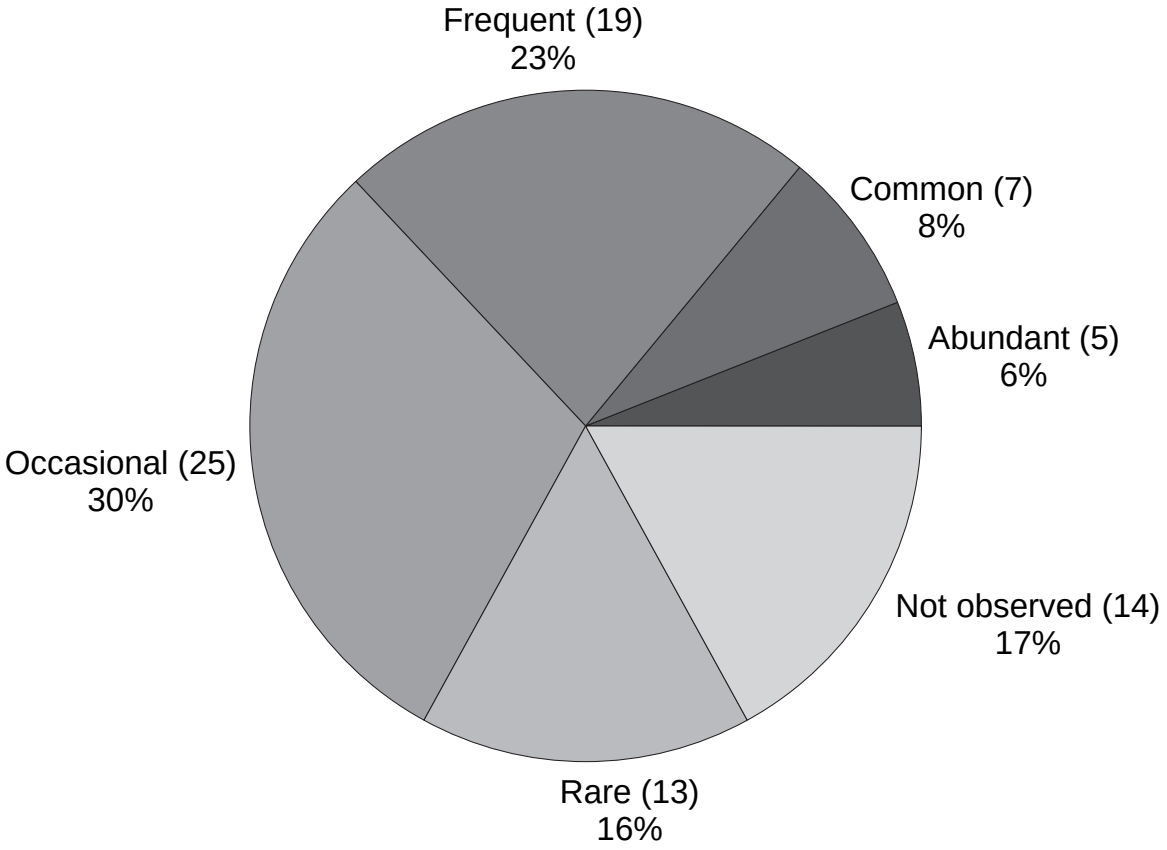

Fig. 3. – Percentage of occurrence of each category of cladocerans known to occur in Flanders. The number of species in each category is presented between brackets.

TABLE 1

Complete list of all different cladocerans that have been reported for Belgian water bodies. For each species, the current status in Flanders as revealed by the present study, and the status during Luyten's days (12) is presented. Species where no status is assigned are indicated by (–).

Species	Current status	Status Luyten
Sididae		
Diaphanosoma brachyurum (Liévin, 1848)	Frequent	Occasional
Sida crystallina (O.F. Müller, 1776)	Frequent	Occasional
Daphniidae		
Ceriodaphnia dubia Richard, 1894	Occasional	Not observed
Ceriodaphnia laticaudata P.E. Müller, 1867	Common	Occasional
Ceriodaphnia megops Sars, 1862	Occasional	Occasional
Ceriodaphnia pulchella Sars, 1862	Abundant	Frequent
Ceriodaphnia quadrangula (O.F. Müller, 1785)	Frequent	Occasional
Ceriodaphnia reticulata (Jurine, 1820)	Frequent	Occasional
Ceriodaphnia rotunda Sars, 1862	Not observed	Not observed
Ceriodaphnia setosa Matile, 1890	Not observed	Rare
Daphnia ambigua Scourfield, 1946 **	Frequent	–
Daphnia atkinsoni Baird, 1859	Rare	Not observed
Daphnia cucullata Sars, 1862	Frequent	Frequent
Daphnia curvirostris Eylmann, 1887 *	Occasional	–
Daphnia galeata Sars, 1864 *	Common	–
Daphnia hyalina Leydig, 1860	Rare	Not observed

TABLE 1

Complete list of all different cladocerans that have been reported for Belgian water bodies. For each species, the current status in Flanders as revealed by the present study, and the status during Luyten's days (12) is presented. Species where no status is assigned are indicated by (–).

Species	Current status	Status Luyten
Daphnia longispina O.F. Müller, 1785	Occasional	Frequent
Daphnia magna Straus, 1820	Frequent	Occasional
Daphnia obtusa Kurz, 1874 *	Common	–
Daphnia parvula Fordyce, 1901 **	Frequent	–
Daphnia pulex Leydig, 1860	Common	Occasional
Megafenestra aurita (Fischer, 1849)	Occasional	Not observed
Moina brachiata (Jurine, 1820)	Occasional	Occasional
Moina macrocopa (Straus, 1820)	Occasional	Not observed
Moina micrura Kurz, 1874	Occasional	Not observed
Moina weismanni Ishikawa, 1896 **	Not observed	–
Scapholeberis mucronata (O.F. Müller, 1785)	Abundant	Frequent
Scapholeberis rammneri Dumont & Pensaert, 1983 *	Occasional	–
Simocephalus exspinosus (Koch, 1841)	Occasional	Occasional
Simocephalus serrulatus (Koch, 1841)	Rare	Not observed
Simocephalus vetulus (O.F. Müller, 1776)	Abundant	Frequent
Bosminidae		
Bosmina coregoni Baird, 1857	–	Occasional
Bosmina longirostris (O.F. Müller, 1785)	Abundant	Common
Bosmina longispina Leydig, 1860	–	Not observed
Macrothricidae		
Acantholeberis curvirostris (O.F. Müller, 1776)	Occasional	Occasional
Drepanothrix dentata (Eurén, 1861)	Rare	Occasional
Ilyocryptus acutifrons Sars, 1862	Not observed	Rare
Ilyocryptus agilis Kurz, 1878	Occasional	Occasional
Ilyocryptus sordidus (Liévin, 1848)	Frequent	Frequent
Lathonura rectirostris (O.F. Müller, 1785)	Not observed	Occasional
Macrothrix hirsuticornis Norman & Brady, 1867	Occasional	Not observed
Macrothrix laticornis (Jurine, 1820)	Occasional	Rare
Macrothrix rosea (Jurine, 1820)	Rare	Occasional
Streblocerus serricaudatus (Fischer, 1849)	Rare	Not observed
Chydoridae		
Acroperus harpae (Baird, 1835)	Frequent	Common
Alona affinis (Leydig, 1860) *	Frequent	–
Alona costata Sars, 1862	Frequent	Frequent
Alona elegans Kurz, 1875	Not observed	Not observed
Alona guttata Sars, 1862	Common	Frequent
Alona intermedia Sars, 1862	Rare	Not observed
Alona phreatica Dumont, 1983 *	Not observed	–
Alona protzi Hartwig, 1900	Not observed	Not observed
Alona quadrangularis (O.F. Müller, 1785)	Occasional	Common
Alona rectangula Sars, 1862	Common	Frequent
Alona rustica Scott, 1895	Not observed	Not observed
Alonella excisa (Fischer, 1854)	Occasional	Frequent
Alonella exigua (Lilljeborg, 1853)	Occasional	Occasional
Alonella hamulata (Birge, 1879) **	Rare	–
Alonella nana (Baird, 1843)	Frequent	Frequent
Alonopsis elongata (Sars, 1861)	Rare	Occasional
Anchistropus emarginatus Sars, 1862	Not observed	Occasional
Camptocercus lilljeborgi Schoedler, 1862	Not observed	Rare

TABLE 1

Complete list of all different cladocerans that have been reported for Belgian water bodies. For each species, the current status in Flanders as revealed by the present study, and the status during Luyten's days (12) is presented. Species where no status is assigned are indicated by (–).

Species	Current status	Status Luyten
Camptocercus rectirostris Schoedler, 1862	Occasional	Occasional
Chydorus gibbus Sars, 1890	–	Not observed
Chydorus latus Sars, 1862	–	Not observed
Chydorus ovalis Kurz, 1874	–	Occasional
Chydorus sphaericus (O.F. Müller, 1785)	Abundant	Abundant
Disparalona rostrata (Koch, 1841)	Frequent	Frequent
Eurycercus glacialis Lilljeborg, 1887	Not observed	Not observed
Eurycercus lamellatus (O.F. Müller, 1785)	Frequent	Frequent
Graptoleberis testudinaria (Fischer, 1848)	Frequent	Frequent
Leydigia acanthocercoides (Fischer, 1854)	Occasional	Occasional
Leydigia leydigi (Schoedler, 1863)	Frequent	Occasional
Monospilus dispar Sars, 1862	Rare	Rare
Oxyurella tenuicaudis (Sars, 1862)	Occasional	Occasional
Paralona pigra (Sars, 1862)	Not observed	Not observed
Pleuroxus aduncus (Jurine, 1820)	Common	Frequent
Pleuroxus denticulatus Birge, 1879 **	Frequent	–
Pleuroxus laevis Sars, 1862	Occasional	Rare
Pleuroxus trigonellus (O.F. Müller, 1785)	Occasional	Occasional
Pleuroxus truncatus (O.F. Müller, 1785)	Frequent	Frequent
Pleuroxus uncinatus Baird, 1850	Occasional	Occasional
Pseudochydorus globosus (Baird, 1843)	Rare	Occasional
Rhynchotalona falcata (Sars, 1862)	Rare	Occasional
Tretocephala ambigua (Lilljeborg, 1900)	Rare	Not observed
Polyphemidae		
Bythotrephes longimanus Leydig, 1860	Not observed	Not observed
Polyphemus pediculus (Linnaeus, 1761)	Occasional	Occasional
Leptodoridae		
Leptodora kindtii (Focke, 1844)	Occasional	Occasional

Note : (12) did not yet identify some taxa as separate species (*); some species were not yet present in Flanders (non-indigenous species, **).

In order to search for trends in the frequency of occurrence of cladocerans over the past 70 years, we made an attempt to compare our results with those of (12) (Table 1). We are fully aware that the interpretation of this comparison should be done with care, as there are differences among both datasets in geographic location and number of sites, number and type of water bodies sampled ([12] sampled in most cases only one water body per site), and frequency of sampling in the habitats. Table 1 illustrates that many species show no or only minor shifts between categories of the ACFOR scale. Only a limited number of species is nowadays more widespread than before (i.e. mostly daphniids such as *Ceriodaphnia* and *Moina*, *Daphnia pulex*, *Megafenestra aurita*, *S. mucronata*, and *S. vetulus*). Their increase in frequency of occurrence may be attributed to the greater number of small water bodies incorporated in our survey, but may also be related to an increased nutrient load in many water bodies during the last decades (15; 16). The increased nutrient load has lead to a higher production of organic material, and as such to an increased availability of food sources. This may explain why (12) found *Daphnia magna* only twice during his survey, whereas it is now frequently observed. Other species of non-indigenous origin (e.g. *Daphnia ambigua*, *Daphnia parvula*, and *Pleuroxus denticulatus*) were only introduced in Europe many years after Luyten's study (6), and are now frequently observed. For a subset of species, a comparison of the frequency of occurrence cannot be made, as (12) did not yet identify them as separate species (*Daphnia curvirostris* probably identified as *D. pulex*; *Daphnia galeata* probably identified as *Daphnia longispina*; *Daphnia obtusa* probably identified as *D. pulex*; *Scapholeberis rammneri* probably identified as *S. mucronata*; and *Alona affinis* probably identified as *Alona quadrangularis*).

The relatively large proportion of species (17%) that were not observed during our intensive survey may indicate the loss of specific habitats. For instance, the degradation of clear, weakly buffered, and oligotrophic water bodies may explain the disappearance of some species. Other species were only recently recorded as isolated cases, and are relict species (e.g. *Eurycercus glacialis*

reported by [17]) or non-indigenous species (*Moina weismanni* reported by [9]). More detailed research in the Campine region would probably result in the rediscovery of some species that were not observed during our study.

Hot spots for cladocerans, identified as sites in which 2 or 3 rare species were observed, are sites which contain clear, weakly buffered, and oligotrophic to mesotrophic water bodies such as Zonhoven (De Teut), and Genk (De Maten and Het Wik) (Fig. 1). The site of Damme is another hot spot, as it contains many small, turbid and often temporary habitats, which are frequented by migrating waterfowl (see further).

NEW SPECIES FOR BELGIUM

Streblocerus serricaudatus

This extremely rare macrothricid was found in a *Sphagnum* rich pond in only one site (De Teut, Zonhoven) during summer 2005. The accompanying cladocerans existed, amongst others, of several species that are typical for clear, weakly buffered, and oligotrophic water bodies (*A. curvirostris*, *Alonopsis elongata*, *Drepanothrix dentata*). The geographic distribution of *S. serricaudatus* is Holarctic, but the species is only rarely observed (6).

Alonella hamulata (synonym *Disparalona hamata* in [18])

This non-indigenous chydorid was observed in two different sites. First, it was observed in Bekkevoort (belongs to site 81, Fig. 1) during summer 2003 in a heavily stocked fish pond. One year later (summer 2004), the species was detected both in a fish pond and a dead arm of the Demer river near Vorsdonkbos (belongs to site 8, Fig. 1). *A. hamulata* has a cosmopolitan distribution, but seems to be largely restricted to tropical and subtropical regions (6). In Europe, the species has most probably been accidentally introduced and is recorded only twice : in Prague (Czech Republic), and in Slovakian lowland abandoned river arms (19). As fish ponds in Flanders are regularly stocked with cyprinids imported from East European countries, it is not unlikely that the species was introduced in Belgium during such translocations. Combined with rising temperatures due to climate change, the species may potentially extend its geographic distribution towards the north.

NEW LOCATIONS OF RECENTLY DISCOVERED SPECIES

Daphnia atkinsoni

The species was observed for the first time in Belgium in a newly created pond in Damme (2002). In this site, it was found to also occur in several other small water bodies in the immediate neighborhood (10). Since then, two nearby sites were found to also harbour this large daphniid. So far, all *D. atkinsoni* populations in Belgium have been found in small and turbid (due to suspended clay particles) fishless ponds in the East Coast Polders region of Flanders : Blankenberge (Uitkerkse Polder), Damme (Oude Stadswallen), and Knokke-Heist (Zwinbosjes). The incidence of the species might be linked to the presence of wintering geese in the region, which may act as dispersal agent for their propagules (10).

Tretocephala ambigua

The extremely rare chydorid *T. ambigua* was first detected as a new record for Belgium (Koolkerke; belongs to site 17, Fig. 1) in summer 2002 in a shadowed ditch with a thick layer of leaf litter on its bottom (10). In summer 2004, the species was found in another location (Honegem; belongs to site 56, Fig. 1) in two neighboring water bodies, i.e. a flooded meadow and a ditch. The species has a pan-European distribution, but is found only accidentally (6).

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APP. 1.

Names (city, municipality or toponym) of the sampled sites in Flanders (Belgium). Sites investigated by (12) are indicated in italics.

No	Site name	No	Site name	No	Site name	No	Site name
1	*Aalst (Moorsel)*	22	*Destelbergen*	45	*Ieper (Dikkebus)*	68	*Nieuwpoort*
2	Aalter	23	Diest	46	Kalmthout	69	Oostkamp
	Aalter (Bellem)		*Diest (Deurne)*	47	Kampenhout	70	Opglabbeek
3	Alken	24	Diksmuide	48	Kasterlee	71	Oud-Heverlee
4	Antwerpen	25	Dilsen-Stokkem	49	Kinrooi		*Oud-Heverlee*
	Antwerpen	26	Genk	50	Knokke-Heist	72	Oud-Turnhout
5	*Arendonk*		*Genk*		*Knokke-Heist (Knokke)*	73	Ranst
6	*Assenede*	27	*Genk (Bokrijk)*	51	*Knokke-Heist (Heist)*	74	*Ravels*
7	Beernem	28	Gent	52	Komen-Waasten	75	*Ravels (Poppel)*
8	Begijnendijk	29	*Gent*	53	Kortessem	76	*Ravels (Weelde)*
9	Berlare	30	*Gent (Drongen)*	54	Kortrijk	77	*Sint-Genesius-Rode*
	Berlare (Overmere)	31	*Gent (Zwijnaarde)*	55	*Lanaken*	78	Temse
10	Bierbeek	32	Geraardsbergen	56	Lede	79	Ternat
11	Bilzen	33	Halen	57	*Leopoldsburg*	80	Tervuren
12	Blankenberge	34	Halle	58	Leuven	81	Tielt-Winge
13	Bonheiden	35	*Ham (Kwaadmechelen)*	59	Lierde	82	Tongeren
14	Bornem	36	*Hamont-Achel (Achel)*	60	Lommel	83	Voeren
15	Boutersem	37	Harelbeke		*Lommel*	84	Waregem
16	Bree	38	Hasselt	61	Lummen	85	Zedelgem
17	Damme	39	Heers	62	Maarkedal	86	Zemst
18	*Deinze (Astene)*	40	Heist-op-den-Berg	63	Maasmechelen	87	Zonhoven
19	*Dendermonde*	41	Herentals	64	Middelkerke	88	Zottegem
20	*Dendermonde (Schoonaarde)*	42	Heusden-Zolder	65	*Mol*	89	Zoutleeuw
21	Dessel	43	Huldenberg	66	*Mol (Postel)*	90	*Zwalm (Nederzwalm)*
	Dessel	44	Ieper	67	*Neerpelt*		

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