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TREMATOCARA ZEBRA (PERCIFORMES; CICHLIDAE), NOUVELLE ESPÈCE DU NORD-OUEST DU LAC TANGANYIKA (ZAÏRE)

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Résumé. *Trematocara zebra,* un nouveau cichlidé de la côte occidentale du lac Tanganyika est décrit du milieu sous-littoral et benthique des fonds rocheux situés entre Luhanga et Pemba, de 12 à 26 km au sud d'Uvira (Zaïre). Ce poisson est particulièrement caractérisé par sa livrée (corps brunâtre à réflets pourprés et avec des taches et des bandes verticales noires irrégulières chez les mâles, livrée similaire des femelles mais avec une large bande foncée horizontale distincte au niveau de la nageoire pectorale), par le nombre restreint de branchiospines sur la partie inférieure du premier arc branchial (12 à 13), par la présence de sept pores sensoriels infraorbitaires bien développés plus un orifice préorbitaire, par le nombre limité de rayons anaux mous (7-8), par les deuxièmes et troisièmes rayons anaux branchus fortement allongés et par la nageoire dorsale particulièrement haute. Une brève comparaison avec les autres espèces du genre ainsi que quelques données biologiques sur la nouvelle espèce sont également présentées.

Mots-clés : Trematocara, Cichlidae, nouvelle espèce, lac Tanganyika, Zaïre.

Trematocara zebra (Perciformes; Cichlidae), a new species from the north west coast of Lake Tanganyika (Zaïre)

Summary. *Trematocara zebra*, a new cichlid fish from the north west coast of Lake Tanganyika is described from the sublittoral and benthic zone of the rocky shores at Luhanga and Pemba situated between 12 and 26 km South of Uvira (Zaïre). The species is characterized by its particular colour pattern (brownish body with purple reflections and irregular dark brown vertical stripes and blotches on the sides in males, a similar pattern in females but with a large distinct horizontal dark bar at the

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level of the pectoral fin), by the relatively low number of gill rakers on the lower part of the first branchial arch (12 to 13), by the presence of 7 large infraorbital and 1 preorbital sensory pores, by the low number of soft anal fin rays (7-8), by the elongated second and third anal finray and by the particularly high dorsal fin. A short comparaison with the other known species of the genus and some biological data on the new species are given.

Key words : *Trematocara*, Cichlidae, new species, Lake Tanganyika, Zaïre.

INTRODUCTION

Le genre *Trematocara* Boulenger, 1899 comprend sept espèces endémiques du bassin du lac Tanganyika qu'on trouve essentiellement dans le milieu lacustre à des profondeurs assez variables. Les représentants de ce genre sont facilement reconnaissables en particulier par la présence de canaux sensoriels céphaliques à orifices hypertrophiés : les os frontaux, nasaux, préorbitaires, et sous-orbitaires ainsi que la mâchoire inférieure et le préopercule sont creusés de larges canaux s'ouvrant par de grands orifices extérieurs en nombre variable. En outre les yeux sont très grands, la ligne latérale supérieure est très courte et unique et la ligne inférieure est absente ou vestigiale (POLL, 1986). COULTER (1991) a avancé l'hypothèse que l'hypertrophie du système sensoriel céphalique augmenterait considérablement les capacités sensorielles à des profondeurs où la luminosité est fort restreinte. Le développement important des yeux indiquerait également une adaptation à la vie dans des endroits peu illuminés. KONINGS (1993) signale que toutes les espèces connues de *Trematocara* vivent à des profondeurs importantes (généralement plus de 100 mètres), et semblent chercher leur nouriture dans la faible lueur pénétrant depuis la surface. Le grand développement de leur système de détection acoustique au niveau crânien est leur caractère physiologique principal. Les pores sensoriels apparaissent comme des bulles sous-cutanées et la membrane de chaque pore fonctionne comme un tympan. Plus grand est le pore, meilleure est la perception. L'association de tous les pores forme un véritable radar grâce auquel le poisson peut détecter et localiser sa proie. Quelques espèces de ce genre ont, en effet, été trouvées dans le lac, à des profondeurs très importantes caractérisées aussi par des faibles concentrations en oxygène : POLL (1952) signale les *Trematocara* parmi les plus représentatifs de la faune benthique "abyssale", c.à.d. à des profondeurs de l'ordre de 100 m. COULTER (1967) mentionne la capture dans le sud du lac de deux spécimens de *T. unimaculatum* Boulenger, 1901 à une profondeur de 205 m, tandis que des individus de *T. nigrifrons* Boulenger, 1906 furent trouvés entre 120 et 140 m de profondeur (COULTER, 1966). Dans le nord du lac où les eaux sont moins profondes que dans le sud, nous avons observé ces deux espèces jusqu'à 80 m de profondeur dans des couches d'eau pratiquement dépourvues d'oxygène. Ces poissons ne vivent vraisemblablement pas à demeure à ces grandes profondeurs; plusieurs espèces effectuent des migrations pendant la nuit pour s'approcher du rivage du lac (POLL, 1952; COULTER,

1991;KONINGS, 1988; KONINGS & DIECKHOFF, 1992). Bien que les *Trematocara* puissent être considérés comme des poissons essentiellement lacustres, *T. variabile* Poll, 1952 a été capturé en milieu fluviatile véritable dans le cours inférieur de la Malagarasi, quelques kilomètres en amont de son embouchure (pêche expérimentale avec TAFIRI Center Kigoma à la fin du mois d'août 1993). A notre connaissance, il n'y a pas d'autres mentions de ce groupe hors du milieu strictement lacustre.

Depuis 1992, le Centre Régional de Recherches en Hydrobiologie Appliquée (CRRHA) de Bujumbura (Burundi), en collaboration étroite avec le Centre de Recherche en Hydrobiologie (CRH) d'Uvira (Zaïre), a organisé des échantillonnages réguliers dans le nord du lac Tanganyika le long des côtes burundaises et zaïroises. Dans des captures provenant des localités de Luhanga (3•30'S-29•08'E) et Pemba (3•40'S-29•10'E), situées sur la côte nord-ouest au Zaïre, nous avons récolté plusieurs spécimens d'une espèce de *Trematocara* fort différente de toutes les espèces connues jusqu'à présent. Ce poisson a été capturé dans les zones sous-littorale et benthique de plusieurs sites rocheux. Nous le décrivons ci-dessous comme nouvelle espèce sous le nom de *T. zebra.*

RESULTS

Trematocara zebra nov. spec.

Matériel examiné

23 exemplaires (15 mâles et 8 femelles) entre 44 et 69,5 mm de longueur standard (58-89 mm longueur totale), récoltés dans le lac Tanganyika à des profondeurs de 10 à 60 m, entre 12 et 26 kilomètres au sud de la cité d'Uvira (Zaïre). Ils ont été capturés à l'aide de filets dormants à mailles de 8 à 16 mm noeud à noeud. Ces spécimens sont conservés dans les collections de la section des Vertébrés du Musée royal de l'Afrique centrale à Tervuren, Belgique (MRAC) et en partie dans les collections du CRRHA et du CRH.

Holotype : MRAC 95-18-P-1; mâle, 66 mm LS; Luhanga II; profondeur 50 m; Nshombo coll.; 17-11-1994.

Paratypes : MRAC 94-68-P-128-129; 2 ex., 58-59 mm LS; Luhanga II, profondeur 30 m; Nshombo coll.; 21-7-1994.

MRAC 94-68-P-130-131; 2 ex., 43-49 mm LS; Luhanga, profondeur 10 et 30 m; De Vos et Nshombo coll.; 15-02-94.

MRAC 94-68-P-132-134; 3 ex., 52-59 mm LS; Luhanga, profondeur 10 m; Nshombo coll.; 26-04-94.

MRAC 95-18-P-2; 1 ex., 57 mm LS; Luhanga II, profondeur 50 m; Nshombo coll.; 17-11-1994.

MRAC 95-18-P-3, 1 ex., 61 mm LS; Luhanga II, profondeur 30 m; Nshombo coll.; 21-7-1994.

MRAC 95-70-P-1-4; 4 ex., 55,5-62,5 mm LS; Pemba, profondeur 30 m; Mboko coll.; 18-08-1995.

MRAC 95-70-P-5-8; 4 ex., 47-69,5 mm LS; Luhanga, profondeur 60 m; Mboko coll.; 28-07-1995.

CRRHA non cat.; 1 ex., 59 mm LS; Luhanga, prof. 30 m; De Vos et Nshombo coll.; 15-01-1994.

CRRHA non cat.; 3 ex., 55-60 mm LS; Luhanga, profondeur 30 m; Mboko coll.; 15-07-1995.

CRH non cat.; 1 ex. 56,5 mm SL; Luhanga; prof. 30 m; De Vos et Nshombo coll.; 15-02-1994.

[Note : la localité «Pemba» est souvent indiquée fautivement par le nom «Bemba» (MARLIER, 1958, KONINGS, 1988, BRICHARD, 1989, ...), mais en Swaheli, la langue locale, «pemba» signifie littéralement «blanc» et fait allusion au sol blanc qu'on peut observer à certains endroits de cette localité.].

Etymologie

Le nom spécifique provient du mot latin «zebra» (zèbre, ongulé à rayures sur la peau) et fait référence à la livrée caractérisée par des lignes et des taches foncées sur les flancs.

L'espèce semble inconnue des riverains locaux et n'a apparemment pas de nom vernaculaire.

Description

Les principaux caractères méristiques avec leur fréquence et les proportions du corps les plus importantes sont indiqués dans le Tableau 1.

Corps allongé (Fig. 1), plus haut que large, hauteur maximum au niveau de l'origine de la nageoire dorsale 3,4-3,7 fois dans la longueur standard, dos légèrement convexe. Pédoncule caudal relativement court, 1,8-2,4 fois plus long que haut. Une ligne latérale supérieure très courte, n'offrant que quelques tubes juste derrière la tête. Une ligne latérale médiane antérieure formée de 8-9 écailles perforées et une deuxième partie postérieure indistincte le long du pédoncule caudal formée d'une dizaine à une douzaine d'écailles. On remarque parfois de vagues traces d'une ligne latérale inférieure au niveau du pédoncule caudal. Tête caractéristique, avec une légère bosse postorbitaire et au museau arrondi; mâchoire inférieure oblique et légèrement proéminente. Oeil large et ovale, 2,9-3,3 fois dans la longueur de la tête. Maxillaire atteignant le bord antérieur de l'oeil. Orifices sensoriels céphaliques bien développés: 7 orifices sensoriels sous-orbitaires, 1 orifice préorbitaire et 2 supraorbitaires. Une fontanelle frontale ovale impaire au milieu du crâne entre les deux orbites. Une légère bosse supraoccipitale juste derrière la fontanelle. Préoperculaire et mâchoire inférieure creusés d'une série d'une douzaine d'orifices sensoriels (Fig. 2). Espace interoculaire 3,7-5 fois dans la longueur de la tête.

TABLEAU 1.

Principaux caractères méristiques et métriques de la série holotype et paratypes de Trematocara zebra *sp. nov.*
(LS = Longueur standard, Lt = Longueur de la tête).

Caractères méristiques:

Rayons de la nageoire dorsale: X + 10 (f9), X + 11 (f13), X +12 (f1)

Rayons de la nageoire anale: III + 7 (f13), III + 8 (f9)

Rayons de la nageoire pectorale: i + 9 (f6), i + 10 (f14)

Rayons de la nageoire pelvienne: I + 5 (f23)

Ecailles en ligne longitudinale: 26 (f2), 27 (f6), 28 (f6), 29 (f7)

Branchiospines en bas du premier arc branchial: 12 (f8), 13 (f14)

Vertèbres abdominales (7 individus): 11 (f2), 12 (f5)

Vertèbres caudales (7 individus): 16 (f1), 17 (f5) , 18 (f1)

Caractères métriques : (holotype et série -paratypes)	*Holotype*	*Min.*	*Max.*	*Moyenne*
Longueur totale (mm):	87	58	87	-
Long. standard (mm):	66	44	66	-
Hauteur du corps(%LS)	30,3	26,9	30,4	28,5
Long.de la tête (%LS)	38,9	36,3	40,7	38,3
L.base dorsale (%LS)	46,1	42,4	48,1	45,8
L. base anale (%LS)	16,8	14,0	17,9	16,2
Dist.prédorsale (%LS)	39,4	36,8	41,4	38,9
Dist. préanale (%LS)	61,4	61,4	66,2	63,4
Dist. prépectorale (%LS)	40,9	37,5	40,9	39,3
Dist. prépelvienne (%LS)	39,5	38,6	41,9	40,0
L. pédoncule caudal (%LS)	23,8	20,5	26,3	23,0
Haut. pédoncule caudal (%LS)	11,1	11,1	11,5	11,3
Long.pectorale (%LS)	38,2	33,9	38,9	36,3
L. pelvienne (%LS)	30,3	26,3	32,4	28,4
Espace interorbitaire (%LT)	26,1	20,1	26,5	23,2
Longueur museau (%LT)	33,8	28,7	36,1	32,2
Diamètre de l'oeil (%LT)	31,5	29,1	34,6	31,6
Distance entre les narines (%LT)	21,0	18,3	21,1	19,6

Branchiospines au nombre de 12 à 13 sur la partie inférieure du premier arc branchial, les 2 à 4 premières nettement plus courtes que les autres. Les branchiospines suivantes de cette série sont relativement minces et allongées. Une branchiospine sur l'angle entre le cératobranchial et l'épibranchial, 3 ou 4 sur la partie supérieure du premier arc.

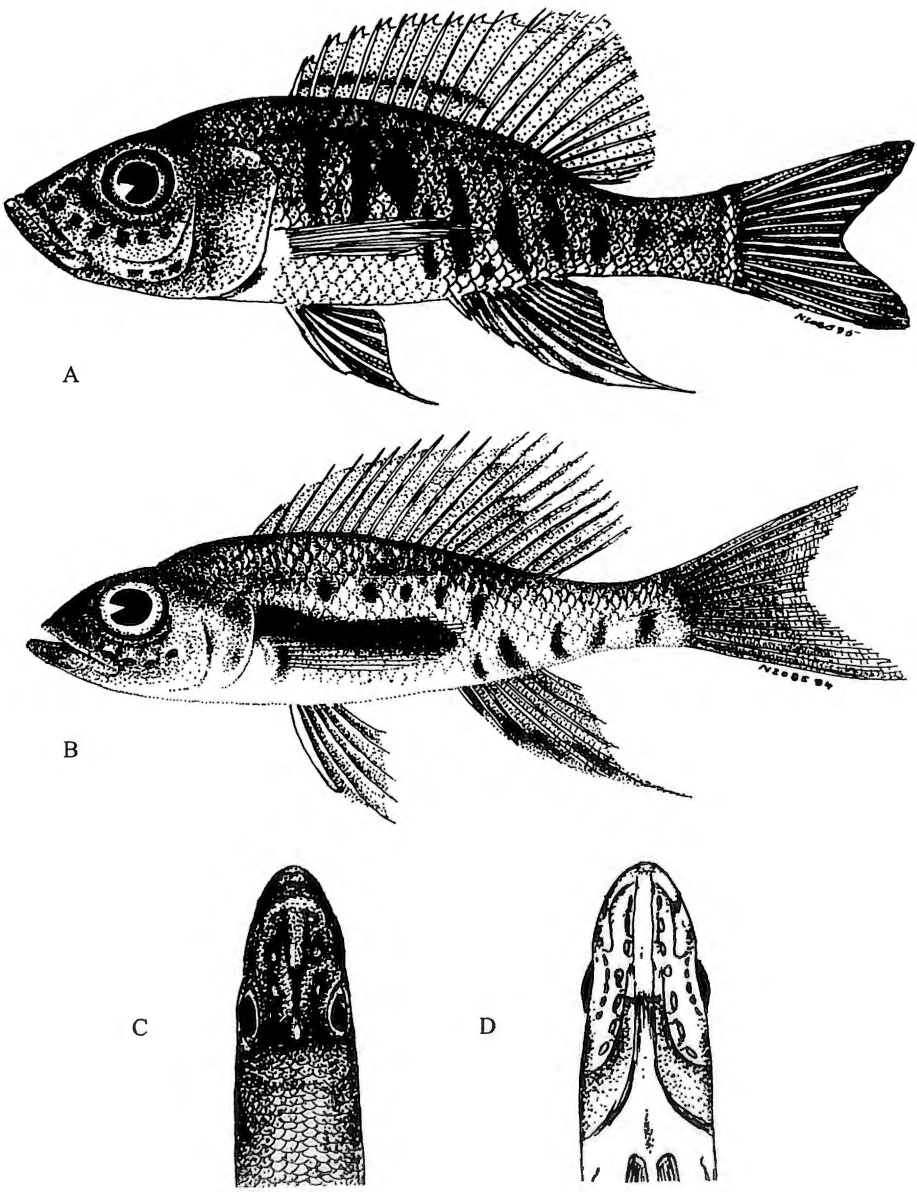

Fig. 1. – *Trematocara zebra* sp. nov. – A. Vue latérale du holotype, un mâle de 66 mm LS. – B. Vue latérale d'une femelle paratype de 60 mm LS. – C. Vue dorsale de la tête du holotype. – D. Vue ventrale de la tête du holotype.

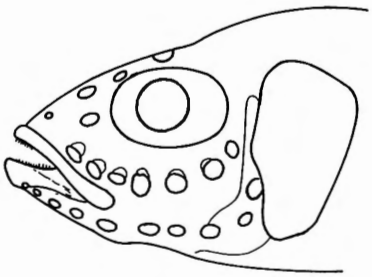

Fig. 2. – *Trematocara zebra* sp. nov. – Schéma de la tête d'un spécimen de 56 mm LS montrant les orifices sensoriels céphaliques hypertrophiés.

Os pharyngien inférieur en triangle à surface dentigère un peu plus large que longue (Fig. 3). La majorité des dents pharyngiennes très petites, avec une cuspide antérieure insignifiante et une cuspide postérieure saillante (type "bevelled" suivant la nomenclature de BAREL *et al.*, 1977). Quelques dents pharyngiennes périphériques sont du type monocuspide.

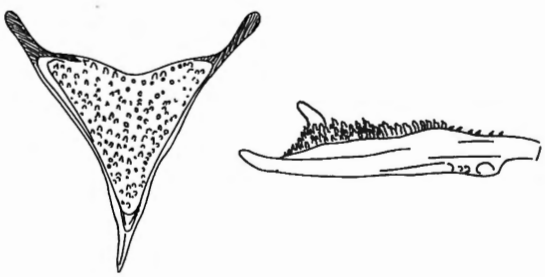

Fig. 3. – *Trematocara zebra* sp. nov. – Vues dorsale et latérale de l'os pharyngien inférieur d'un paratype de 56,5 mm LS.

Vertèbres au nombre de 28-29 (la vertèbre terminale complexe urostylaire exclu), comprenant 11-12 vertèbres abdominales et 16-18 vertèbres caudales.

Nageoire dorsale X + 10-12, haute et arrondie, avec une hauteur maximale au niveau du deuxième rayon mou (voir Fig. 1). Dernier rayon de la dorsale n' atteingnant pas la base de la caudale. Anale III + 7-8, le deuxième et troisième rayon mou fortement alongés. Nageoire pectorale avec un simple rayon supérieur et 9-10 rayons branchus mous, dépassant nettement le niveau de l'origine de la nageoire anale. Nageoire pelvienne I + 5, effilée, le rayon mou externe le plus long, atteignant au moins l'origine de l'anale. Nageoire caudale échancrée, à lobes pointus et 1 + 14 + 1 rayons mous.

Mâchoires garnies de nombreuses petites dents externes (jusqu'environ une centaine), coniques, fines et serrées. Deux à trois rangées de minuscules dents coniques internes. Les dents ne garnissent pas les côtés externes des lèvres.

Ecaillure cycloïde, écailles des flancs grandes, au nombre de 26-29 en ligne longitudinale. Ecailles ventrales plus petites. Joue et opercule nus, sauf pour le bord supérieur de l'opercule qui présente parfois quelques écailles cycloïdes. Douze écailles autour du pédoncule caudal. Tête non écailleuse sauf dans la zone occipitale qui est couverte de petites écailles cycloïdes.

Coloration (Fig. 4) : spécimens fraîchement capturés : Mâles: livrée brunâtre avec réflets métalliques rose-pourpre; ventre plus clair; une série de bandes ou taches verticales irrégulières brun foncé (jusqu'à une dizaine), de longueur variable, et plusieurs petites taches bleu-clair sur les flancs; nageoire caudale gris-brun avec une tache noire à la base, pectorales incolores; dorsale brun-clair bordée d'une fine ligne noire et pourvue d'une large bande noire juste au-dessus de la base de la dorsale (absente chez la femelle); nageoires anale, dorsale et pelviennes bordées de noir.

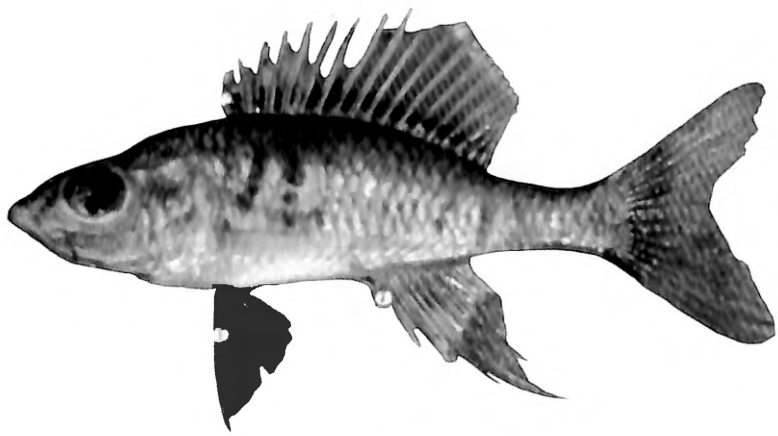

Fig. 4. – *Trematocara zebra* sp. nov. – Un mâle d'environ 55 mm LS de Luhanga, Zaïre (photo L. De Vos).

Femelles : livrée ressemblant celle des mâles mais avec une large bande foncée horizontale très tranchée sur les flancs au niveau de la nageoire pectorale (absente ou peu distincte chez le mâle), suivie d'une série de petites bandes verticales foncées. Quelques lignes verticales foncées au-dessus de la bande pectorale. Une ligne noire au milieu de l'anale, se prolongeant jusqu'au plus long rayon. Deux courtes lignes noires parallèles au milieu des pelviennes.

Spécimens préservés : brun-pâle, la tête un peu plus foncée; une série de bandes ou taches verticales brun foncé sur les flancs; nageoires gris-brun, l'anale et la pectorale plus foncées. Une large bande horizontale au niveau de la pectorale chez la femelle, suivie de quelques lignes verticales sur l'arrière-train du corps. Une longue bande noire juste au-dessus de la base de la dorsale chez les mâles.

Un dimorphisme sexuel concernant la livrée a également été observé dans toutes les autres espèces du genre, les mâles étant typiquement ornés de 2-3 bandes noires longitudinales et les nageoires étant pourvues de bandes ou taches mélaniques suivant l'espèce; la coloration de la femelle est en général beaucoup plus claire ce qui ne semble pas le cas chez la nouvelle espèce.

Taille : petite espèce. Taille maximale observée : 69,5 mm de LS (89 mm de LT).

Caractères distinctifs et comparaison avec d'autres *Trematocara*

Trematocara zebra se distingue particulièrement des autres espèces du genre par sa coloration. Les *Trematocara* décrits auparavant (Fig. 5) sont de couleur blanc-argenté, ornés typiquement de 2-3 bandes noires longitudinales chez le mâle, les nageoires pourvues de bandes ou taches mélaniques suivant l'espèce (POLL, 1986). *Trematocara zebra* par contre est brunâtre, avec des reflets olivâtres, et montre des bandes verticales brun foncé chez les mâles; le ventre est pâle et la base de la nageoire dorsale est noire. Les femelles ont une livrée similaire mais sont ornées en outre d'une large bande foncée horizontale au niveau de la nageoire pectorale. La Fig. 5 montre des individus mâles des différents *Trematocara* connus, permettant une comparaison facile de leur livrée. La nouvelle espèce se caractérise également par la présence de 7 orifices sensoriels infraorbitaires et 1 orifice préorbitaire, par une nageoire dorsale particulièrement haute, par les deuxième et troisième rayons anaux branchus fortement allongés et par le nombre relativement restreint de branchiospines (12 ou 13) sur la partie inférieure du premier arc branchial. Chez les autres *Trematocara* (sauf chez *T. marginatum* Boulenger, 1899 qui en possède 9 à 12) il y a au moins 15 branchiospines sur la portion ventrale du premier arc branchial. Il y a également peu de rayons anaux mous (7-8) par comparaison avec d'autres *Trematocara* qui, en général, en possèdent au moins 9 (sauf chez *T. kufferathi* Poll, 1948 qui en présente seulement 8-9). Les mâchoires de *T. zebra* sont garnies de nombreuses petites dents externes, coniques, fines et serrées. On compte deux à trois rangées de minuscules dents coniques internes. Les dents ne garnissent pas les côtés externes des lèvres mais chez quelques individus certaines dents externes sont placées fortement vers l'extérieur. Nous avons observé une situation identique chez plusieurs individus de *T. marginatum* et de *T. stigmaticum* Poll, 1943. Pourtant, ce caractère des dents garnissant la surface externe des lèvres au niveau des prémaxillaires et des dentaires figure parmi les critères importants pour séparer le genre monospécifique *Telotrematocara* Poll, 1986 du genre *Trematocara*. Sans vouloir faire une analyse détaillée du problème, notre observation pourrait apporter un certain doute quant à la validité du genre *Telotrematocara*. La seule espèce de ce genre se distingue d'ailleurs fortement de *T. zebra* e.a. par des données méristiques et une livrée fortement différentes et par un développement extraordinaire de la bouche et des orifices sensoriels céphaliques (BAILEY & STEWART, 1977; POLL, 1986).

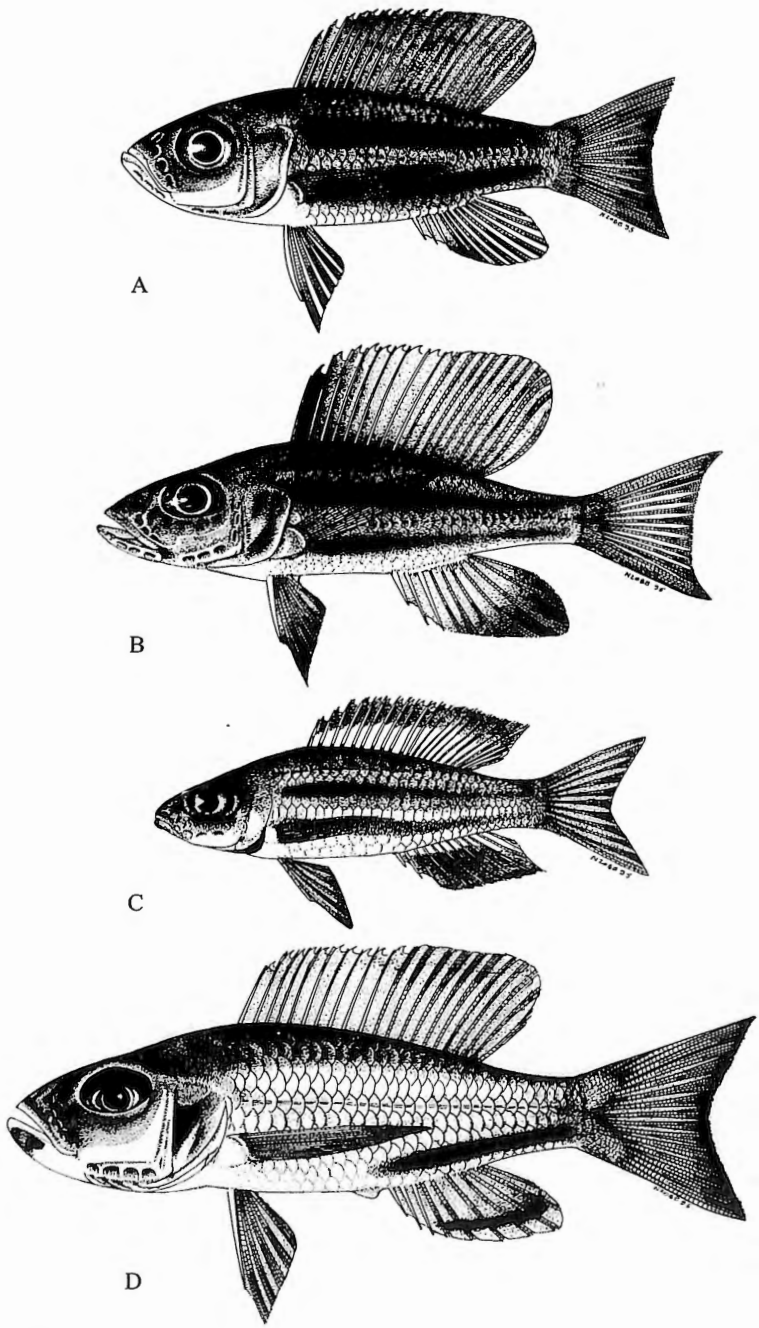

Fig. 5. - Individus mâles des différentes espèces connus du genre *Trematocara* – A. *T. caparti* (Karema, Tanzania: 45 mm LS) – B. *T. kufferathi* (Karema, Tanzania: 51 mm LS) – C. *T. marginatum* (Nyanza-Lac, Burundi: 62 mm LS) – D. *T. nigrifrons* (Rumonge, Burundi: 61 mm LS).

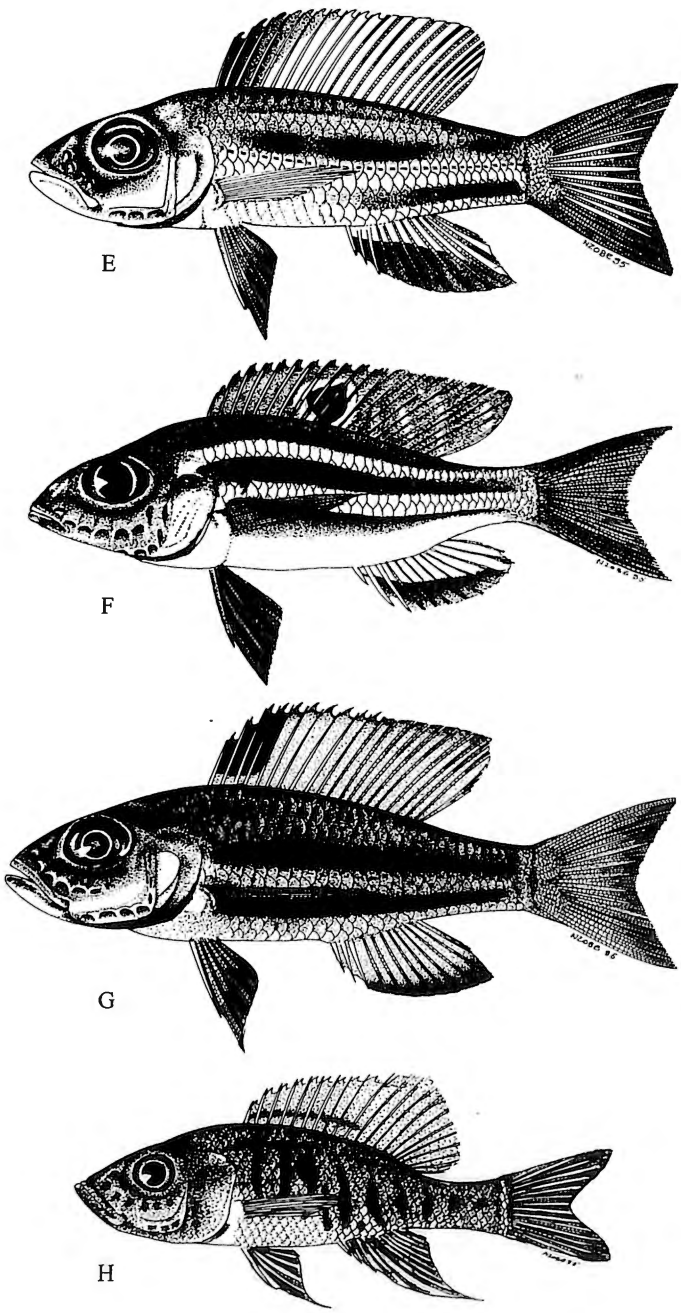

Fig. 5 (cont.) – E. *T. stigmaticum* (Gatumba, Burundi: 45 mm LS) – F. *T. unimaculatum* (Gitaza, Burundi: 83 mm LS) – G. *T. variabile* (Gatumba, Burundi: 56 mm LS) – H. *T. zebra* (Luhanga, Zaïre: 66 mm LS).

Le Tableau 2 donne une comparaison de quelques caractères méristiques des différentes espèces de *Trematocara*. Nous avons pris en considération le nombre de branchiospines sur la partie inférieure du premier arc branchial, le nombre total de vertèbres et de rayons aux nageoires dorsale et anale, le nombre d'écailles en ligne longitudinale ainsi que le nombre d'orifices sensoriels préorbitaires et sous-orbitaires

TABLEAU 2

Tableau comparatif de quelques caractères méristiques des différentes espèces de Trematocara. *Ces données ont été prises à partir des descriptions de Poll (1956) et de Bailey & Stewart (1977). (Branchiosp. premier arc = nombre de branchiospines sur la partie inférieure du premier arc branchial; Vertèbres = nombre total de vertèbres, l'élément urostylaire exclus; Ecailles ligne long. = nombre d'écailles en ligne longitudinale; Orif. pré + sous-orbit. = nombre total des orfices sensoriels pré- et sousorbitaires).*

	Branchiosp. prem. arc.	Vertèbres N total	Rayons dorsaux	Rayons Anaux	Ecailles ligne long.	Orif. pré- + sous-orbit.
Trematocara caparti Poll,1948	21-25	-	IX-XI+10-12	III+9-10	27-29	4
T. kufferathi Poll,1948	16-19	30	VIII-X+10-12	III+8-9	28-29	3
T. marginatum Boulenger,1899	9-12	29	X+11-12	III+9-11	28-30	8-9
T. nigrifons Boulenger,1906	17-21	29-30	IX-XI+11-13	III+9-11	28-30	5
T. stigmaticum Poll, 1943	15-20	28-30	IX-X+11-13	III+10-12	28-30	4
T. unimaculatum Blgr,1901	16-19	29-30	X-XII+11-13	III+9-10	29-31	9
T. variabile Poll, 1952	15-17	29-30	IX-XI+10-13	III+9-11	28-30	5-7
T. zebra spec. nov.	12-13	28-29	X+10-12	III+7-8	26-29	8

Données biologiques et écologiques

Régime alimentaire : l'examen qualitatif du contenu stomacal de deux individus a montré des débris de crevettes ainsi que du sable et du phytoplancton (Cyanophytes et Chlorophytes du groupe *Oocystis*). Il est possible que le sable et le phytoplancton soient ingérés accidentellement avec les crustacés car en général les *Trematocara* benthiques sont considérés comme des fouilleurs du fond à régime microphage vorace, se nourrissant d'une variété d'invertébrés et de diatomées (POLL, 1956; MATTHES, 1960; KONINGS, 1988; COULTER, 1991). La dentition conique ainsi que la disposition et le nombre restreint des branchiospines suggèrent une nutrition sélective sur le fond et les rochers. Le tube digestif d'un individu de 56 mm de LS mesurait 35 mm.

Reproduction : Fécondité : l'ovaire d'une femelle paratype de 60 mm LS contenait 41 oeufs d'un diamètre approximatif de 1,5 mm. Ce nombre relativement limité d'œufs suggère qu'il s'agit d'une espèce à incubation buccale. L'incubation buccale maternelle a

d'ailleurs été constaté chez quelques autres espèces du genre: *T. nigrifrons*, *T. stigmaticum* et *T. caparti* Poll, 1948 (POLL, 1956; KONINGS, 1988; KRÜTER, 1991).

Sex-ratio : dans l'échantillon de 23 spécimens, il y a 15 mâles et 8 femelles, donc presque deux fois plus de mâles. Ce phénomène est assez curieux mais notre échantillon est trop restreint pour tirer des conclusions certaines concernant la répartition numérique des sexes.

Communauté de poissons associés : le Tableau 3 présente une liste d'espèces prises à Luhanga et Pemba dans l'étage sous-littorale (10-40 m de profondeur) et dans le milieu benthique (captures sur des profondeurs de 40-60 m) lors de douze nuits de pêches expérimentales effectuées entre février 1994 et août 1995. Cette liste ne représente sans doute pas un inventaire complet de l'ichtyofaune sous-littorale et benthique de cette côte mais elle donne néanmoins une idée assez précise de la communauté de poissons vivant en association avec *T. zebra* dans la zone concernée.

TABLEAU 3

Poissons capturés ensemble avec Trematocara zebra
dans l'étage sous-littoral (10-40 m de profondeur) et dans le milieu benthique (prises entre 40 et 60 m de profondeur) du lac Tanganyika à Luhanga et Pemba au Zaïre.

Milieu sous-littoral (10-40 m)	**Milieu benthique (40-60 m)**
Clupeidae :	**Clupeidae :**
- *Limnothrissa miodon* (Boulenger, 1906)	+
Cyprinidae :	
- *Acapoeta tanganicae* (Boulenger, 1900)	
Claroteidae :	**Claroteidae :**
	- *Auchenoglanis occidentalis* (Valenciennes, 1840)
- *Bathybagrus tetranema* Bailey & Stewart, 1984	+
- *Chrysichthys brachynema* Boulenger, 1900	+
- *C. graueri* Steindachner, 1911	+
- *C. sianenna* Boulenger, 1906	+
- *C. platycephalus* Worthington & Rickardo, 1936	+
- *Phyllonemus filinemus* Worthington & Rickardo, 1936	+
Clariidae :	**Clariidae :**
- *Dinotopterus cunningtoni* Boulenger, 1906	
- *Tanganikallabes mortiauxi* Poll, 1943	+
Mochokidae :	**Mochokidae :**
- *Synodontis granulosus* Boulenger, 1900	+
- *S. multipunctatus* Boulenger, 1898	+
- *S. petricola* Matthes, 1959	+

- *S. polli* Gosse, 1982

Poeciliidae :

- *Lamprichthys tanganicanus* (Boulenger, 1898)

Cichlidae :	**Cichlidae :**
- *Altolamprologus compressiceps* Boulenger, 1898)	+
	- *Auloncranus dewindti* (Boulenger, 1899)
- *Bathybates fasciatus* Boulenger, 1901	+
- *B. vittatus* Boulenger, 1914	
- *Benthochromis tricoti* (Poll, 1948)	+
- *Boulengerochromis microlepis* (Boulenger, 1899)	
- *Cyathopharynx furcifer* (Boulenger, 1898)	+
- *Cyphotilapia frontosa* (Boulenger, 1906)	+
- *Cyprichromis microlepidotus* (Poll, 1956)	+
- *Eretmodus cyanostictus* Boulenger, 1898	
- *Gnathochromis pfefferi* (Boulenger, 1898)	
- *Grammatotria lemairii* Boulenger, 1899	
- *Greenwoodochromis christyi* (Trewavas, 1953)	+
- *Haplochromis benthicola* Matthes, 1962	+
- *Haplotaxodon microlepis* Boulenger, 1906	+
- *Julidochromis marlieri* Poll, 1956	
- *J. transcriptus* Matthes, 1958	
- *Lamprologus callipterus* Boulenger, 1906	+
- *L. lemairii* Boulenger, 1899	+
- *Lepidiolamprologus attenuatus* (Steindachner, 1909)	
- *L. elongatus* (Boulenger, 1898)	+
- *L. profundicola* (Poll, 1949)	
- *Limnochromis auritus* (Boulenger, 1901)	+
- *Limnotilapia dardennii* (Boulenger, 1899)	
- *Lobochilotes labiatus* (Boulenger, 1898)	+
- *Neolamprologus brevis* (Boulenger, 1899)	
- *N. brichardi* (Poll, 1974)	+
- *N. fasciatus* (Boulenger, 1898)	
- *N. furcifer* (Boulenger, 1898)	
- *N. leleupi* (Poll, 1956)	
- *N. mondabu* (Boulenger, 1906)	+
- *N. niger* (Poll, 1956)	
- *N. savoryi* (Poll, 1949)	+
- *N. toae* (Poll, 1949)	

- *N. tretocephalus* (Boulenger, 1899) +
- *Neolamprologus wauthioni* (Poll, 1949)
- *Ophthalmotilapia nasuta* (Poll & Matthes, 1962)
- *O. ventralis* (Boulenger, 1898)
- *Perissodus microlepis* Boulenger, 1898 +
- *P. orthognatus* Matthes, 1959
- *P. polyodon* Boulenger, 1898
- *P. trewavasae* Poll, 1948 +
- *Plecodus paradoxus* Boulenger, 1898 +
- *P. straeleni* Poll, 1948 +
- *Pseudosimochromis curvifrons* (Poll, 1942)
- *Simochromis diagramma* (Günther, 1893)
- *Tanganicodus irsacae* Poll, 1950
- *Telmatochromis temporalis* Boulenger, 1898
- *Trematocara unimaculatum* Boulenger, 1901
- *T. variabile* Poll, 1952
- *T. zebra* spec. nov.
- *Tropheus duboisi* Marlier, 1959
- *T. moorii* Boulenger, 1898 +
- *Xenochromis hecqui* Boulenger, 1899
- *Xenotilapia flavipinnis* Poll, 1985
- *X. nasus* De Vos, Risch & Thys van den Audenaerde, 1995
- *X. ochrogenys* (Boulenger, 1914)
- *X. sima* Boulenger, 1899

Centropomidae : **Centropomidae :**

- *Lates angustifrons* Boulenger, 1906
- *L. mariae* Steindachner, 1909 +
- *L. microlepis* Boulenger, 1898

Lates stappersii (Boulenger, 1914)

Mastacembelidae : **Mastacembelidae :**

- *Afromastacembelus cunningtoni* (Boulenger, 1906) +
- *A. ellipsifer* (Boulenger, 1899) +
- *A. flavidus* (Matthes, 1962)
- *A. moorii* (Boulenger, 1899)
- *A. ophidium* (Günther, 1893)
- *A. platysoma* (Poll & Matthes, 1962)

Habitat : *Trematocara zebra* est un poisson benthique lacustre que nous avons capturé sur des fonds rocheux dans les zones sous-littorale et benthique entre 10 et 60 m de profondeur. Les échantillonnages se faisaient pendant la nuit. Des observations répétées, effectuées en plongée pendant la journée entre 10 et 40 m de profondeur, n'ont pas permis d'observer cette espèce dans son milieu naturel. Il est possible qu'elle reste dans des eaux plus

profondes durant la journée. Des déplacements nocturnes verticaux pour s'approcher du rivage, vraisemblablement pour se nourrir ou pour échapper aux prédateurs de fond nocturnes, ont également été signalés pour d'autres *Trematocara* (POLL, 1952; KONINGS, 1988; KONINGS & DIECKHOFF, 1992).

A chaque capture de la nouvelle espèce, nous n'avions que quelques exemplaires. Elle semble donc peu abondante. En outre, dans nos captures à Luhanga et Pemba les autres *Trematocara* étaient également particulièrement rares. Pourtant, nous prenons des centaines, parfois des milliers de *Trematocara* dans nos pêches sur les fonds vaseux-sablonneux de la côte burundaise. D'après KONINGS (1988) et COULTER (1991), du point de vue écologique, on peut distinguer deux groupes de *Trematocara* : un premier groupe comprend des espèces benthiques vivant en grandes bandes sur des fonds vaseux-sablonneux. Il s'agit de *T. nigrifrons*, *T. variabile*, *T. stigmaticum*, *T. marginatum* et *T. unimaculatum*. Deux autres espèces au contraire fréquentent le milieu bathypélagique où elles sont à la recherche de zooplancton (*T. caparti* et *T. kufferathi*). *Trematocara zebra* occupe une niche écologique bien différente et, apparemment, cette espèce est limitée au milieu benthique rocheux ou mixte rocheux-sablonneux. Sa livrée originale brun foncé avec des bandes et taches obscures permet peut-être un mimétisme avec le décor des fonds rocheux profonds et peu illuminés. Quant aux «caractères abyssaux» du genre, *T. zebra* se différencie de la majorité des autres espèces par le nombre d'orifices crâniens sensoriels élevé (Tableau 2), tandis que le développement de l'oeil et du système de la ligne latérale sont plutôt typiques du groupe. Malheureusement, nous manquons totalement de données écologiques concrètes, illustrant l'importance spécifique du nombre d'orifices crâniens des différentes espèces, et nous ne pouvons pas avancer d' explications plausibles pour ces différences numériques. Quoi qu'il en soit, *T. zebra* illustre une fois de plus la radiation adaptative d'un groupe de Cichlidés du lac Tanganyika qui était jusqu'à présent seulement connu par des représentants vivant sur des fonds vaseux-sablonneux ou dans le milieu bathypélagique.

Distribution

Trematocara zebra est endémique du lac Tanganyika. Jusqu'à présent ce poisson n'a été récolté que dans les localités types sur la côte rocheuse du nord-ouest du lac. Contrairement aux autres *Trematocara*, tous à répartition circumlacustre, *T. zebra* présente vraisemblablement une distribution très localisée et restreinte. Certains éléments de l'ichtyofaune de cette côte nord-ouest semblent assez particuliers. En effet, à Luhanga, on trouve une population isolée de *Lamprologus leleupi* Poll, 1956 qui, suivant KONINGS & DIECKHOFF (1992), fréquente uniquement la moitié nord du lac, mais qui serait également représentée par des populations conspécifiques ou très voisines dans la partie sud (KONINGS, 1993). Le seul spécimen connu de l'espèce *Trematochromis schreyeni* Poll, 1987 provient également de Luhanga (voir POLL, 1987). En outre, à Pemba, on rencontre une population isolée de *Tropheus duboisi* Marlier, 1959. Jusqu'à présent quatre populations de cette dernière espèce seulement ont été trouvées dans le lac, les trois autres populations étant connues de la côte tanzanienne près de Kigoma, au sud du delta de la rivière Malagarasi et en face de Bulu Point (KONINGS, 1988).

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ENDOPARASITIC HELMINTHS OF THE WHITESPOTTED RABBITFISH (*SIGANUS SUTOR* (Valenciennes,1835)) OF THE KENYAN COAST: DISTRIBUTION WITHIN THE HOST POPULATION AND MICROHABITAT USE

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Summary. The parasitic fauna of the alimentary tract of adult whitespotted rabbitfish, *Siganus sutor*, sampled in December 1990 at the Kenyan coast, was investigated. Five endoparasites were found: the digenean trematodes *Opisthogonoporoides hanumanthai*, *Gyliauchen papillatus* and *Hexangium sigani*, the acanthocephalan *Sclerocollum rubrimaris* and the nematode *Procammalanus elatensis*. No uninfected fish, nor single species infections occurred. Parasite population data showed very high prevalences for all endoparasites, ranging from 68.18 % to 100 %. *G. papillatus* occurred with the highest mean intensity, 201.68 ± 12.54 parasites per infected fish. The parasites were overdispersed within their host's population and frequency distributions generally fitted the negative binomial function. The relationship between host size and parasite burden showed that smaller fish were more heavily infected. The infection with *O. hanumanthai* and *H. sigani* decreased significantly with total length of *S. sutor*. Study of the associations between parasites showed that the intensities of the three digenean species were significantly positively correlated. Possible transmission strategies of the digenea and impact of the feeding habits of *S. sutor* are discussed. Microhabitat preferences of the five endoparasites indicated a selective site segregation of all species.

Keywords: *Siganus sutor*, endoparasites, microhabitat, Indian Ocean.

INTRODUCTION

Siganidae or rabbitfish have a wide distribution in the Indo-Pacific, ranging from East-Africa to French Polynesia (WOODLAND,1983). Siganids are, together with the Lethrinidae, the most important fish in the local artisanal marine fisheries along the East African coast (NTIBA & JACCARINI, 1988). Fifty percent of the total catch is made up of rabbitfish (NZIOKA, 1984). *Siganus sutor* (Valenciennes, 1835) is the dominant species at the Kenyan coast, followed by *S. luridus* (Ruppell, 1828), *S. argenteus* (Quoy and Gaimard,1825) and *S. stellatus* Forsskål, 1775 (see NTIBA & JACCARINI, 1988). Siganids are widely recognised as potentially suitable species for mariculture (BEN-TUVIA *et al.*,

1973, LAM, 1974, HORSTMANN, 1975, LICHATOWICH & POPPER, 1975, POPPER & GUNDERMANN, 1975, VON WESTERNHAGEN & ROSENTHAL, 1976).

In view of their maricultural potential, LAM (1974) stated that "proper studies on diseases in siganids are badly needed". Most studies on parasites of marine fish in tropical regions are limited to taxonomic or zoogeographical descriptions. Ecological studies on endoparasitic helminths of fish in tropical seas and especially in the Indian Ocean are scarce. DIAMANT & PAPERNA (1986) listed the parasites of wild Red Sea rabbitfish, with special attention to the potential pathogens in mariculture. The ecology of the acanthocephalan *Sclerocollum rubrimaris* was reported in three species of rabbitfish, *Siganus rivulatus* (Forsskål, 1775), *S. argenteus* and *S. luridus* by DIAMANT (1989).

Several investigations have documented the distribution of ecto- and endoparasites of fish in the host population and their microhabitat use within their host (CROMPTON, 1973, HOLMES, 1973; 1990a, ROHDE, 1984, BATES & KENNEDY, 1991, SUKHDEO, 1990, BUCHMANN, 1991). Most of these studies present data on fish of temperate waters. The present work presents data on the distribution of five endoparasitic helminth species of *S. sutor* within the host population and on their microhabitat selection. The investigation is part of a wider research project on the parasitic fauna of *S. sutor* of the Kenyan coast. The study on ectoparasites will be presented in a separate paper (GEETS *et al.*, in prep).

MATERIAL AND METHODS

In December 1990, a sample of adult whitespotted rabbitfish *Siganus sutor* (Valenciennes, 1835) caught in the seagrass areas off the Mombasa coast, were obtained from local fishermen. Cabbage baited dematraps (traditional bottom traps) were used. They were placed in shallow water (<14 m depth) at low tide and removed at the next low tide. Upon being landed, fish were intraperitoneally injected with formaldehyde solution 7%. Total length (TL) to the nearest 0.1 cm and wet weight (WW) to the nearest 0.1 g were measured.

Twenty two specimens of *S. sutor* (24.9 ± 1.9 cm mean TL, 249.2 ± 62.1 g mean WW) were examined for endoparasites. The alimentary tract was divided into stomach and intestine. From the pyloric sphincter up to the anus, the intestine was cut into four equal parts and parasite load was counted for each section. For identification purposes, additional live parasite specimens were obtained from *S. sutor* from the same area and immediately fixed using the methods described by BERLAND (1984) and PRITCHARD & KRUSE (1982). The acanthocephalan was identified using the the description of SCHMIDT & PAPERNA (1978).

The terms prevalence, intensity, mean intensity and abundance were used as indicated by MARGOLIS *et al.* (1982).

The frequency distributions of the parasites were compared with the theoretical Poisson and negative binomial functions. The number of frequency classes was determined using the following equation $Q = 1 + 3.3 \log N$, with Q the number of classes and N the maximal number of a particular parasite recorded (ROBERT *et al.*,1990). The expected values for the Poisson series were calculated in Lotus 123R4 for Windows with the @POISSON function, those for the negative binomial function were calculated using

PROBNEGB in SAS version 6.08. The k values were calculated with the maximum likelihood equation (ELLIOTT, 1977). Goodness of fit was tested using the chi square test. Variance to mean ratio as well as k values were used as indices of dispersion. Variance to mean ratio will approximate unity if there is an agreement with a Poisson series. If variance to mean ratio is greater than 1, a contagious or aggregated distribution is suspected. The H_0 hypothesis that parasite species (total number) were equally distributed over the four different areas of the intestine was tested using G and chi square tests (SOKAL & ROHLF, 1987; 1995). Analysis of correlations of occurrence of two parasite species was done using Spearman's rank correlation coefficient r_s since data on intensities of infection were not normally distributed. Statistica software (release 4.1) was used for most statistical analysis.

RESULTS

Five endoparasite species were found in the alimentary tract of *Siganus sutor*: the digenean trematodes *Opisthogonoporoides hanumanthai* Madhavi, 1971, *Gyliauchen papillatus* (Goto & Matsudaira, 1918), *Hexangium sigani* Goto & Okazi, 1929, the acanthocephalan *Sclerocollum rubrimaris* Schmidt & Paperna, 1978 and the nematode *Procammalanus elatensis* Fusco & Overstreet, 1979.

In Table 1 the quantitative data on the five endoparasites are given. Prevalence was very high for all species, ranging between 68.2 % for *H. sigani* up to 100 % for *G. papillatus*. The digenean *G. papillatus* reached the highest mean intensity, 201.68 ± 12.54 parasites per infected fish, the acanthocephalan *S. rubrimaris* was the least common with a mean intensity of 2.72 ± 0.12. *G. papillatus* accounted for 52.2 % of the total of 8496 parasites recovered from the 22 rabbitfish. Of all fish examined, 54.5 % were infected with all five endoparasites. Neither uninfected fish, nor single-species infections were recorded (Table 2).

TABLE 1

Quantitative data on the endoparasites of Siganus sutor

Parasite species	Total number of parasites (%)	Maximum intensity	Prevalence (%)	Mean intensity ± s.d.	Abundance ± s.d.
O. hanumanthai	3254 (38.3)	691	81.8	180.77±11.01	147.91±8.73
G. papillatus	4437 (52.2)	1317	100.0	201.68±12.54	201.68±2.54
H. sigani	317 (3.7)	125	68.2	21.13±2.27	14.41±1.35
S. rubrimaris	49 (0.01)	9	81.8	2.72±0.12	2.22±0.10
P. elatensis	439 (5.2)	53	90.9	21.95±0.64	19.95±0.63

TABLE 2

Relative proportion (%) of Siganus sutor *infected with 0 to 5 endoparasite species. (Total number of fish examined = 22)*

Number of parasite species	Percentage *S. sutor* infected
0	0
1	0
2	9.1
3	13.6
4	22.7
5	54.5

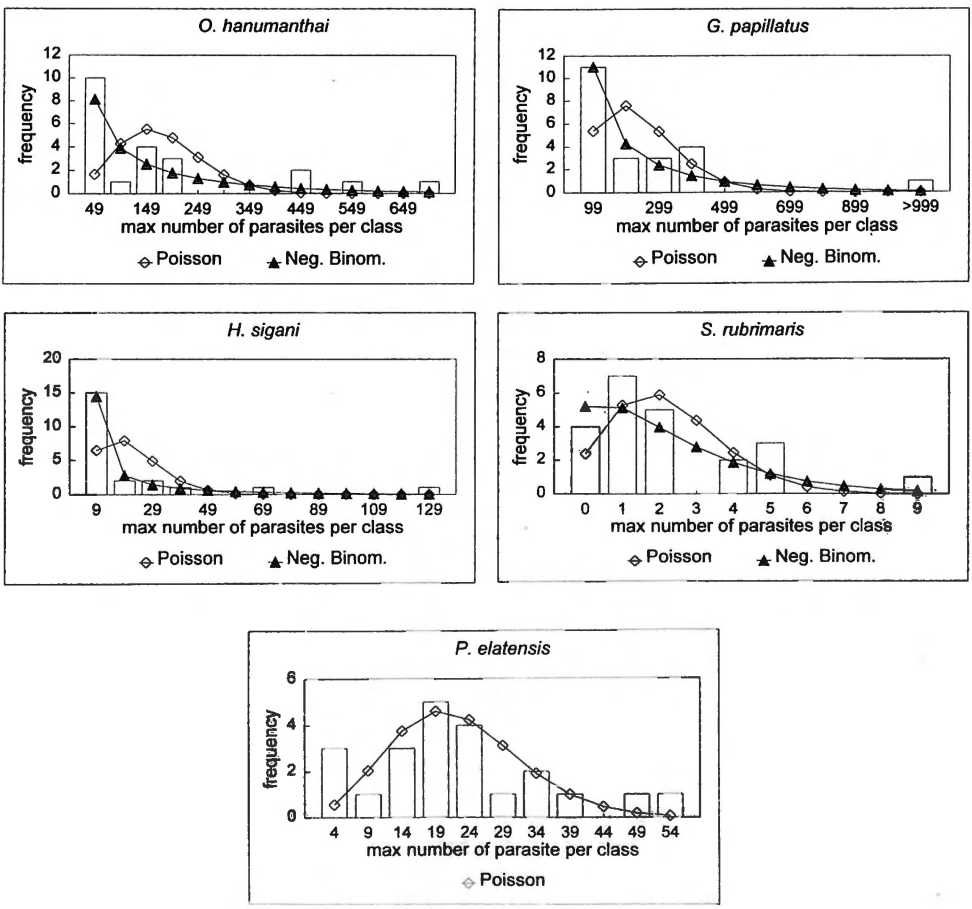


Fig. 1. – Frequency distribution of five endoparasite species of *Siganus sutor* with fitting of Poisson and negative binomial distribution.

The distribution of the endoparasites in the host population is shown in Fig. 1. Variance (s^2) to mean (m) ratio and k-values (Table 3) are both used as indices of dispersion: $s^2/m > 1$ and 1/k are both measures for clumping of the individuals in the host population. All five endoparasite species were overdispersed (aggregated) throughout the rabbitfish population. *O. hanumanthai* and *G. papillatus* showed a very high aggregation in the host's population. A few hosts carried an enormous amount of parasites, eg. the maximum intensity of infection in one host was 1317 for *G. papillatus* and 691 for *O. hanumanthai*.

TABLE 3

Indices of dispersion, s^2/m and k-value, for the five endoparasite species of Siganus sutor

Parasite species	s^2/m	k-value
O. hanumanthai	249.58	0.59
G. papillatus	377.64	0.53
H. sigani	52.31	0.28
S. rubrimaris	2.26	0.74
P. elatensis	9.40	2.36

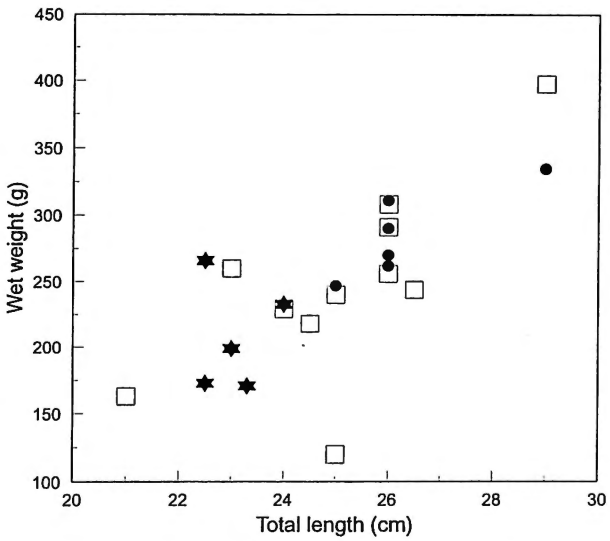
Poisson series and negative binomial distribution were fitted to the frequency distributions. A goodness of fit chi square test showed that none of the parasites were randomly distributed in the host population ($P<0.001$ for the Poisson series). Almost all endoparasite distributions could be described by the negative binomial distribution ($P>0.05$), except for the distribution of the nematode *P. elatensis*. Although an aggregated distribution was expected from the high variance to mean ratio for *P. elatensis*, the negative binomial distribution was not a good theoretical function to describe this frequency distribution.

A Spearman 's rank correlation (Table 4) between total length and number of parasites of each species showed significant negative correlations for *O. hanumanthai* and *H. sigani*. The total parasite load was also significantly negatively correlated with the size of the host. Of the total number of parasites found, *O. hanumanthai* accounted for 38.3 % and *H. sigani* for 3.7 %, so this could partly explain the negative correlation of the total number of parasites with the total length. But, on the other hand, *G. papillatus*, which accounts for 52.2 % of all the parasites, did not show a significant negative correlation between intensity of infection and host size. The relation between total length and wet weight of the fish with an indication of the six least parasitized specimens (total number of parasites < 100) and of the five most heavily parasitized hosts (total number of parasites > 600) is shown in Fig. 2. Mean weight and mean total length were considerably lower for the heavily parasitized siganids.

TABLE 4

Spearman's Rank Correlation (r_s) between total length (TL) of Siganus sutor *and intensities of infection. Significant correlations are indicated in bold*

Pair of variables	r_s	p-level
TL-*O. hanumanthai*	**-0.74**	**<0.001**
TL-*G. papillatus*	-0.12	>0.05
TL-*H. sigani*	**-0.69**	**<0.001**
TL-*S. rubrimaris*	-0.27	>0.05
TL-*P. elatensis*	-0.37	>0.05
TL-*total n of parasites*	**-0.53**	**<0.05**



	< 100 parasites ●	> 600 parasites ★
Mean total length ±SD (cm)	26.3 ±1.4	23.1 ±0.6
Mean wet weight ±SD (g)	285.7 ±32.5	208.4 ±40.8

Fig.2.–Relation between total length and wet weight of *Siganus sutor* with indication of the least parasitized individuals (solid circle) (< 100 parasites / fish) and the heaviest parasitized fish (star) (>600 parasites / fish). Mean total length and mean wet weight and their standard deviations are indicated in the accompaning table and are significantly different for both groups (T-test, P<0.001).

The interrelationship between parasites is demonstrated in Table 5 by the Spearman 's rank correlation coefficients (r_s). For this comparison, only rabbitfish containing at least one of the two parasite species involved, were included. All three digenean parasites showed significant positive correlations with each other. Thus, there seems to be an asso-

ciation between the numbers of digenean parasites found in *S. sutor*. No correlation between the numbers of the acanthocephalan *S. rubrimaris* or the nematode *P. elatensis* and the other parasite species could be found.

TABLE 5

Correlation between intensities of infection of five endoparasites of Siganus sutor *(r_s = Spearman's rank correlation coefficient). Significant correlations are indicated in bold.*

Number of pairs r_s Probability	*O. hanumanthai*	*G. papillatus*	*H. sigani*	*S. rubrimaris*
G. papillatus	22 **0.437** **< 0.05**			
H. sigani	18 **0.691** **< 0.01**	22 **0.633** **< 0.01**		
S. rubrimaris	22 0.147 > 0.05	22 -0.154 > 0.05	21 -0.33 > 0.05	
P. elatensis	21 0.328 > 0.05	22 0.313 > 0.05	20 0.382 >0.05	22 0.011 > 0.05

Microhabitat preferences of the five parasite species in the alimentary tract of *S. sutor* are shown in the histograms in Fig. 3. The stomach of *S. sutor* harboured no parasites. The H_0 hypotheses for the chi square as well as for the G test stated that the total number of parasites of each species was equally distributed over the four (equal) parts of the intestine. Both tests rejected this hypothesis ($P<0.001$). The distribution of each of the parasite species was not equal over the intestine but showed a distinct pattern of preference.

The three digenean species preferred the two most posterior parts, I3 and I4, but each species showed a distinct microhabitat preference within this posterior region. *O. hanumanthai* was found in the I3 region in 71.5 % of the cases, *G. papillatus* showed a preference for the I4 region (95.4 %) and *H. sigani* was distributed over both areas but with a slight preference for the I4 region (41.6 % in I3, 58.4 % in I4).

The acanthocephalan *S. rubrimaris* attached most frequently (65.3 %) to the first quarter of the intestine (I1), with decreasing numbers towards the rectum. *P. elatensis*, the nematode, seemed the least site specific of the five species. It was abundant in the first three quarters of the intestinal tract, but rarely occurred in the most posterior part (4.8 %). Each of the four intestinal sectors was characterized by the abundant presence of different parasite species: I1 by *S. rubrimaris*, I2 by *P. elatensis*, I3 by *O. hanumanthai* and I4 by *G. papillatus*, which suggests a spatial niche segregation of the species.

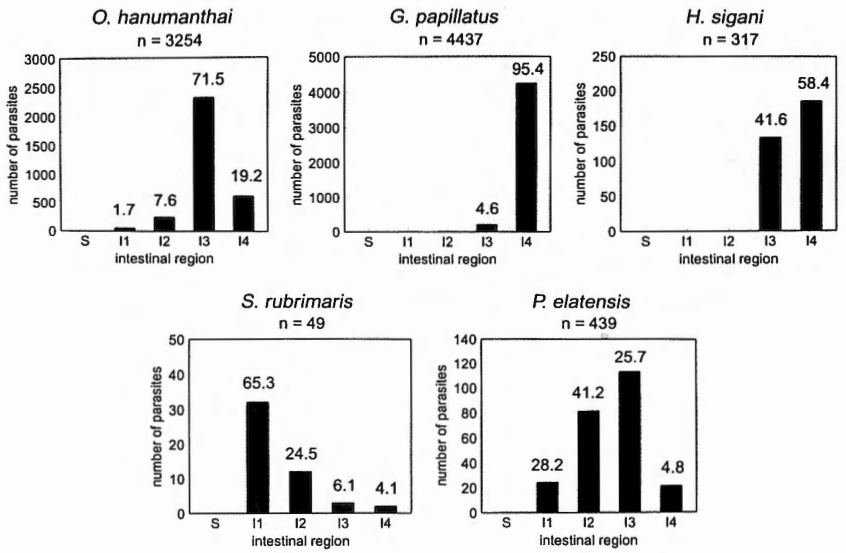


Fig. 3. – Distribution of five helminth species along the alimentary tract of *Siganus sutor*. Relative proportion (%) of the total number of parasites are given on top of each bar. (S = stomach, I1-I4 = four sections of the intestine from pyloric sphincter to anus.).

DISCUSSION

Siganids are herbivorous fish which incidentally take in invertebrate food items (LAM, 1974, SUYEHIRO, 1942). *Siganus sutor* lives in schools on reef flats and seagrass beds. Although herbivorous fish are generally considered to harbour less intestinal parasites than carnivorous or omnivorous fish, this is certainly not the case for *S. sutor*, considering the high prevalences and mean intensities of parasites found in our study.

Up to now, most parasitological studies on siganids were carried out on species of the Red Sea (DIAMANT & PAPERNA, 1986), the Philippines (JONES & HINE, 1983) and the Seychelles (TOMAN, 1977; 1989). DIAMANT & PAPERNA (1986) list thirty five parasite species (12 ecto- and 23 endoparasites) on three rabbitfish species, *Siganus argenteus*, *S. luridus* and *S. rivulatus* (Forsskål, 1775) of the Red Sea. All five endoparasites considered in our study were also collected on the siganid species investigated by DIAMANT & PAPERNA (1986), except for *H. sigani* on *S. luridus* and *P. elatensis* on *S. argenteus*. Infection levels were marked by DIAMANT & PAPERNA (1986) on a nominal scale from "not found" to "rare", "fairly common" and "abundant". Heavy infections with *O. hanumanthai* and *Gyliauchen papillatus* were not noted in the Red Sea siganids. *H. sigani* and *G. papillatus* were also found in *Siganus* spp. (probably *S. oramin* and *S. fluvescens* resp.) from the Seychelles (TOMAN, 1977; 1989).

The mean intensities of digenean trematodes found in *S. sutor* are remarkably high. Stomach analysis only occasionally revealed remains of small crustaceans and gastropods, which could have served as intermediate hosts (pers. obs.). VON WESTERNHAGEN (1973) also reported small invertebrates to occur as part of the food of *S. oramin* and *S. striolata.* The life cycles of the three digenean species, *O. hanumanthai*, *G. papillatus* and *H. siganni* have not yet been elucidated. Since *S. sutor* does not feed selectively on invertebrate prey, infection probably occurs by incidental ingestion of either encysted metacercariae on the plant material or small epiphytic invertebrates which are intermediate hosts. For digenean-dominated communities, (such as in *S. sutor*), the molluscan fauna determines which lifecycles will be possible in the ecosystem (KØIE, 1983, ESCH *et al.*, 1990). Those potential intermediate host species found in the stomach of *S. sutor* could be examined for metacercariae as a first step to elucidating some of the life cycles of the digeneans found as adults in the whitespotted rabbitfish.

The acanthocephalan *Sclerocollum rubrimaris* was described by SCHMIDT & PAPERNA in 1978 from *Siganus rivulatus* and *S. rostratus*. DIAMANT (1989) studied the infection of the species in *S. argenteus*, *S. rivulatus* and *S. luridus*. Although the prevalence of *S. rubrimaris* in *S. sutor* (81.8 %) was higher than in any of the three *Siganus* species investigated by DIAMANT (1989), the mean intensity noted in *S. sutor* was lower (2.72 ± 0.12). DIAMANT (1989) also suggested that the infection route would be by accidental ingestion of the infected intermediate host (mostly crustaceans).

The nematode *Procamallanus elatensis*, previously recorded in *S. luridus* and *S. rivulatus* from the Gulf of Eilat (FUSCO & OVERSTREET, 1979) is also presumably acquired through the ingestion of infective larvae in crustaceans.

All five endoparasites displayed an aggregated distribution in the host population. CROFTON (1971) pointed out that the infection process of parasites tends to lead to an overdispersed distribution, with heavily infected hosts often killed by the parasites. An overdispersed distribution ensures that the infection is kept at a low level with only a few hosts becoming heavily infected. In fact, the parasite species acts as a regulator of the host population, the degree of the regulatory function being related to both host and parasite population densities. The dynamic equilibrium of host and parasite populations is essential to the continuous association between both. DIAMANT (1989) found an overdispersion of *Sclerocollum rubrimaris* in both *Siganus rivulatus* and *S. argenteus* populations and the frequency distribution could be fitted to the negative binomial function. Several other examples of overdispersed trematode distribution patterns have been recorded (CANNON, 1972, WANSTALL *et al.*, 1990).

Although an overdispersed distribution is noted, the negative binomial is not always the right theoretical function to be fitted. HINE & KENNEDY (1974) noticed this for *Pomphorhynchus laevis* in dace (*Leuciscus leuciscus*). In our study, the frequency distribution of the nematode *P. elatensis*, although clumped according to the variance to mean ratio, could not be described by the negative binomial function. The distribution seemed to be bimodal.

Changes in the abundance of parasites with host size is a well known phenomenon. DIAMANT (1989) noted increasing numbers of *Sclerocollum rubrimaris* with total length of *Siganus rivulatus*, but a decreasing trend in *S. argenteus*. He suggested that the acantho-

cephalan infection is associated with feeding on deeper, adjoining algal patches where red sea weeds are abundant. *S. rubrimaris* infection showed a slight, but not significant, negative correlation with the size of *S. sutor*. In a study on the incidence of trematode parasites of long rough dab (= American plaice) (*Hippoglossoides platessoides*) in relation to fish length, SCOTT (1975) pointed out that changes in the fish 's diet were the main cause for the observed differences (increasing as well as decreasing numbers of parasites with increasing host size). CANNON (1972) came to the same conclusions for intestinal trematodes of perch (*Perca flavescens*). The observed negative correlations between the intensities of the digeneans *O. hanumanthai* and *H. sigani* and total length of *S. sutor* might be caused by a change in the fish's diet, but a preliminary analysis of the food content of the stomachs did not show remarkable differences between smaller or bigger specimens (pers.obs.). DIAMANT (1989) relates the increasing spring abundance of *S. rubrimaris* with the accelerated feeding rates of *S. rivulatus* and *S. argenteus* during gonadal development and maturation. In our study, all *S. sutor* which were screened for parasites were adult and ready for spawning in January - February (NTIBA & JACCARINI, 1990). Migration movements associated with spawning behaviour and possible temporal changes in feeding habits of the adult fish have not yet been investigated for *S. sutor*, but they may provide valuable information to explain the observed differences in parasite load.

Mass infections of parasites can negatively influence the host condition and growth (ROHDE, 1984). This may provide an alternative explanation for the observed differences in length and weight of lightly and heavily parasitized rabbitfish. The high infections of *S. sutor* with both endo- and ectoparasites (Monogenea, Isopoda, Copepoda) (GEETS, in prep.) and the lack of knowledge on their life cycles and impact on their hosts, make the interpretation and detection of causative agents for this phenomenon impossible at this stage.

Analysis of interspecific relationships between the five endoparasites demonstrated that only the three digenean species significantly occurred together and that their infection intensities were positively correlated. Positive associations were found between a number of intestinal helminths in brown trout (*Salmo trutta*) (THOMAS, 1964). HINE & FRANCIS (1980) studied interspecific associations between three digeneans and two nematodes of New Zealand freshwater eels (*Anguilla dieffenbachii* and *A. australis*) and attributed the positive association between two of the digeneans to their use of the same intermediate host. To explain positive associations, THOMAS (1964) suggested two hypotheses. The association could be of mutual benefit for both parasites, or the observed association could be the result of ecological factors outside the host. HINE & FRANCIS (1980) stated that positive associations are unusual and that they rather result from the distribution of the infectious larval stages of the helminths in relation to feeding habits of the host than from any cooperation between the helminths. The importance of the intermediate host was also emphasized by SOUSA (1994). Helminth parasites which share the same intermediate host will often co-occur in individual vertebrate hosts since they are acquired simultaneously when the final host feeds on this intermediate host.

The correlated response of different parasites to certain heterogenous qualities of the host eg. age, sex, host size, was also suggested to result in positive associations of parasites. For *O. hanumanthai* and *H. sigani* infections in *S. sutor*, this last assumption could be

true. Intensities of infection of both species are negatively correlated with host size. Hence, their apparent interspecific interaction might be a result of independent negative correlation with host size. Since *G. papillatus* infections are independent of host size (within the examined range), the positive association between *O. hanumanthai* or *H. sigani* and *G. papillatus* is not explained by this hypothesis. It is striking, however, that positive associations were only found between the three digenean species and no associations involved the acanthocephalan or the nematode species. A detailed study of *S. sutor*'s feeding habits would probably provide a suitable explanation.

The relationship between interspecific interaction and site selection by parasites has been widely discussed (HOLMES, 1973; 1990b). Assemblages of parasites consist of varying numbers of potentially interacting species (SOUSA, 1994). BUSH & HOLMES (1986) defined a parasite infracommunity as being all parasites species in a single host individual, and the component community (ESCH *et al.*,1990, SOUSA, 1994) as all parasite species in a population of a certain host species. Parasite infracommunities are classified as interactive or isolationist (HOLMES & PRICE, 1986). Most infracommunities will be situated somewhere in between these two extremes. The parasite infracommunity of *Siganus sutor* tends to be more interactive than isolationist. Characteristics are: high colonization rates, high average densities of parasite infrapopulations and high species diversities (SOUSA, 1994). In an interactive parasite infracommunity, as in *S. sutor*, frequent interspecific interactions amongst the parasites will occur, which will eventually lead to a reduced spatial overlap and a tendency towards site specificity. Site selection is an active and continuing process on the part of the parasite (HOLMES, 1973). The presence of other parasites may modify the microhabitat selected by a particular species. It is a generally accepted rule in ecology that species which coexist in the same habitat, at the same time, will either compete or interact to specialize and hence segregate their niches. HOLMES (1973) hypothesized that interactive segregation, in which the realized niche of a species is reduced by the presence of another species, would gradually be replaced by (genetically based) selective segregation, in which the realized microhabitat does not change in the presence or absence of another species.

When we consider the microhabitat uses of the three digenean parasites found in the intestinal tract of *S. sutor*, more evidence is found for selective than for interactive site segregation. Since no single species infections were recorded in present study, possible shifts in microhabitat use could only be compared in fish with high or low intensities of infection of a certain parasite. *G. papillatus*, in 95 % of the cases found in the last quarter of the intestine (I4), did not change its distribution when it was the only digenean species present (observed in 4 fish). When infections of *G. papillatus* were low and outnumbered by *O. hanumanthai* or by *H. sigani*, no changes in the distribution along the intestine were observed for the last two species, which again is an indication of selective site segregation.

Site segregation along the intestine of fish is well documented (MACKENZIE & GIBSON, 1970, CANNON, 1972, MÖLLER, 1974, HINE, 1980a; 1980b, KENNEDY & MORIARTY, 1987, DIAMANT, 1989, WANSTALL *et al.*,1990, BUCHMANN, 1991). Our observations on the overall distribution of the five endoparasites of *S. sutor* along the intestinal tract, coincide with

the distributions indicated by DIAMANT & PAPERNA (1986) for the same endoparasites in three other siganid species of the Red Sea.

CROMPTON (1973) stated that the distribution of helminths in the intestine of vertebrates is related to the different conditions in different parts of the tract, to the effect of digestion and to the nature of the diet. Digenean parasites feed mainly on mucosa and epithelial tissue. Therefore, they will be browsing in the intestine in places where the epithelial turnover rate is high (CROMPTON, 1973). The fact that the three digenean parasites in our study were most frequently found in the posterior part of the gut (I3 and I4) could support this hypothesis. HALTON & ARME (1971) pointed out that body surfaces of trematodes are fragile. The intestinal wall of I3 and I4 of *S. sutor* is characterized by dense villi which could act as a protection for the digeneans. When a mixed parasite population is present, the relative size of the parasite bodies is also of importance (CANNON, 1972). Closely related sympatric parasite species (or ecological equivalents) were generally separated by at least a 10 % linear measurement and the smaller species was a more specialized feeder (KEAST, 1968, *fide* CANNON,1972). *O. hanumanthai* is the smallest of the three digeneans, followed by *G. papillatus* and *H. sigani* is the largest (pers.obs.). Both smaller species have a more restricted (specialized?) microhabitat than *H. sigani*. Furthermore, it was noted that the villi in the I3 part were much smaller than in the I4 part. *G. papillatus* (generally found in I4) was immediately noticed when it was present in I3, since its body partly projected into the lumen. *O. hanumanthai* specimens were more frequently found completely enclosed between the small villi of the third part of the intestine.

The microhabitat of the acanthocephalan *Sclerocollum rubrimaris* in *Siganus sutor*, coincides with the distribution reported for the same species in *S. rivulatus* and *S. argenteus*, namely the anterior 5-25 % of the gut (DIAMANT, 1989). Acanthocephalans feed by absorbing nutrient molecules through the trunk. CROMPTON (1973) suggests that the attachment sites of adult acanthocephalans are therefore limited to regions of the intestine which are specialized for the absorption of nutrients.

Nematoda are equipped with an alimentary canal and would thus be more free to roam around the intestine. *Procamallanus elatensis* also seemed to be the least site specific of the five endoparasites of *S. sutor*. Nevertheless, certain sites seem to be preferred by certain nematode species. HINE (1980b) described the distribution of three nematode species in two New Zealand freshwater eels. He noted that *Paraquimpera sp.* and *Spirocamallanus sp.* were mostly found in the anterior or mid intestine, depending on the host species. *Cucullanus sp.* occurred more throughout the intestine. Migrations of this nematode with food was a possible explanation.

SOMMERVILLE (1963, *fide* HOLMES, 1973) stated that "each species of nematode tends to be located about the region of greatest abundance as a normal frequency distribution." This can also be noticed for the distribution of *P. elatensis* in the gut of *S. sutor* (Fig. 3).

The site segregation of the endoparasites of *S. sutor* described in the present study, is probably not the only mechanism of niche restriction involved for the parasites. Since only adult fish of one sampling occasion were investigated, temporal segregation for instance could not be studied. Infection with different parasites at different times of the year was demonstrated in previous studies to be a potential mechanism to avoid competition. Parasites may also utilise different age classes of the host, which is a special kind of tem-

poral niche segregation, or may show a reproductive segregation in time. Furthermore, spatial segregation, as described here within the intestine of a host, is not the only aspect of differential use of 'space'. Geographical segregation (not discussed here) is an other mechanism to avoid niche overlap.

Deeper understanding of the observed infection patterns of *Siganus sutor* endoparasites will have to await studies on the ecology of the fish and elucidation of the life cycles of its parasites.

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THREE ECHINODERM INHABITING FLATWORMS (PLATYHELMINTHES, RHABDOCOELA) FROM WESTERN AUSTRALIA

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Summary. Three new species of flatworms symbiotic in echinoderms from Western Australia are described. *Pterastericola rottnestensis* n.sp. belongs to the Pterastericolidae. *Syndesmis cannoni* n. sp. and *Wahlia westbladi* n. sp. are members of the Umagillidae.

Key words: *Ammotrophus*, *Astropecten,* Echinodermata, Platyhelminthes, *Pterastericola*, Pterastericolidae, Rhabdocoela, *Stichopus*, symbionts, *Syndesmis*, Umagillidae, *Wahlia*, Western Australia

INTRODUCTION

The rhabdocoel flatworms of the Pterastericolidae and Umagillidae are endosymbionts of echinoderms. Eastern Australian representatives of the two groups were treated by CANNON in numerous papers (1974, 1978, 1982, 1986, 1987 and 1990). To-date there are no Australian records of the groups outside Queensland and New South Wales.

The present paper reports the first new species of the Umagillidae and Pterastercolidae (Platyhelminthes, Rhabdocoela) occurring on the Australian west coast.

MATERIAL AND METHODS

Specimens of host echinoderms were collected by SCUBA diving (*Astropecten preissi* Müller and Troschel, 1843, *Stichopus mollis*, (Hutton, 1872)) or dredging (*Ammotrophus arachnoides* H.L. Clark, 1938) off Rottnest Island, Western Australia in January 1991. The echinoderms were dissected, the intestine and the rest of the body placed in separate dishes and inspected for flatworms with the aid of a dissecting microscope. Flatworms were studied live under a compound microscope. Fixation of worms for serial sections was in Bouin's fluid. Sections were cut at 5-6 μm and stained with hematoxylin-eosin or Mallory's trichrome stain.

RESULTS

Pterastericola rottnestensis n.sp.

Type material

Holotype (SMNH 4841) one longitudinally sectioned specimen from oesophagus of *Astropecten preissi* from sandy bottom off Rottnest Island, Western Australia, January 1991. Paratypes, 8 longitudinally and cross sectioned specimens, from oesophagus and stomach of host, same data as holotype. The worm was present in all five specimens of *A. preissi* dissected. A total of 29 specimens were found. This is the species referred to as *Pterastericola* sp Rottnest by JONDELIUS (1992a; 1992b).

Description

Body broad, rounded and flattened anteriorly, tapering posteriorly, unpigmented. Length of live specimens 1.0 mm (0.8 - 1.2 mm, n = 6).

Pterastericola with tripartite vitellarium, two anteriorly and one posteriorly directed branches (Figs 1 A, 2 E). Single egg capsule in ootype. Gonopore immediately posterior to pharynx, somewhat left of body midline.

Epidermis uniformly ciliated, cilia about 5 µm long. Numerous eosinophilic gland necks penetrate epidermal cells on the ventral anterior surface (Fig. 2A, B). Mouth opening to anterior ventral surface, leading to anteriorly directed sub-spherical pharynx, which is 55 - 60 µm long (n = 5), 52 - 60 µm wide (n = 4) and 68- 72 µm high (n = 3). Brain immediately anterior to pharynx. Intestine sac-like, dorsal, extending posteriorly from pharynx.

Testis a thin-walled sac extending posteriorly from copulatory bulb. copulatory bulb bean shaped 65 - 78 µm long and 25 - 38 µm wide (n = 5), divided into two compartments holding seminal and prostatic vesicles (Fig. 2 D). Male armature consisting of stylet and accessory piece which is blunt and much shorter than the stylet (Fig. 1 B, C). Muscular male antrum joins ciliated common genital atrium, which reaches ventral body surface at common gonopore.

Single ovary on left side anterior to testis, posterior to gonopore, anteriorly with larger, mature oocytes (Fig. 2 C). Oviduct anteriorly joined first by seminal receptacle and then vitelline ducts and shell glands. No pseudovagina was observed. Egg capsules were only observed in the ootype; none were seen in the parenchyma.

Comments and differential diagnosis

Tripartite vitellaria with two anteriorly directed branches occur in *P. sinensis*, Jondelius 1992 a species that, like *P. rottnestensis,* occurs in the oesophagus and cardiac stomach of an asteroid of the genus *Astropecten.* However, in *P. sinensis* mouth and gonopore are widely separated.

The male armature of *Pterastericola rottnestensis* differs from other known species of the genus since the accessory piece is much shorter than the stylet, and blunt-ended.

Etymology

The species name is derived from the type locality.

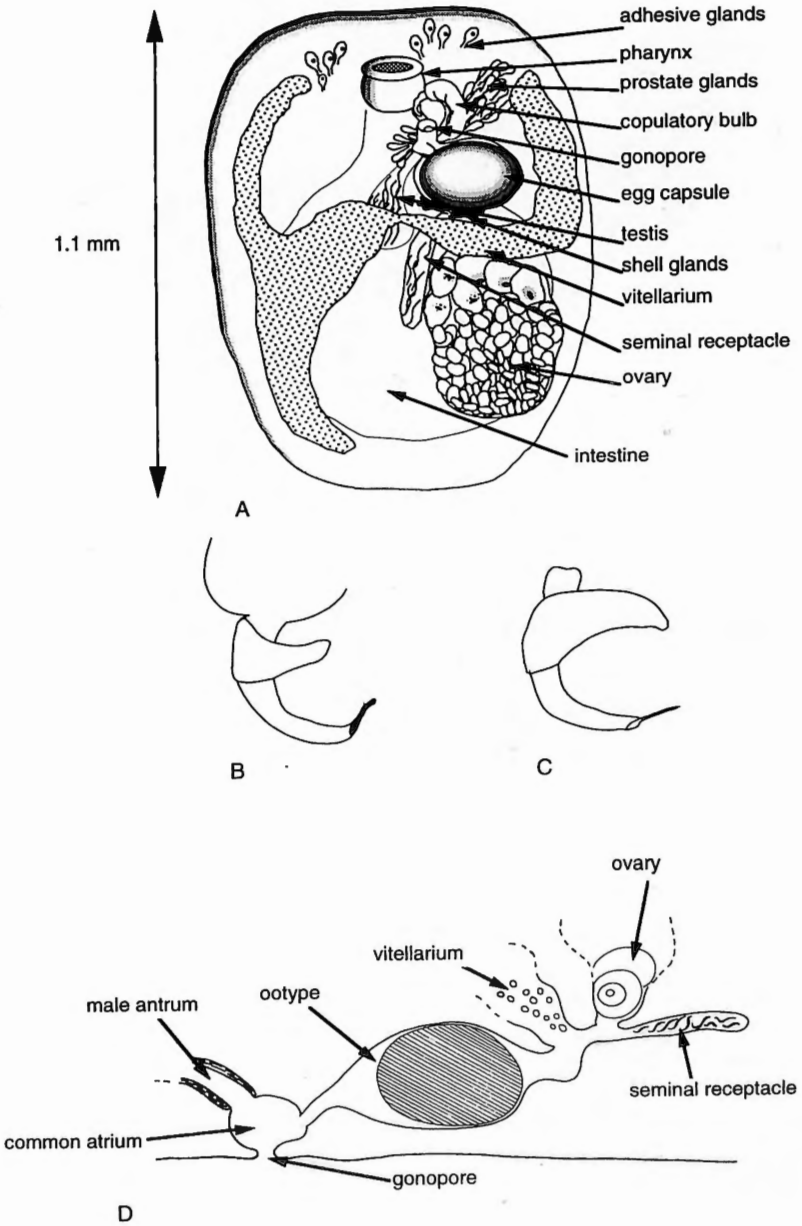

Fig. 1. – *Pterastericola rottnestensis* n.sp. – A. ventral view of general anatomy; reconstruction from live animals and serial sections – B, C. copulatory stylets drawn from life – D. diagram of genital system in lateral view; reconstruction from serial sections.

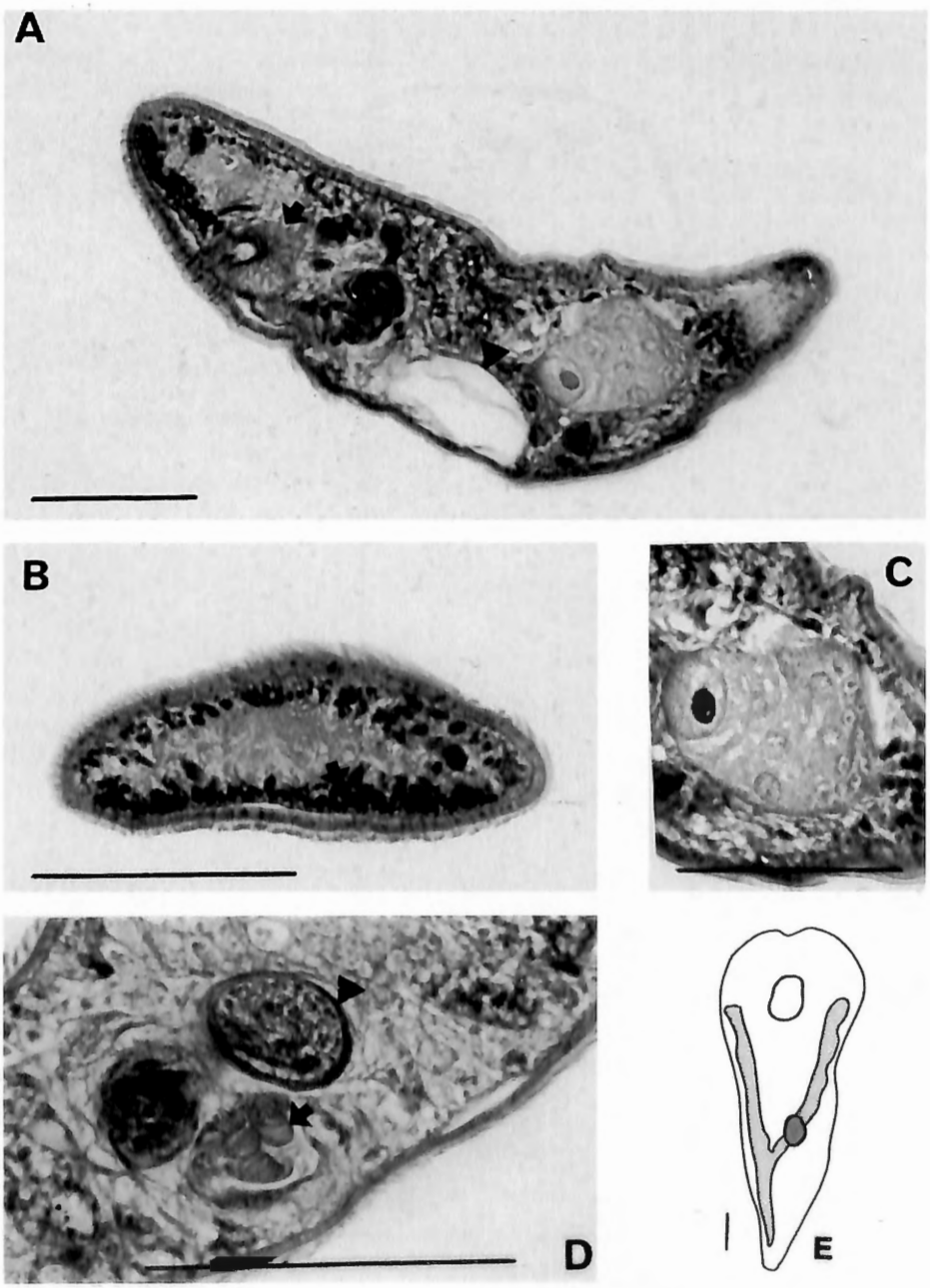

Fig. 2. – *Pterastericola rottnestensis* n.sp. – A. median longitudinal section showing pharynx (arrow), uterus (arrowhead) and ovary – B. cross section of anterior part at the level of the brain showing eosionophilous adhesive glands (arrow) penetrating the ventral epidermis – C. horizontal section of ovary with mature oocytes anterior – D. cross section showing male antrum (arrow), prostate glands and copulatory bulb (arrowhead) – E. free hand drawing of the habitus of live worm. (A-D photomicrographs from serial sections; scale bars: 100 μm).

Syndesmis cannoni n. sp.

Type material

Holotype (SMNH 4815) one longitudinally sectioned specimen from intestine of *Ammotrophus arachnoides* from sandy bottom off Rottnest Island, Western Australia, January 1991. Paratypes, 5 serially sectioned specimens, same data as holotype.

The worm was present in all five specimens of *A. arachnoides* dissected with a total of 23 specimens found.

Description

Anteriorly and posteriorly blunt-ended, 0.9 - 1.2 mm long (live specimens), red-pigmented *Syndesmis* with short stylet wholly within male antrum. Ejaculatory duct without coils (Figs 3 A, C).

Lateral edges of body curved towards the ventral surface (Fig. 4 C). Dorsal epidermis densely ciliated, cells 7 - 11 µm high, cilia 3-4 µm long. Ventral epidermis 3-4 µm high with sparse, 5-7 µm long cilia. Ventral surface medially with clusters of club-shaped cells that protrude 9-12 µm (n=4) (Figs 4 A, C).

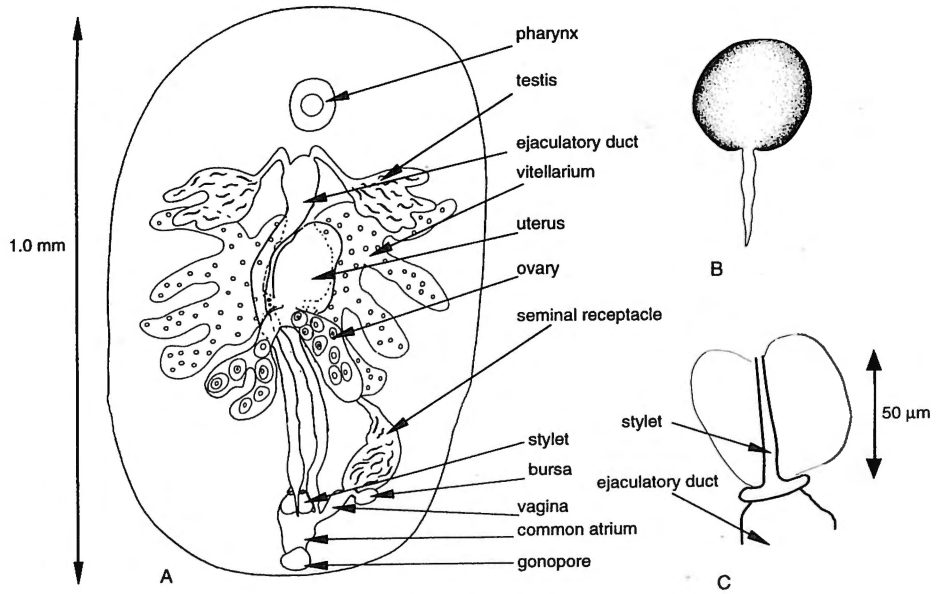

Fig. 3. — *Syndesmis cannoni* n.sp. - A. Dorsal view of general anatomy. Reconstruction from live animals and serial sections.- B. Egg capsule. - C. Camera lucida drawing of stylet surrounded by lobes of pellucid (glandular?) tissue.

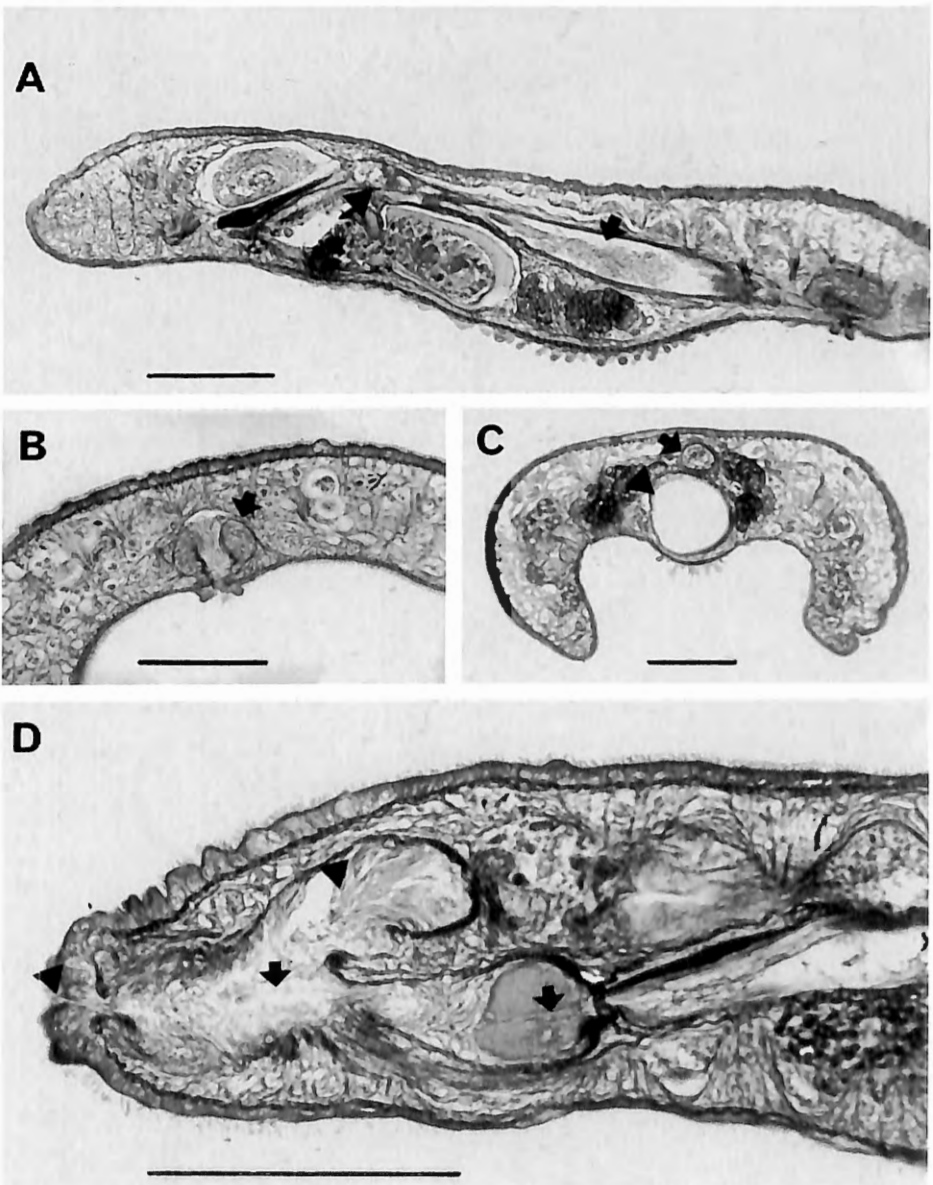

Fig. 4. — *Syndesmis cannoni* n.sp.: photomicrographs from serial sections - A. median longitudinal section showing ejaculatory duct (arrow), uterus (arrowhead) with egg capsule and vitellarium; note papillate epidermal cells ventrally and pharynx anterior to ejaculatory duct - B. cross section showing pharynx (arrow) and dorsoventral musculature - C. cross section showing ejaculatory duct (arrow), uterus (arrowhead) and vitellaria, note curved lateral parts of body and papillate epidermal cells ventrally - D. longitudinal section of posterior part, showing stylet (arrow) connected to walls of male antrum, common atrium (arrow), gonopore and vagina (arrowhead). (Scale bars: 100 μm).

Mouth opening to ventral surface one-fifth of the body length from the anterior end. Ventrally directed pharynx, sub-spherical 40-50 µm high, 65-82 µm wide (n=4) (Figs 4 A, B). Saclike, dorsal intestine extending posteriorly from pharynx.

Paired testes on both sides of body midline behind pharynx reach vitellaria posteriorly. Sperm ducts extend anteriorly, turn posteriorly and join common ejaculatory duct. There is no seminal vesicle outside the 600-700 µm long ejaculatory duct, which has no coils, but is sheathed by a 3-5 µm layer of circular muscles. Lumen of ejaculatory duct conspicuous, filled with sperm (Figs 4 A, C, D).

The male antrum has a folded lining (Fig. 4D). Sclerotized straight penis stylet 47-50 µm long (n=4), with proximal collar, positioned in male antrum and attached to the antrum walls through lobes of pellucid (possibly glandular) tissue (Figs 3A and C).

Vitellaria posterior to testes with 4-6 main branches with distal lobes enter uterus/ootype at about half body length. Paired ovaries posterodorsal to vitellaria, each with two lobes. Ovaries enter uterus/ootype posterodorsally to vitellaria. Uterus ventral to ejaculatory duct occupies a large proportion of body cross section when containing an egg capsule (Figs 3AD and 4C). Seminal receptacle enters uterus/ootype posterodorsally, posteriorly tapering to short sclerotized duct entering copulatory bursa. Distally to the bursa a prominent vagina enters the common atrium (Fig. 4 D). Common gonopore postero-terminal.

Comments and differential diagnosis

The short stylet distiguishes *S. cannoni* from seventeen of the previously described species of *Syndesmis* and *Syndisyrinx* (see MOENS *et al.* (1994) for a complete list of species and authors). The remaining species *S. aethopharynx* Westervelt and Kozloff, 1990 has a short stylet, but egg capsules are anterior and the pharynx is club-shaped.

The distinction between the genera *Syndesmis* Silliman and *Syndisyrinx* Lehman seems arbitrary. Presence of a bursal valve was regarded by LEHMAN (1946) and CANNON (1982) as the distinguishing feature of *Syndisyrinx*. However, KOZLOFF and WESTERVELT (1987) found a bursal valve in *Syndesmis echinorum*, the type species of *Syndesmis*, thus this distinction is not valid. Electron microscopy of the bursal valve in *S. longicanalis* showed it to be a derivative of basal lamina developed to a varying degree in different mature specimens (GEVAERTS *et al.*, 1995). A wide male antrum where the stylet is connected to the walls through tissue is possibly an apomorphy of *Syndesmis* (WESTERVELT and KOZLOFF, 1992), the male antrum being narrower and the stylet free-moving in *Syndisyrinx*. However, data on these features are lacking for many of the *Syndesmis* /*Syndisyrinx* species. Classification must depend on hypotheses of phylogeny, and such are not available at present for the species of the *Syndesmis* - *Syndisyrinx* complex, hence there is no reason to split them into separate genera. I thus concur with MARCUS (1949), WESTBLAD (1953), HYMAN (1960) and MOENS *et al.* (1994) that *Syndisyrinx* should be suppressed and all the species referred to *Syndesmis*.

Etymologie

The species is named after Lester R. G. Cannon in recognition of his contribution to the knowledge of symbiotic flatworms.

Wahlia westbladi n. sp.

Type material

Holotype (SMNH 4816) one longitudinally sectioned specimen from papillose anterior part of intestine in *Stichopus mollis* from bottom off Rottnest Island, Western Australia, January 1991. Paratypes, one longitudinally sectioned and one cross sectioned specimen, same data as holotype. Several specimens studied live. A total of 23 specimens were found in the four specimens of *S. mollis* dissected.

Description

Red-pigmented, with broad anterior end, tapering posterior. Body length 1.7 mm (1 - 2.5 mm, n = 6), relaxed specimens longer than 2 mm. Pharynx one-sixth body length from anterior. Anterior end of worms often positioned between papillae of host intestine with posterior protruding upwards from the epithelium.

One dorsal and two lateral longitudinal ciliated bands present, remainder of epidermis unciliated. Mouth opening large, about 75 µm wide, to ventral surface. Pharynx ventrally directed, thin-walled but wide 150-200 µm (anterior- posterior) by 150-160 µm (lateral) (n=3). Intestine a simple sac wholly posterior to pharynx, lined with vacuolated cells (Fig. 6A).

Paired lateral testes without prominent lobes from level of pharynx posterior to about two-thirds of body length (depending on stage of maturity). Sperm ducts from posterior part of testes extend anteriorly and unite to form common sperm duct before entering seminal vesicle positioned anterior to pharynx. Seminal vesicle curved, proximally bulbous 50 µm by 60 µm in longitudinal section on holotype. Long sclerotized copulatory stylet runs through body near midline, enters bulbous penis papilla with muscle fibres and vacuolate tissue (85 x 95 µm in longitudinal section of paratype), and makes a 360° loop. Common genital pore postero-terminal (Figs 5 A, C, 6 B, C, D).

Paired ovaries unbranched but with distal lobes, curving posteriorly from ootype. Vitellaria lateral, two main branches on each side of the body extend anteriorly from ootype to level of pharynx with numerous small lateral branches. Vagina dorsal from common atrium joining seminal receptacle (Figs 5 A, C, 6 D) through a short and narrow sclerotized tube (Fig. 5 C). Seminal receptacle dorsal, with sperm-filled vacuoles (Fig. 6D) connected to ootype through unsclerotized duct with sphincter. Bursal valve absent. Distally the primary uterus (about 200 µm long), which contains one untanned egg capsule in mature specimens, joins the secondary uterus (about 180 µm long in sectioned paratype), which normally contains two tanned egg capsules.

The tetrahedral tanned egg capsules are often released when live worms are transferred from the host intestine to a glass dish. Tail piece of egg capsules distally split into multiple coiled threads (Fig. 5B).

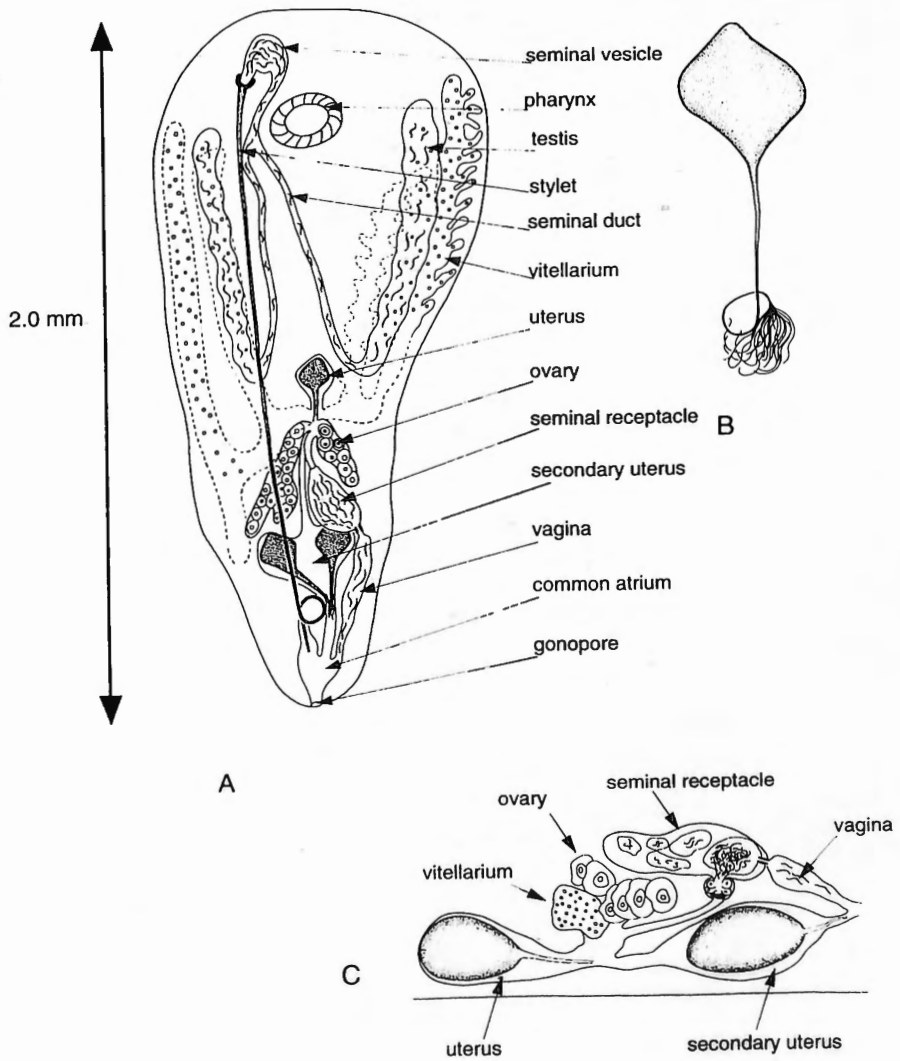

Fig. 5. – *Wahlia westbladi* n.sp. – A. dorsal view of general anatomy; reconstruction from live animals and serial sections – B. egg capsule – C. organisation of female system in lateral view.

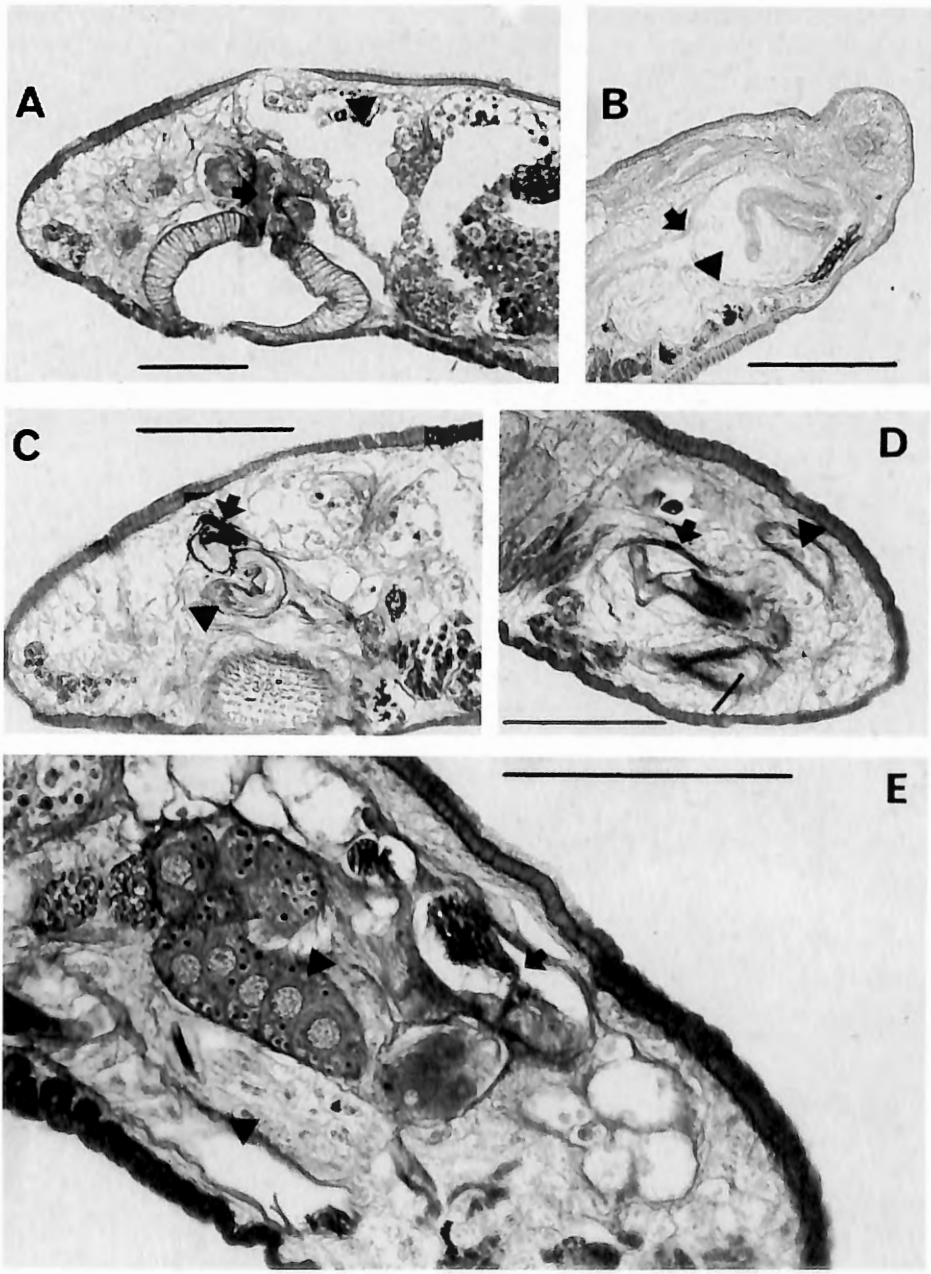

Fig. 6. – *Wahlia westbladi* n.sp.: photomicrographs from serial sections – A. longitudinal section through pharynx, oesophagus (arrow) and intestine – B. longitudinal section showing stylet (arrow) entering loop tissue (arrow head) – C. longitudinal section showing tangentially sectioned pharynx, seminal vesicle (arrowhead) and proximal part of stylet (arrow) – D. longitudinal section showing stylet in loop tissue (arrow), vagina (arrowhead) and distal part of uterus (line) – E. longitudinal section showing seminal receptacle (arrow), ovary (arrowhead) and uterus (arrowhead); note ciliated dorsal part of epidermis. (Scale bars: 100 μm).

Comments and differential diagnosis

The copulatory stylet in *Wahlia westbladi* is longer relative to the length of the body than the stylets of *Seritia striata* (Hickman, 1955), *W. arbora* (Ozaki, 1932), *S. elegans* (Westblad, 1953) and *W. pulchella*, Kozloff and Shinn, 1987. In *W. arbora* the testes are lobulate and vitellaria reach posteriorly to the level of the common atrium. In *W. elegans* the pharynx is antero-terminal.

The species that most closely resembles *W. westbladi* is *W. macrostylifera* Westblad, 1930 from the intestine of *Parastichopus tremulus* (Gunnérus, 1767) on the Norwegian west coast. *W. macrostylifera* and *W. westbladi* have similar body to stylet length ratios. However, *W. macrostylifera* possesses H shaped vitellaria. Examination of Westblad's material (SMNH) reveals the uterus wall to be more muscular, and the proximal, sclerotized part of the vagina is longer than in *W. westbladi*. Furthermore, WESTBLAD (1930) reports the body length of fully extended specimens to 1.5-1.6 mm whereas *W. westbladi* reaches 2.5 mm.

The nature of the bursa copulatrix, the bursal valve and the seminal receptacle in *Wahlia* and related genera was discussed by CANNON (1982) and KOZLOFF and SHINN (1987). The latter authors concluded that a seminal bursa is absent in the genus *Wahlia* (also including the species previously referred to *Ozametra*) and referred species with a distinct bursa to the genus *Seritia* Cannon. Unfortunately they provided no micrographs to illustrate the differences in the female systems of the two genera. Perhaps the situation in *Wahlia westbladi* could be regarded as intermediate: a discrete bursa with sclerotized excurrent nozzle and sheath is absent, but there is a sclerotized proximal part of the vagina opening into the seminal receptacle and a sphincter surrounding the proximal parts of the seminal receptacle.

Etymology

The species is named in recognition of Einar Westblad for his contribution to platyhelminth research.

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MICROSCOPIC OBSERVATION OF THE RETINAL PHOTORECEPTOR LAYER OF THE COMMON BARBEL (TELEOSTEI: CYPRINIDAE)

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Summary. Light and electron microscopic observations show that cones belonging to four types are present in the retina of the common barbel *Barbus barbus* (L.): short single cones, long single cones, twin cones, and unequal double cones. They do not exhibit any particular arrangement. Estimates of cone density suggest that the common barbel has multichromatic vision but of low acuity and that no difference exists between the lower and the upper parts of the retina. Both cone density (approximately 3000 units/mm^2) and the proportion of double cones to single cones (approximately 1:3) are low. The view that the barbel has an inferior colour vision is in good agreement with the fact that this species is active mainly at twilight, but with diurnal activity during spawning.

Key words: *Barbus barbus*, Teleostei, Cyprinidae, retina, photoreceptors, colour perception.

INTRODUCTION

From most studies concerning the visual perception of fish (see for review WAGNER, 1990), the retinal morphology of these animals appears mainly to reflect the functional requirements imposed upon the visual system by ecological and ethological factors. Photoreceptors are regarded as key determinants of the visual performance of a species. Visual communication, considered an important factor controlling fish behaviour (LEVINE *et al.*, 1980; WAGNER *et al.*, 1992), often involves colour patterns and hence a well-developed colour vision depending on the abundance and distribution of cones.

In barbels, reproductive behaviour is diurnal, though the animals tend to be active at dusk and at dawn (BARAS & CHERRY, 1990; BARAS, 1992; PONCIN *et al.*, 1994). During the spawning act - sometimes preceded by "forehead swim" in which two fishes swim together, at high speed, head against head - the female and some accompanying males (approximately 8 to 30 fishes) rise from the gravel and move their genital papillae in the gravel while releasing ova or sperm. Such spawning behaviour implies visual and vibrational cues which may be regarded as important factors controlling barbel reproduction as reported in streamwaters species, e.g. *Oncorhynchus nerka* (Walbaum, 1792)(SATOU *et al.*, 1987). The influence of such stimuli is of great interest when considering the mating pro-

cess in fish, particularly in the genus *Barbus* where hybridisation is possible between fishes of different size and colour (e.g. *Barbus barbus* (L., 1758) and *Barbus meridionalis* (Risso, 1826)(PONCIN *et al.,* 1994).

The aim of the present study was to observe the retinal photoreceptors of the common barbel by light and electron microscopy, with special emphasis on cone variety and distribution. This preliminary approach will allow determination of the visual capabilities of the barbel. Hypotheses thus formed could be tested by an experimental approach using dummies of different sizes and colours.

MATERIALS AND METHODS

Preparation of the material

Barbus barbus (L., 1758) individuals were taken from a stock originated from the River Ourthe (Belgium). They had been reared in tanks from the egg stage to maturity (PHILIPPART *et al.,* 1989), and kept in an aquarium for 2 years. One male (118 g) and two females (177 and 263 g) were chosen for microscopic observation of the retina. These fish were kept for 48 hours in the dark to limit the retino-motor reflex and anaesthetised by addition of up to 0.4‰ 2-phenoxyethanol to the water. From this time on, all handling took place under low-intensity red light. The eyes were excised from the orbits and opened by removal of the iris and the crystalline lens. Then, the detachment of the retina was induced by incubation for 2 hours in Ca^{2+}-free Ringer solution at pH 7.4 (composition: 113.5 mM NaCl, 11.9 mM $NaHCO_3$, 3.3 mM NaH_2PO_4, 3.4 mM KCl, 21 mM $MgCl_2$ and 11.1 mM glucose) according to LEVINE *et al.* (1980). The eyes were then fixed by immersion for 45 min in the dark at room temperature in 2.5% glutaraldehyde in the Ringer solution. Back in the daylight, one sector from the upper and one from the lower part of the detached retina were isolated. The fragments were fixed again for 30 min in glutaraldehyde-Ringer solution, then postfixed for 1 hour at 4°C in 1% osmium tetroxide in Ringer solution. After washing in distilled water and dehydration in a graded ethanol series and in propylene oxide, they were embedded in epoxy resin (Glycidether 100, Serva); flat rubber moulds or Beem capsules were used, respectively, for producing transverse or tangential sections. Semithin and ultrathin sections were obtained with a Reichert-Jung (Ultracut E) ultramicrotome equipped with a diamond knife. Semithin sections were stained with a 1% toluidine blue solution at pH 9.0 and observed under the light microscope. Ultrathin sections contrasted with uranyl acetate and lead citrate were observed in a Jeol JEM 100-SX transmission electron microscope under an accelerating voltage of 80 kV.

Photoreceptor cell count

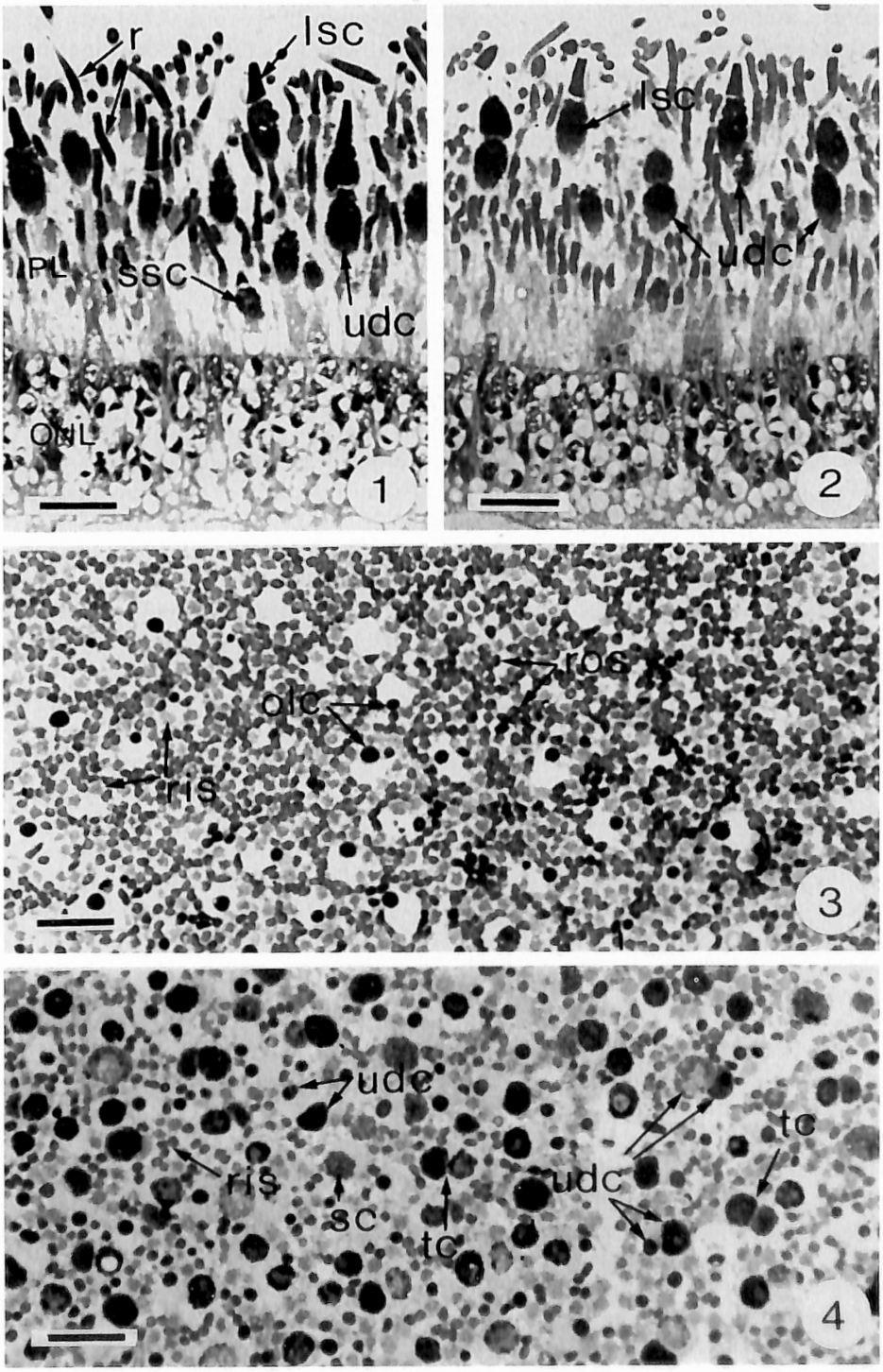
The cones were counted on serial semithin sections cut as parallel as possible to the retina surface. According to the section level, two counting zones were determined to count separately the outer segments of the long cones (long single cones and long partners of unequal double cones) and the inner segments of single cones, equal double cones, and unequal double cones. In each zone, the cone segments were counted in three to eight areas using a

clear chamber on an Olympus BH2 microscope. The mean number of cones of each type per surface unit (mm^2) was calculated for all three fish, separately for the sectors in the upper and lower parts of the eye. No distinction was made between the left and right eyes. The proportion of short and long single cones was calculated first for each fish before calculation of the mean values. For this evaluation, we considered the number of long single cones to be equal to the number of long cone segments minus the number of unequal double cones observed on the sections, and the number of short single cones to be equal to the number of single cones observed on the sections minus the calculated number of long single cones.

RESULTS

Microscopic observation of semithin (Figs 1-4) and ultrathin (Figs 5-6) sections of the common barbel retina shows that, in addition to numerous rods, many cones are present in the retina of both the upper and lower parts of the eye. In transverse sections (Figs 1, 2), the photoreceptor layer is approximately 100 mm thick, representing about half of the overall thickness of the retina. Cones are easy to distinguish from rods by the larger size of their inner segment. Four types of cones are observed : short single cones, long single cones, twin cones, and unequal double cones. Tangential semithin sections cut parallel to the retina surface show that the cones are randomly distributed; different types can be distinguished according to the section level. At the first level toward the outer side of the retina, the outer segment tops of long cones are easily distinguishable among those of the rods (Fig. 3). They appear more intensely toluidine blue-stained, their diameter is usually greater, and they are surrounded by a small rod-free area. They most likely belong to long single cones or to the long partners of unequal double cones. At the second level, deeper toward the external limiting membrane (Fig. 4), single cones appear as large, round, dark blue sections mostly corresponding to the sclerad pole of the inner segment also known as the "ellipsoid". These inner segments of cones are readily identified in transmission electron micrographs by the abundance of large mitochondria (Figs 5, 6). Similarly, double cones with identical partners *(i.e.* "twin cones") appear as two closely juxtaposed round or crescent-shaped sections of similar size and/or staining intensity. In contrast, the unequal double cones exhibit two sections of different size. Both partners of double cones are always located in the same surrounding rod-free area.

As reported in Table I, the estimates of the number of cones of the different types per surface unit do not reveal any significant difference between retina sectors in the upper and the lower parts of the eyes (Mann-Whitney U test values > 0,05). In both parts, the total cone density reaches approximately 3000 units per mm^2 and the relative proportion of the different cone types appears to be quite similar. Single cones constitute the great majority. The proportion of double cones to single cones reaches only about 1:3. Moreover, with a density between 1600 and 1700 units per mm^2, short single cones amount to over half of the total number of cones and over two-thirds of the number of single cones. The calculated results given for each fish do not show obvious differences between individuals. The large standard deviations on the mean values of cone densities may be due to the very low number of cones in the counting areas, e.g. the number of twin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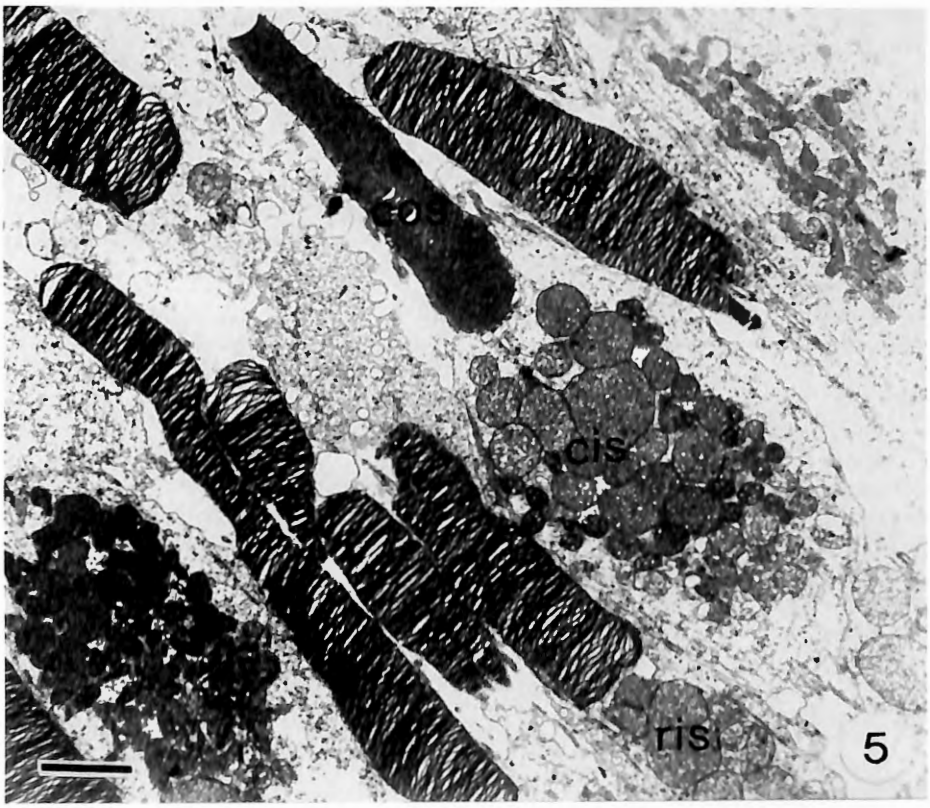
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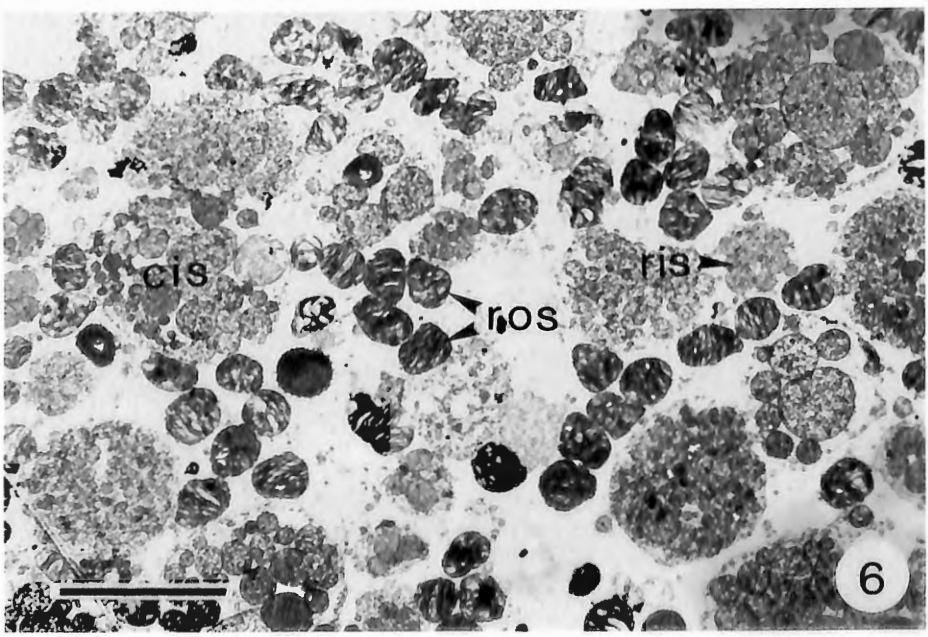
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cones or unequal double cones is often below 10 units and sometimes below 5 units in a microscopic field covering approximately 0.015 mm².

TABLE I

Density of cones of the different types in the upper and lower sectors of the retina of the common barbel, Barbus barbus. The results are reported for each fish as cone numbers per unit (mm2) of retinal surface (fish n°1: female of 177 g; fish n°2: female of 263 g; fish n°3: male of 155 g). The mean numbers in the retina sectors are calculated with standard deviation (n=3) and statistically compared by the Mann-Whitney U test.

	Upper sector of the retina				Lower sector of the retina				
Cone types	Fish n° 1	Fish n° 2	Fish n° 3	Mean ±SD	Fish n° 1	Fish n° 2	Fish n° 3	Mean ±SD	U test
Cones (total number)	2997	3200	3625	3274±320	3568	3215	3233	3339±199	24
Long cones	1240	1227	1200	1222±20	1200	800	2000	1333±611	3.5
Single cones	2133	2167	2750	2350±347	3048	2615	2289	2651±381	2
Long single cones	673	360	975	669±307	936	538	1644	1040±560	3
Short single cones	1460	1806	1775	1680±191	2112	2077	644	1611±837	3
Double cones	864	1033	875	924±95	520	600	944	688±226	2
Twin cones	297	167	650	371±250	256	338	589	394±173	4
Unequal doubles cones	567	867	225	553±321	264	262	356	294±54	3

DISCUSSION

Concerning the visual capabilities of *B. barbus*, the present microscopic observations of the retinal photoreceptor layer and our cone density estimates suggest that this species perceives colours rather poorly; this tallies with the crepuscular activity of the species (BARAS, 1992). Given the thickness of the photoreceptor layer, the size of the outer seg-

Figs 1 - 4. – Vertical semithin sections of the common barbel retina as observed by light microscopy after staining with toluidine blue – Figs 1-4: vertical sections.lsc, long single cones; ONL, outer nuclear layer; PL, photoreceptor layer; r, rod outer segments; ssc, short single cones; udc, unequal double cones. – Figs 3-4: Horizontal sections at the outer (Fig. 3) and inner (Fig. 4) levels in the photoreceptor layer; olc, outer segment of long cones including long single cones and the long patner of unequal double cones; ris, rod inner segments; ros, rod outer segments; tc, outer or inner segments of twin cones; udc, outer and/or inner segments of unequal double cones. (Scale bars : 20 mm). Figs 5 - 6 – Vertical (Fig. 5) and horizontal (Fig. 6) thin sections of the common barbel retina observed by transmission electron microscopy after staining in uranyl acetate and lead citrate; cis, cone inner segments or "ellipsoids"; cos, cone outer segments; ris, rod inner segments; ros, rod outer segments. (Scale bars in 5 : 2 mm; in 6 : 10 mm).

ments of rods, and the abundance of cones of four different types, the barbel retina seems to be half way between that of diurnal species and that of species living in low-light-level habitats (see for review WAGNER, 1990). The most obvious difference between the retina of the common barbel and that of fish living in low-light habitats is the presence of abundant cones of different types and lengths, as mostly develop in the retina of diurnal species. Since cones of different types are known to contain visual pigments sensitive to light of different wave-lengths (for review, see MARC & SPERLING, 1976; BOUWMAKER, 1990), the four types of cones in the barbel retina can be assumed to ensure multichromatic perception. A more detailed comparison reveals that the barbel possesses less developed rods with shorter outer segments and a thicker neural retina than fish living with a low light level. The relative thickness of the photoreceptor layer is approximately 50% of the overall retinal thickness, whereas it reaches over 80% in deep-sea fishes (MUNK, 1964, 1966) and in the glass catfish *Kryptopterus bicirrhis* (Cuvier & Valenciennes, 1839)(WAGNER, 1990). By comparison with diurnal species, however, the barbel retina appears more simply organised and can be assumed to have a colour vision of lower acuity. It contrasts with that of species with superior visual capabilities such as *Aequidens pulcher*(Gill, 1858), a diurnal cichlid predator, the roach *Rutilus rutilus* L. 1758, which has tetrachromatic colour vision, or the Salmonidae (AHLBERT, 1976; WAGNER, 1990): the cone density is lower, the proportion of single cones is higher, the cone densities are similar in different parts of the retina, and there is no regular or mosaic arrangement of cones. The total cone density of approximately 3000 units/mm2 in the barbel retina can be compared, for instance with values recorded by AHLBERT (1976) in the retina of the sea trout *Salmo trutta* L. 1758: lower value: 6,750 units/mm^2, *i.e.* twice as much; higher value: 28,500 units/mm^2, or twelve times the barbel value). Similarly, double cones are the most abundant type of cone in true diurnal species, whilst in the barbel they are but poorly represented. Double cones are 4 to 18 times less numerous in the barbel retina (approximately 700-800 units/mm^2) than in the retina of the sea trout (163 to 686 units/0.05 mm^2: AHLBERT, 1976) and 4 to 16 times less numerous than in salmon retina (153 to 598 units/0.05 mm^2: AHLBERT, 1976). Thus, the ratio of double to single cones is about 0.3 in the barbel, which is much lower than in diurnal salmonidae (1.07 in the sea trout and up to 1.80 in salmon).

In conclusion, the organisation of the retina of the common barbel suggests that the multichromatic perception of this fish is most probably poor, in keeping with the mainly crepuscular activity of this species. The barbel is most probably able to discriminate colours due to the presence of four different types of cones in its retina, but the rather large cone spacing suggests that it could have a low colour vision acuity. Nevertheless, the presence of cones suggests that the barbel retina can perceive colours; this may be related to the barbel's diurnal spawning behaviour, described by BARAS (1992).

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SCARIDIUM ELONGATUM N. SP., A NEW MONOGONONT ROTIFER FROM BRAZIL

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Abstract. A new species of *Scaridium*, *S. elongatum* n. sp., is described from Broa reservoir, São Paulo, Brazil. The species is probably a neotropical vicariant of the palaeotropical *S. grande* SEGERS, 1995.

Key words: *Scaridium elongatum* n. sp., Scaridiidae, Brazil.

INTRODUCTION

In a recent paper revising the Scaridiidae Manfredi (SEGERS, 1995), it was noted that no neotropical *Scaridium* Ehrenberg, 1830 had so far been found, although such could be expected considering the biogeography of the known representatives of the genus, and the generally high level of endemicity in the South American rotifer fauna. Therefore, it came as no surprise to find an undescribed *Scaridium* species in a sample from an otherwise well-investigated reservoir near São Paulo. A more elaborate report on the rotifer fauna of this locality by SEGERS & DUMONT (1995) was based on samples collected during August 1994. The new *Scaridium* occurred in additional samples collected during December 1994 and January 1996, and was also found in a sample from Minas Gerais, Brazil.

MATERIAL AND METHODS

Samples were collected using a standard zooplankton net (mesh width 50μm), and fixed in formalin. Animals were selected under a Wild M10 dissecting microscope, and examined with an Olympus CH2 microscope equipped with a camera lucida. Scanning electron microscopy was performed with a JEOL-JSM 840 microscope, on trophi isolated and treated following a method after SEGERS (1993). The samples from Minas Gerais (Brazil) were collected by M.B. Dabés.

RESULTS

One of the samples collected during December 1994 contained, amongst other species (see Table 1), several specimens of a hitherto undescribed rotifer. Its description is as follows.

TABLE 1

Rotifera (Monogononta) accompanying Scaridium elongatum *n. sp.*

Collotheca ornata (Ehrenberg, 1832) f. *natans* Tschugunoff, 1921
Dicranophorus epicharis Harring & Myers, 1928
D. prionacis Harring & Myers, 1928
Euchlanis dilatata Ehrenberg, 1832
Euchlanis triquetra Ehrenberg, 1838
Filinia novaezealandiae Shiel & Sanoamuang, 1933
Lecane bulla (Gosse, 1851)
L. leontina (Turner, 1892)
L. lunaris (Ehrenberg, 1832)
L. monostyla (Daday, 1897)
L. quadridentata (Ehrenberg, 1832)
L. signifera (Jennings, 1896)
Lepadella patella (O.F. Müller, 1786)
Monommata maculata Myers, 1930
Mytilina ventralis (Ehrenberg, 1832) f. *macracantha* (Gosse, 1886)
Polyarthra sp. near *vulgaris* Carlin, 1943
Ptygura libera Myers, 1934
Scaridium elongatum n. sp.
Testudinella ohlei Koste, 1972
Tetrasiphon hydrocora Ehrenberg, 1840
Trichocerca capucina (Wierzejski & Zacharias, 1893)

Scaridium elongatum n. sp.

Material

Two parthenogenetic females (holotype and paratype) deposited in the Royal Belgian Institute for Natural Sciences (K.B.I.N., reg. nr. IG 28274 RIR 61, 62: paratype), Brussels, Belgium; One parthenogenetic female and one light microscopy trophi preparation in the 'Instituto Nacional de Pesquisas da Amazônia (I.N.P.A., reg. INPA ROT-0550a-b), Manaus, Amazonas, Brazil; one parthenogenetic female, one light microscopy trophi preparation and one S.E.M. trophi preparation in the Laboratory for Animal Ecology, University of Gent, Belgium. In total, 12 specimens were found in a sample from 14 December 1994, and one in a sample collected on 20 January 1996, both from Broa

reservoir, Itirapira, São Paulo, Brazil. One female specimen in a sample from Gameleira river (Afl. Rio Grande), Uberaba, Minas Gerais, Brazil (leg. M.B. Dabés).

Diagnosis

Scaridium elongatum n. sp. is a close relative of *S. grande*[1] Segers, 1995, by its generally similar trophi shape. The species differs mostly by its rounded allulae, different from the prominent hook-shaped allulae of *S. grande*. There are several additional differences in trophi structure (slightly larger ramus teeth, more slender unci teeth, different epipharynx) (Figs 1-5). Also, the species is noticeably smaller than *S. grande* (Fig. 6), and has a relatively longer toe and third foot pseudosegment (Fig. 7).

Following the key by SEGERS (1995) and NOGRADY *et al.* (1995), the species keys out to *S. longicaudum* (O.F. Müller, 1786) and *S. bostjani* Daems & Dumont, 1974. *S. elongatum* n. sp. differs from these by its smaller allulae, more slender unciteeth, larger manubria and overall size, and by its relatively elongate toe and third foot pseudosegment.

Description

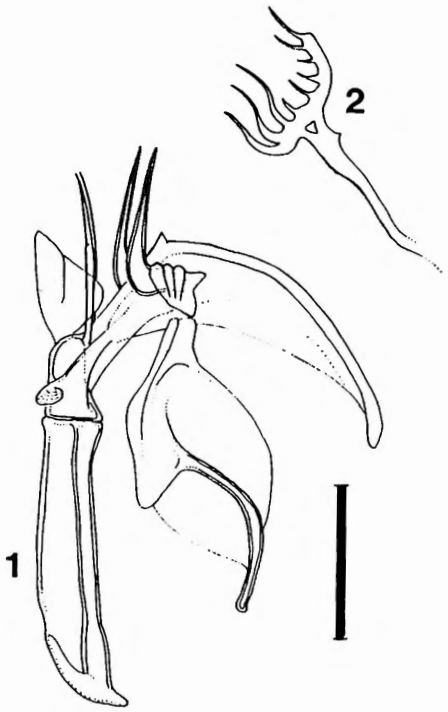
Animal large, second foot pseudosegment and toes relatively long. Head large, trunk with distinct fold antero-ventrally. Ventral pair of head lobes with minute notch. Fulcrum with low midventral crest, basal plate well-developed. Rami teeth relatively small, acutely pointed, triangular. Allulae small, rounded in lateral view. Basal part of unci triangular. Unci teeth large, elongate, slender, accessory teeth indistinct or absent. Manubrium relatively wide and transversally divided proximally. Ventral margin convex. Ventroposterior projection large, triangular. Anterior lamella well developed, posterior lamella large, weak. Terminal rod-shaped part elongate, connected with posterior lamella. Epipharynx teeth elongate, especially the posterior group. Male, and parthenogenetic and resting eggs unknown.

Dimensions (see Table 2, Figs 6, 7): Total length 402-446 µm, body 119-144 µm, second foot pseudosegment length 31-47 µm, third foot pseudosegment length 95-108 µm, toe length 155-168 µm. Fulcrum length 36-38 µm.

Etymology

The species name *elongatum* is a Latin adjective, and refers to the elongate foot and toe shape in this species and, in fact, of all *Scaridium* species.

[1]Emendation of *Scaridium grandis*: incorrect Latin termination of the adjectival species name, in disagreement with the gender of the genus name (see ICZN 31(b), (c)(ii)).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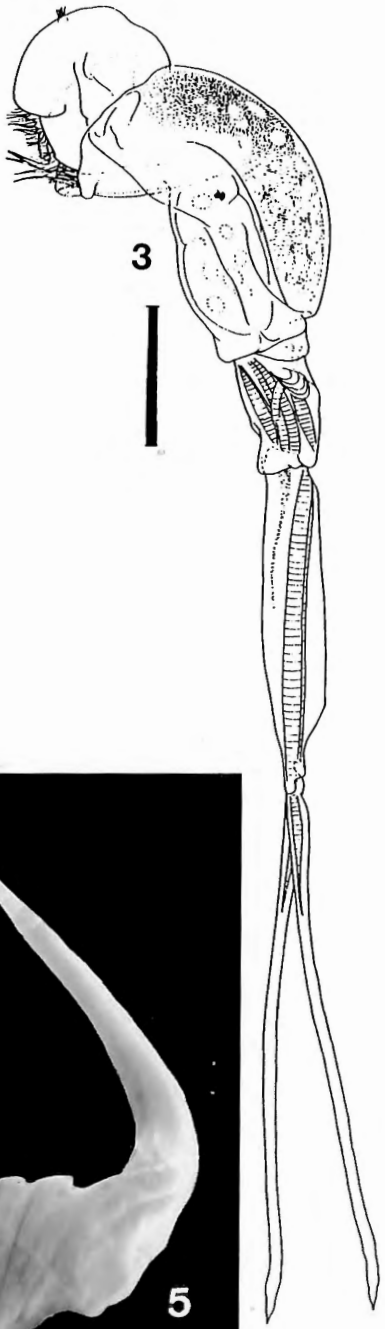
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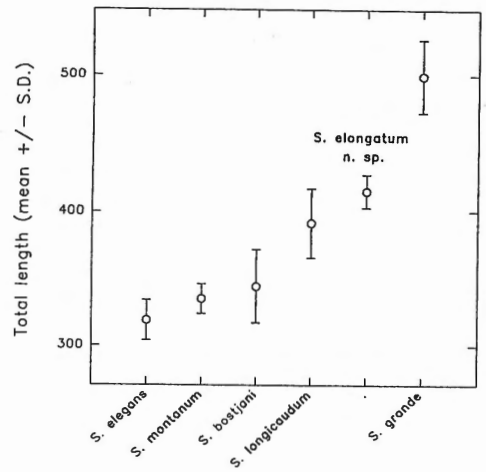

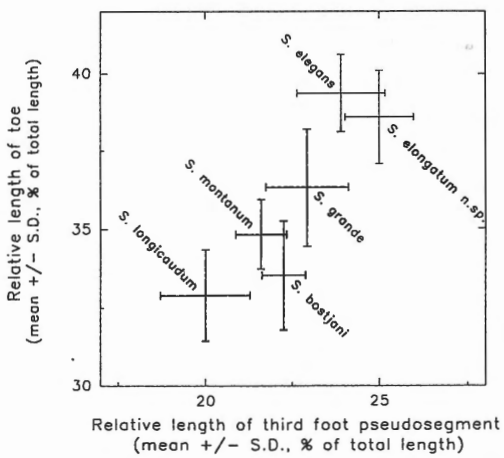

Fig. 6. – A comparison of the total length of all *Scaridium* species (after SEGERS, 1995; modified)
Fig. 7– Relative length of the third foot pseudosegment versus relative length of the toe in *Scaridium* species (after SEGERS, 1995; modified).

Figs 1-5. – *Scaridium elongatum* n. sp. – 1, 4. trophi, lateral view – 2. epipharynx – 4. habitus, lateral view – 5. uncus – 4, 5. S.E.M. photographs. (Scale bars: 1, 2: 25μm – 3: 50μm – 4: 10μm; 5: 5μm).

TABLE 2

Measurements on Scaridium elongatum *n. sp. (in μm)*

Specimen	1	2	3	4	5	6	7	8	9	10	11	12	mean±S.D
- Total length	407	405	412	410	415	405	402	446	420	415	418	428	415±12.17
- Body length	126	129	129	134	122	119	129	144	121	129	124	142	132±9.59
- first foot pseudo segment l.	36	39	36	41	34	36	44	46	44	36	31	36	38±4.81
- second foot pseudosegment l.	103	108	106	96	101	106	101	106	106	106	103	108	104±3.70
- Toe length	157	168	157	160	162	160	157	162	156	160	168	155	106±4.20
- Toe l./total l. (%)	38.6	41.4	38.1	39.1	39.0	39.5	39.1	36.3	37.1	38.5	40.2	36.2	38.6±1.52
- 3rd pseudos.l./ tot. l. (%)	25.3	26.7	25.6	23.3	24.2	26.1	25.0	23.6	25.1	25.4	24.7	25.3	25.0±0.97

Biogeography and ecology

As aforementioned, *Scaridium elongatum* n. sp. resembles, and is probably most closely related to *Scaridium grande*, of all its congeners. Whereas the latter species appears to have a palaeotropical distribution, the former is neotropical, as it is being described from Brazil. The Brazilian fauna now contains four representatives, *S. elongatum* n. sp., *S. bostjani* (see SEGERS, 1995), *S. elegans* Segers & De Meester, 1994 (three specimens in a sample from the Rio Paraguai, Pantanal region, leg. A.L. de Oliveiro-Neto), and *S. longicaudum* (one specimen in a marginal lagoon of São Francisco river, Januária, Minas Gerais, 28 Februari 1995, leg. M.B. Dabés).

The sample containing the majority of the specimens of *Scaridium elongatum* n. sp. was from a littoral habitat. Apart of the new species, 20 additional monogonont Rotifera could be identified from that sample (see Table 1), which is a relatively low number. As the majority of the Rotifera accompanying the new species, and all other *Scaridium* species are littoral, it can be assumed that also *S. elongatum* n. sp. lives in this type of habitat. Remarkable are *Testudinella ohlei* Koste, 1972 a neotropical species, an unidentifiable *Polyarthra* sp. (near *P. vulgaris* Carlin, 1943), and *Filinia novaezealandiae* Shiel & Sanoamuang, 1993. The latter record is the first of this species from the New World (SEGERS *et al.*, in press).

The discovery of a new *Scaridium* species in the neotropical region is particularly noteworthy, as it was hypothesised that such a South American *Scaridium* exists (SEGERS, 1995), an assumption derived from the contemporary knowledge on the biogeography of the taxon. This case therefore illustrates that our knowledge in this field has reached a point where predictions on the distribution and diversity of certain rotifer taxa, at the least at the level of large-scale, regional biogeography, become possible. As such, it is an example of the predictive potential of biogeography.

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IDENTIFICATION OF CATECHOLAMINERGIC CELL GROUPS IN THE BRAINSTEM OF THE CANARY, ZEBRA FINCH, WHITE-THROATED SPARROW AND BUDGERIGAR BY TYROSINE HYDROXYLASE IMMUNOCYTOCHEMISTRY

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Summary. In Passeriformes and Psittaciformes, the learning and production of complex learned vocalizations is controlled by a network of telencephalic, diencephalic and mesencephalic nuclei, the so-called song-control system. Specialized telencephalic song control nuclei such as the high vocal center, nucleus robustus archistriatalis and area X in the basal ganglia receive dense and discrete catecholaminergic inputs. Catecholaminergic fibers also innervate the telencephalon of other birds and other vertebrate species but there appear to be unique specializations of the catecholaminergic inputs in songbirds. In this paper the distribution of tyrosine hydroxylase-immunoreactive cell groups in the brainstem is described in 4 avian species that are known to be vocal learners. The goal of this work is to identify whether novel cell groups are present in the brainstem that may give rise to the specialized catecholaminergic projections in the telencephalon of these vocal learners. These brainstem cell groups are known to be the source of most telencephalic catecholaminergic input in birds and other vertebrates. Three songbird species, the zebra finch, the canary and the white-throated sparrow and one psittaciform, the budgerigar were analyzed. Immunocytochemical analysis identified in the brainstem of the 4 species the same groups of tyrosine hydroxylase-immunoreactive cells that were previously described in the brain of other non-song birds. These were located in the area ventralis of Tsai (A10), around the substantia nigra (A9) and its caudal extension (retroruberal field, A8), and in the nucleus ceruleus and subceruleus (A6). No additional cell group could be detected in these species by comparison with the other species of non oscines studied previously. This suggests that both in song birds and in budgerigars specialized innervations by catecholaminergic neurones of the telencephalic song control nuclei are not associated with the evolution of novel catecholaminergic cell groups in the brainstem as compared to those that are present in species that do not display these specializations.

Key words: Song system, song control nuclei, song birds, oscines, chemical neuroanatomy, dopamine, noradrenaline.

INTRODUCTION

The catecholamines dopamine (DA), norepinephrine (NE) and epinephrine (E) are known to play a crucial role in the control of various brain functions related to reproduction such as the synthesis and release of gonadotrophin releasing hormone (GnRH) and the activation of male and female sexual behavior (CROWLEY & ZEMLAN, 1981; CROWLEY *et al.*, 1989; MEYERSON *et al.*, 1979; MEYERSON *et al.*, 1985; BITRAN & HULL, 1987; BARCLAY & CHENG, 1992). The activity of catecholaminergic neurons is itself modulated by steroids so that changes in DA and NE turnover rate are often hypothesized to be a part of the cascade of biochemical events that are triggered by steroids in the brain and are responsible for the physiological and behavioral effects of these steroids (MEISEL & SACHS, 1994; PFAFF *et al.*, 1994). Alternative explanations have however been suggested (BALTHAZART & BALL, 1992).

In birds, specific vocalizations are associated with reproduction and activated by steroids (NOTTEBOHM 1975). Three avian orders, the Passeriformes (songbirds), Psittaciformes (parrots, budgerigars) and Trochiliformes (hummingbirds) have independently evolved the ability to learn and produce complex vocalizations. These vocalizations are learned, either during an early age or throughout the entire life of the bird. In two of these orders (Passeriformes, Psittaciformes), it has been demonstrated that the learning and production of these vocalizations is controlled by a complex network of telencephalic, diencephalic and mesencephalic nuclei, the so-called song-control system (NOTTEBOHM, 1980; KONISHI, 1985; STRIEDTER, 1994; BRAUTH *et al.*, 1994; BALL, 1994).

In songbirds, several lines of evidence indicate that the specialized telencephalic song control nuclei such as HVC (High vocal center, formerly Hyperstriatum ventral pars caudale), RA (nucleus robustus archistriatalis), the lateral part of the magnocellular nucleus of the anterior neostriatum (lMAN), and area X of the parolfactory lobe receive dense catecholaminergic inputs. For example, fibers immunoreactive for tyrosine hydroxylase have been described in HVC, RA, lMAN and area X of zebra finches (BOTTJER, 1993; SOHA *et al.*, 1995), and high densities of noradrenergic receptors of the α_2 and $\beta 1/\beta 2$ subtypes (HVC, RA and area X) (BALL *et al.*, 1994; BALL, 1994) or of dopaminergic D1 receptors (Area X) (CASTO & BALL, 1994a) have been described in several song birds species. High concentrations of NE and DA have also been measured in the song control nuclei of zebra finches and the baseline levels and/or turnover rates of these amines appears to be modulated by steroids and during development (BARCLAY & HARDING, 1988, 1990; HARDING *et al.*, 1995). NE also appears to play a role in the control of vocal behavior of zebra finches (BARCLAY *et al.*, 1992).

Less information is available for Psittaciformes, another avian order of vocal learners, but it is already clear at present that species such as the budgerigar possess telencephalic specializations that are analogous (and probably not homologous) to the HVC and RA of song birds (STRIEDTER, 1994; BRAUTH *et al.*, 1994; BALL 1994) and at least one of these nuclei, the magnocellular nucleus of the parolfactory lobe (LPOm) also receives a dense catecholaminergic input as indicated by the presence of high densities of receptors (BALL, 1994).

NE or DA-containing fibres also innervate the telencephalon of other birds and other species of vertebrates but the highly specific, dense catecholaminergic inputs received by HVC and RA appear to be unique to songbirds. This therefore raises the question of the origin of these projections. One obvious hypothesis is that these dense neuroanatomically discrete inputs originate from the same catecholaminergic brain areas that have been described in other vertebrate species including birds (REINER *et al.*, 1994). Alternatively, specific cell groups may have evolved in vocal learners in order to support this specialized innervation of the telencephalon. In the brain, the catecholamines, NE and E are synthesized solely in the brainstem of all vertebrate species including birds (REINER *et al.*, 1994). DA is primarily synthesized in the brainstem, however, cells synthesizing DA have also been identified in the diencephalon (REINER *et al.*, 1994). The available data do not suggest that novel cell groups are present in the brainstem of songbirds and psittaciforms. One study by Bottjer described the distribution of tyrosine hydroxylase (TH) immunoreactive cells in the zebra finch brain (BOTTJER, 1993). This study did not appear to identify catecholaminergic cells groups that would be specific to song birds but the generality of this finding should be tested in other members of the oscine family. In addition, because similar telencephalic specializations have independently evolved in psittaciforms and because some of these nuclei also receive dense catecholaminergic inputs, it is also appropriate to ask whether the brainstem catecholaminergic nuclei in these species also follow the common vertebrate pattern. Previous work utilizing histofluoresence methods to describe the distribution of catecholamines in a psittaciform species, the budgerigar (*Melopsittacus undulatus*) does not suggest that novel catecholaminergic cell groups are present in this species (TAKATSUKI *et al.*, 1981; SHIOSAKA *et al.*, 1981; TOHYAMA *et al.*, 1974). A single study utilized immunocytochemical methods for the localization of TH and other enzymatic markers of monoamines but this study focussed on monoaminergic cells that either contain or do not contain immunoreactive L-amino acid decarboxylase and did not provide an overview of cells containing catecholamines in the budgerigar brain (SAKAI *et al.*, 1992).

It is difficult to make accurate inter-specific comparisons based on studies utilizing different methods and carried out in different laboratories. Therefore, in the present study questions concerning the presence of catecholaminergic cell groups are addressed consistently in 3 species of songbirds and one psittaciform, the budgerigar. These studies focus on the brainstem, the site of noradrenergic and adrenergic synthesis and of the greater part of dopaminergic synthesis (REINER *et al.*, 1994).

MATERIAL AND METHODS

Subjects and fixation

The immunocytochemical experiments described here were carried out on three species of song birds, namely canaries (*Serinus canaria* Linné, 1758; n=6), zebra finches (*Taeniopygia guttata* Vieillot, 1817; n=8), white-throated sparrow (*Zonotrichia albicollis* Gmelin, 1789; n=1) and on another species of vocal learning bird that does not belong to the passerine order, the budgerigar (*Melopsittacus undulatus* Shaw, 1805; n=2). Subjects

were obtained from a breeding colony established at the University of Liège (Zebra finches) or were bought from local breeders in Liège (canaries, budgerigars). The white-throated sparrow was caught in the wild in North Carolina, kindly provided by Dr. Stephen Nowicki, Duke University. In the laboratory, all birds were maintained under a photoperiod simulating long days (16 hours of light, 8 hours of dark per day) with food and water available at libitum. All subjects were male and sexually mature as evidenced by the presence of fully developed testes.

Birds were deeply anesthetized with Hypnodil (Janssen Pharmaceutica, Beerse, Belgium, 50 mg/kg body weight) and intravenously injected with 50-100 µl of heparin solution (Sigma H-7005, 20 mg/ml or 3340 units/ml). They were then perfused through the heart with a saline solution (9 g/l; 0.15 M NaCl) until the return blood in the atrium was clear, followed by 100-300 ml of fixative (paraformaldehyde 4% and 0.1% glutaraldehyde in 0.15 M phosphate buffer, pH 7.2). Brains were immediately dissected out of the skull, post-fixed one hour in the fixative solution without glutaraldehyde and placed overnight in a 20% sucrose solution in 0.1 M phosphate buffer-saline, pH 7.2. Brains were then frozen on powdered dry ice and stored in a freezer at -75° C until used. This same procedure was used for all species except for the white-throated sparrow which was anesthetized with chloral hydrate and perfused with a fixative containing 4% paraformaldehyde without any glutaraldehyde.

Brains were cut with a cryostat in the coronal plane at 30 µm thickness starting at the rostral end. The plane of section was adjusted to match as closely as possible the atlas of the canary brain (STOKES *et al.*, 1974). Sections used in this study were collected through the mesencephalon from the level of the occulomotor nerves to the level of the nucleus vestibularis. Every fifth section at least (one section every 150 µm or more) was stained by immunocytochemistry for tyrosine hydroxylase.

Tyrosine Hydroxylase Immunocytochemistry

The distribution of tyrosine hydroxylase (TH) immunoreactive (ir) cells was visualized in free floating sections by a standard indirect immunocytochemical procedure using peroxidase as reporter enzyme and diaminobenzidine as the chromogen. This method has been fully described and validated previously (BAILHACHE & BALTHAZART, 1993). Briefly, after two rinses in phosphate buffer 0.01 M-saline (PBS), sections were treated for 15 min with 0.6% H_2O_2 in methanol to block endogenous peroxidase, rinsed in PBS and placed overnight at 4° C in the primary TH antiserum (mouse anti-TH, Incstar cat.Nbr.22941, dilution 1/1000 in PBS containing 0.1% Triton X-100). This antibody was raised against TH purified from rat PC12 cells and recognizes an epitope in the mid-portion of the TH molecule that has been well conserved through evolution so that cross-reactivity is observed over a wide range of species. This antibody does not cross-react with dopamine β-hydroxylase, phenylethanolamine-N-methyltransferase, phenylalanine hydroxylase or tryptophan hydroxylase (Incstar specification sheets). We have also shown that this antibody exclusively recognizes in the quail brain cell groups that are known to be catecholaminergic and that omission of the primary antibody eliminates all immunocytochemical

staining (BAILHACHE & BALTHAZART, 1993). Its specificity for the TH molecule is therefore firmly established.

Sections were then rinsed in PBS and incubated with a goat anti-mouse peroxidase-conjugated antibody (peroxidase-conjugated affinity-isolated goat immunoglobulins to mouse immunoglobulins, DAKO P-447) at a dilution of 1/200 for one hour. The peroxidase was finally revealed by placing the sections for 6 min in a solution of diaminobenzidine (DAB; 20 mg in 50 ml PBS containing 0.1% Triton X-100 and 20 µl of H_2O_2 at 30%). Sections were then mounted on microscope slides and coverslipped.

RESULTS

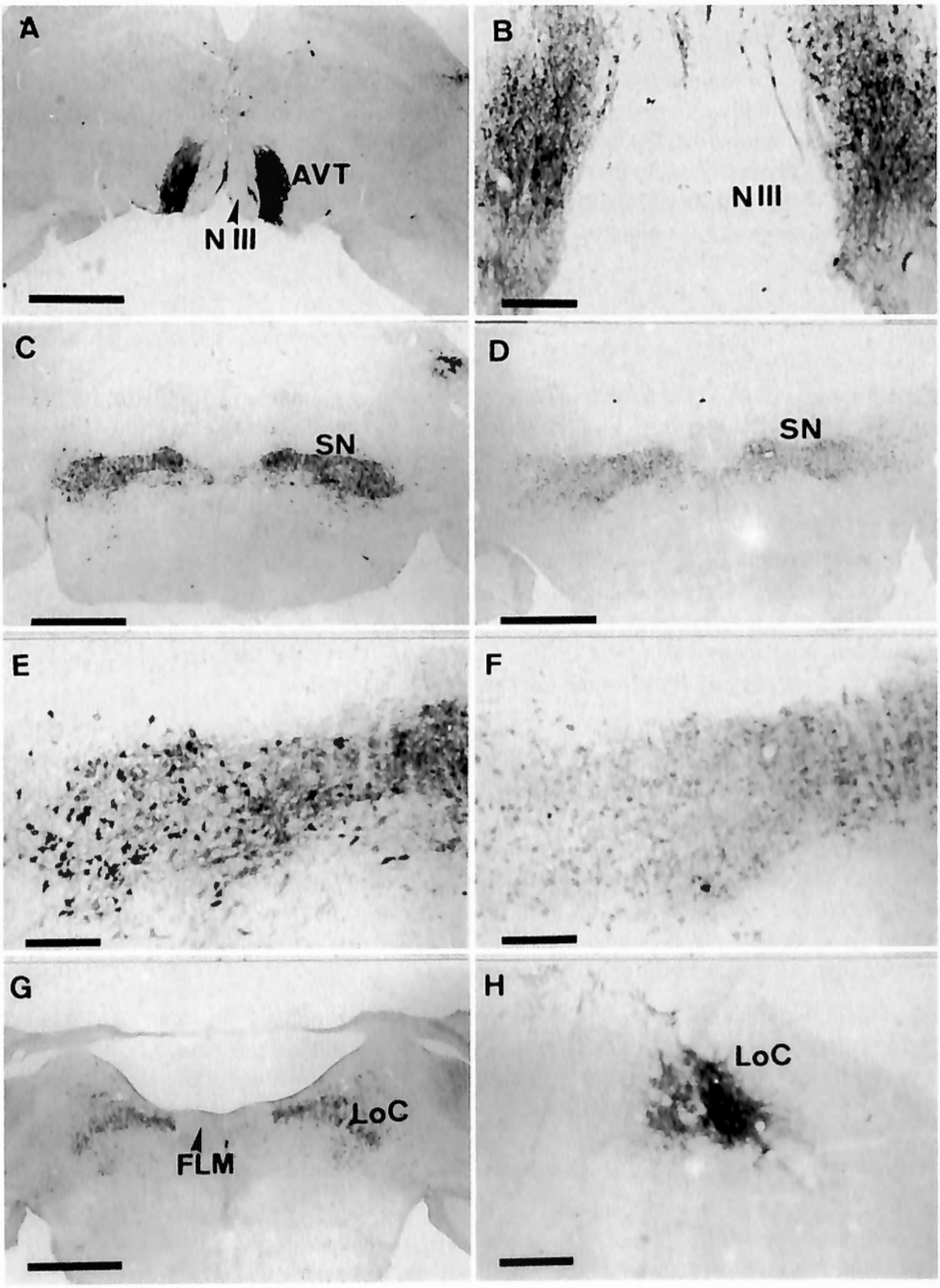
Dense groups of TH-ir perikarya were observed throughout the rostral to caudal extent of the brainstem in the three song birds species that were considered (see Fig. 1 for the zebra finch and white-throated sparrow, Fig. 2 for canary). They are described below as they appear in a rostral to caudal order.

First a numerous group of TH-ir cells bodies is present in the area ventralis of Tsai (AVT), just lateral to the roots of the third nerve, which indicate the transition from the hypothalamus to the mesencephalon (Fig. 1A-B). This cell group was visible in several consecutive sections taken along the rostral to caudal extent of the mesencephalon. It is systematically accompanied by a smaller number of TH-ir cells that are located medially to the third nerves just dorsal to the nucleus interpeduncularis.

At its most caudal level, the AVT group of TH-ir perikarya progressively expands in a dorso-lateral direction (Fig. 2A-B) and invades a very wide area at the level of the substantia nigra (SN). The AVT group of TH-ir cells then disappears as the new group reaches its maximal extent (Figs 1C-D, 2C). The SN contains by far the largest number of TH-ir cells in the brains of the 3 species considered here and the density of these cells is so high that they can barely be discriminated in 30 µm-thick sections (Figs 1E-F, 2B). At even more caudal levels, the SN group of TH-ir cells progressively thins out leaving only a small group of positive cells in a more dorsal position that presumably corresponds to the mammalian A8 group (retroruberal).

This cell group disappears at the mesencephalic-metencephalic junction and a compact but smaller and less dense group of TH positive cells appears in a dorso-lateral position at the ventro-lateral corner of the aqueduct (Figs 1H, 2D) at the level of the locus ceruleus. At its rostral end, this group of TH-ir cells is less dense and does not form such a clearly recognizable cell group. These TH-ir cells located just lateral to the fasciculus longitudinalis medialis are, however, usually considered as the rostral end of the locus ceruleus (Fig. 1G).

At similar levels in the rostral to caudal axis, a scattered population of TH-ir positive perikarya is also present in a more ventral and lateral position. These TH-ir cells are distributed over a wide area and do not appear to be specifically associated with any specific cell group as identified in classical histology stains although they clearly overlap with the dorsal and ventral parts of the nucleus subceruleus.


A
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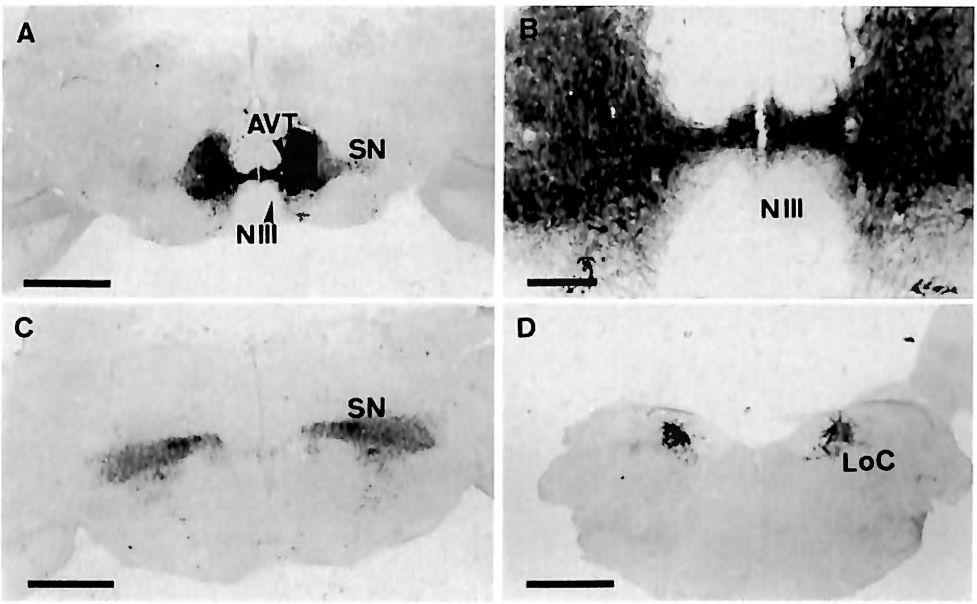

Fig. 2. – Photomicrographs illustrating the distribution of TH-ir cells in the midbrain of the canary. A: Group of TH-ir cells located at the caudal end of the area ventralis of Tsai where it expands in a dorsolateral direction to merge with the substantia nigra. B: Higher magnification of the same area. C: Group of TH-ir cells in the substantia nigra. D: TH-ir cells located at the level of the locus ceruleus. Magnification bar = 1 mm in A, C, D and 200 μm in B. AVT= area ventralis of Tsai, LoC= locus ceruleus, NIII= nervus oculomotorius (third nerve), SN= substantia nigra.

The caudal end of the pons is then essentially devoid of any TH-positive perikarya but small additional populations of immunoreactive cells are located in the medulla oblongata during a fairly long rostral to caudal extension of its more rostral part. Two subgroups of positive cells are recognizable at this level. One is located in dorsomedial position at the level of the nucleus tractus solitarii while the other is more ventral and is centered around the complex of the nucleus reticularis.

A similar distribution of TH-ir cells was observed in the midbrain of budgerigars (Fig. 3). The most rostral group of positive cells occurs at the level of the occulomotor nerves and is clearly identified as the AVT (Fig. 3A-B). It is followed at more caudal levels by the very abundant population of TH-ir cells corresponding to the SN (Fig. 3C-D).

Fig. 1. – Photomicrographs illustrating the distribution of TH-ir cells in the midbrain of the Zebra finch (left; A, C, E, G) and of the white-throated sparrow (right; B, D, F, H). The top panels illustrate the TH-ir cells located in the area ventralis of Tsai (A, B) and in the substantia nigra at low (C, D) and high (E, F) magnification. The two bottom panels illustrate the TH-ir cells located in the locus ceruleus at its rostral end at the level of the fasciculus longitudinalis medialis (G) or more caudally when TH-ir cells form a dense cluster that outlines the locus ceruleus (H). Magnification bar = 1 mm in A, C, D, G and 200 μm in B, E, F, H. AVT= area ventralis of Tsai, FLM= fasciculus longitudinalis medialis, LoC= locus ceruleus, NIII= nervus oculomotorius (third nerve), SN= substantia nigra.

Scattered TH-ir cells then appear at a slightly more caudal level in dorsal position, just lateral to the fasciculus longitudinalis medialis. At more caudal levels, the dorsal part of this cluster becomes very dense and clearly identifies the locus ceruleus located at the ventrolateral edges of the aqueduct as can be observed in sections stained by classical histology techniques (Fig. 3E-F).

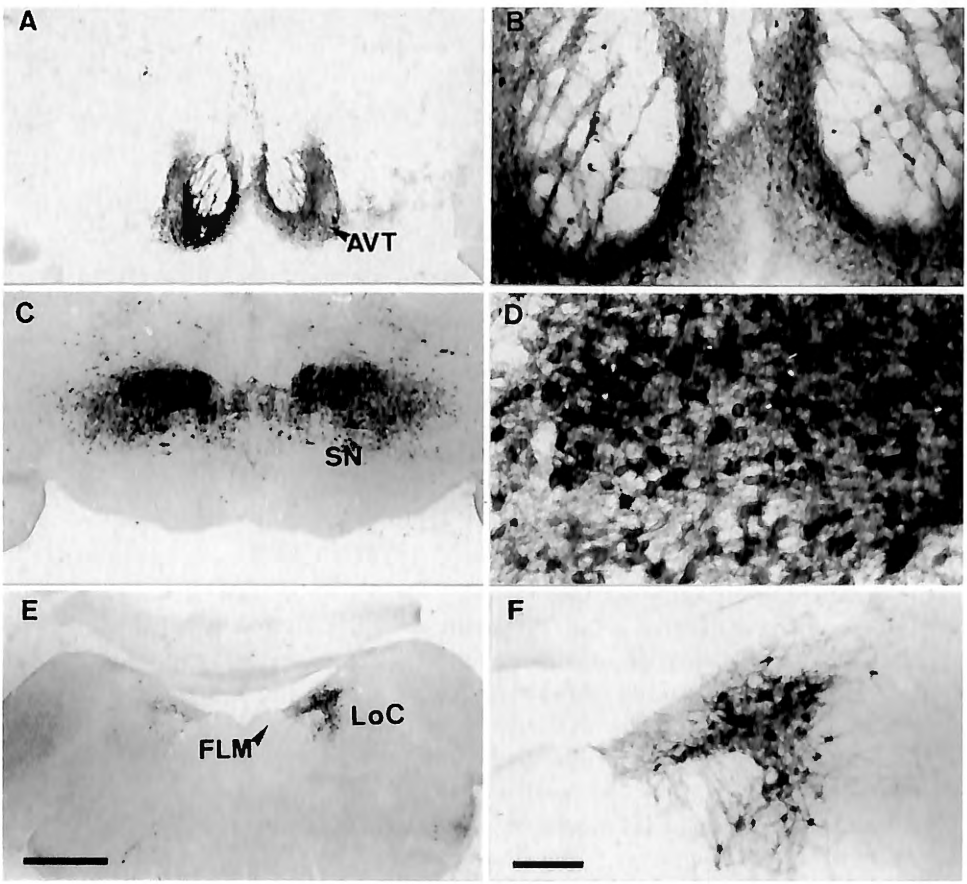

Fig. 3. – Photomicrographs illustrating the distribution of TH-ir cells in the midbrain of the budgerigar. The same areas are shown at low and high magnification in the left and right columns respectively. Panels are arranged in a rostral to caudal order from the top to the bottom. A-B: TH-ir cells at the level of the area ventralis of Tsai. C-D: TH-ir cells at the maximal extension of the substantia nigra. E-F: dense group TH-ir cells outlining the locus ceruleus. A few immunoreactive cells are also visible in a ventrolateral position. These cells belong to the subceruleus cell group. The magnification bar in the left column (panels A, C, and E)=1 mm; magnification bar in the right column (panels in B, D, and F)= 200 μm. AVT= area ventralis of Tsai, FLM= fasciculus longitudinalis medialis, LoC= locus ceruleus, NIII= nervus oculomotorius (third nerve), SN= substantia nigra.

DISCUSSION

Anatomical findings and homologies

The neurochemical studies described in the introduction demonstrate that the telencephalic nuclei of the song system receive a dense catecholaminergic innervation that is not visible in the corresponding brain areas of other birds (non-song birds). To our knowledge, the origin of these projections has not been investigated in detail. The presence of a connection between midbrain dopaminergic cells groups and the newly formed neurons of the dorsal telencephalon (in and around HVC) in the canary brain has, however, been reported in abstract form (BURD *et al.*, 1986).

As a first step in trying to establish the origin of these catecholaminergic pathways innervating the song control nuclei, we analyzed here the TH-ir cells groups in the mesencephalon and the metencephalon of 3 species of songbirds and one non-oscine vocal learner the budgerigar. We have identified in the brainstem of all 4 species the same groups of TH-ir cells that were previously described in the brain of the quail (BAILHACHE & BALTHAZART, 1993) and in other non-oscine species (REINER *et al.*, 1994). These cell groups had also been previously described in the brain of zebra finches (BOTTJER, 1993) and to some extent in budgerigars (TAKATSUKI *et al.*, 1981; SHIOSAKA *et al.*, 1981; TOHYAMA *et al.*, 1974).

A leading group of TH-ir cells was located at the edges of the third nerve in the area ventralis of Tsai (AVT), the avian homologue of the ventral tegmental area of mammals and it can therefore be considered as the homologue of the A10 dopaminergic cell group (REINER *et al.*, 1994) as defined in the nomenclature of Dahlström and Fuxe (DAHLSTRÖM & FUXE, 1964; BJÖRKLUND & LINDVALL, 1984). It extended in the dorso-lateral direction into the substantia nigra (SN), which can be considered as the avian homologue of the A9 dopaminergic cell group (DALHSTRÖM & FUXE, 1964; BJÖRKLUND & LINDVALL, 1984; REINER *et al.*, 1994) and more caudally into the retroruberal field (A8). These cell groups have also been described in other avian species (BAILHACHE & BALTHAZART 1993; MOONS *et al.*, 1994; REINER *et al.*, 1994).

The TH-ir cell group located at the mesencephalic-metencephalic junction at the ventro-lateral corner of the aqueduct is also found in other species in which it also reacts with antibodies directed against dopamine β-hydroxylase (quail: BAILHACHE & BALTHAZART, 1993; European starling, *Sturnus vulgaris*: BALL G.F. and BERNARD D.J., pers. comm.). This indicates the noradrenergic nature of this cell group that can be considered as the homologue of the locus ceruleus (noradrenergic cell group number A6: DAHLSTRÖM & FUXE 1964; MOORE & CARD, 1984) even if some avian brain atlases do not locate this nucleus in this exact position (KUENZEL & MASSON, 1988) or do not mention it at all (BAYLÉ *et al.*, 1974). The more scattered TH-ir populations that overlap partly with the nucleus subceruleus are also noradrenergic in other avian species (BAILHACHE & BALTHAZART, 1993; BALL G.F. and BERNARD D.J., pers. comm.) and may be the homologue of the mammalian A7 group but this conclusion can only be considered as tentative at present (REINER *et al.*, 1994).

No additional cell group could be detected in these species of vocal learners in comparison with the other species of non-oscines that have already been studied. This sugge-

sts that both in oscines and in psittaciforms the specialized innervation of the telencephalic song control nuclei have developed from catecholaminergic cell groups that are already present in species that do not display these specializations. This suggestion can be made more strongly for noradrenergic and adrenergic projections, the two catecholamines that appear to be synthesized solely in the brainstem, than for dopaminergic projections (REINER *et al.*,1994). Although, DA is primarily synthesized in the brainstem, cells synthesizing dopamine have also been identified in the diencephalon (REINER *et al.*,1994; MOONS *et al.*,1994). Therefore, the neuroanatomical and neurochemical specializations of the telencephalic song control nuclei seem to involve variations of projections from existing catecholaminergic cell groups rather than the evolution of novel cell populations. This analysis does not preclude the possibility that a more detailed quantitative study within these midbrain cell groups would reveal inter-specific differences indicative of the neural specialization associated with vocal learning. This study, however, clearly shows that the neurochemically specialized song control nuclei that are characterized by the unique presence of steroid receptors and of dense populations of catecholamine receptors (BALL, 1990; 1994) are not associated with the presence of new catecholaminergic cell groups in the brainstem.

Functional interpretations

The functional significance of the noradrenergic and dopaminergic inputs to the song control nuclei remains unclear at present. The steroidal activation of many reproductive behaviors is known to involve the modulation of noradrenergic transmission (NOCK & FEDER, 1981; CROWLEY *et al.*,1989; ETGEN *et al.*,1992). In particular the density of α_2 - adrenergic receptors has been shown to be modulated by steroids in brain areas important for the activation of reproductive behavior in mammals and in nuclei involved in the control of vocalizations in non-songbird species such as the Japanese quail (JOHNSON *et al.*,1988; BALL & BALTHAZART, 1990). Many of the song control nuclei are characterized by high levels of norepinephrine and these are known to be modulated by steroids (BARCLAY & HARDING 1990). It is therefore possible that the activation of song by steroids is also mediated by local changes in noradrenergic (and dopaminergic) transmission. This notion is supported by one recent experiment showing that treatment of male zebra finches with the noradrenergic neurotoxin DSP4 significantly decreases male courtship song and the behavioral deficit is correlated with the depletion of norepinephrine in some song control nuclei such as RA (BARCLAY *et al.*, 1992). The behavioral impairment appears to result from an attentional rather than a motor deficit: latency to intitiate singing during behavioral tests increases in drug-treated animals but once behavior is initiated it is identical to that produced by untreated controls. This correlates well with previous research that relates norepinephrine to attention and memory processes (MCGAUGH, 1985; SARA, 1985).

On the other hand, recent data indicate that the catecholaminergic innervation of the telencephalic song control nuclei, identified by TH immunocytochemistry (SOHA *et al.*,1995) or by direct assay of the NE and DA content in micropunched nuclei (HARDING *et al.*,1995) or by quantitative autoradiography of specific receptor subtypes (CASTO & BALL, 1994b), progressively develops during the first two to three months post-hatch in male zebra finches, that is during the period when song is learned. This raises the possi-

bility that the noradrenergic and/or dopaminergic neurotransmission could play a role in the development of the song system or in song learning.

These studies suggest potential roles for catecholamines in the development and activation of song but these now need to be established experimentally. In particular, it is important to evaluate the morphological and behavioral consequences of electrolytic or neurochemical lesions aimed at the noradrenergic or dopaminergic cell groups that innervate the song control nuclei. The present study clearly suggests that, in songbirds and psittaciforms as in other avian species, these should be located in the midbrain but the specific connections should now be investigated by retrograde tract tracing. Such studies would provide a sound basis for the lesion investigations and would also determine whether there is any variation in the pattern of catecholaminergic innervation between the two types of vocal learners. Tracing studies would also be essential to ascertain definitely whether the focal innervation of the song control nuclei derives from the same catecholaminergic cell groups that innervate, in a less dense and non specific manner, the general brain areas where these specialized nuclei have developed.

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SHORT NOTE

FIRST BELGIAN RECORD OF *COTESIA VESTALIS* (HALIDAY, 1834) (HYMENOPTERA, BRACONIDAE), PARASITE OF PROCLOSSIANA EUNOMIA (ESPER, 1799) (LEPIDOPTERA, NYMPHALIDAE), NEW HOST SPECIES

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Key-words : parasitoid, parasitism rate, *Cotesia vestalis*, *Proclossiana eunomia*, *Polygonum bistorta.*

The genus *Cotesia* Cameron, 1891 (Hymenoptera, Braconidae) was redefined by MASON (1), and contains the species of the *glomeratus*-group as defined by NIXON (2, 3), formely a part of the huge genus *Apanteles* Foerster, 1862. *Cotesia vestalis* (HALIDAY, 1834) appears to be the prioritary synonym of *C. cynthiae* (Nixon, 1974) (Van Achterberg, pers comm.). *C. vestalis* is only known from the Alps and Central Europe (Switzerland, Austria, Hungary and Bulgaria) (2, 3). *C. vestalis* , like many braconids, is an endoparasitoid species, larvae developing in caterpillars. Its only known host is the butterfly *Hypodryas cynthia* (Denis & Schiffermüller, 1775) (Lepidoptera, Nymphalidae) (2). In this note we report on the first observation of *C. vestalis* in Belgium, on a previously unknown host species, the bog fritillary, *Proclossiana eunomia* (Esper, 1799) and present some data on the effect of parasite larvae on their host.

We collected 112 last-instar caterpillars of *P. eunomia* from the Pisserotte peat bog (50°13'N, 5°48'E) from 26 May to 2 June 1995. These caterpillars were reared under various conditions. Caterpillars were grown in individual plastic bottles. They were fed every two days with fresh leaves of *Polygonum bistorta*, the only host plant in the study area. Caterpillars were weighed at each food replacement on a Sauter type MDT 160/0001 balance, 0.001 g precision.

During rearing of *P. eunomia* caterpillars, we recorded the presence of *C. vestalis.* Of the 112, 103 caterpillars (92%) were parasitized. The mean number of parasite cocoons per host caterpillar was 35.4 (SD=16.3). Larvae within the same caterpillar left their host synchronously. Caterpillar behaviour was strongly affected by this event: in all our observations, caterpillars stood motionless during the emergence of parasite larvae (Fig. 1, left). Larvae emerged along the whole body of the caterpillar and immediately spun individual bright yellow cocoons, forming a muff around the caterpillar. After larval emergence, caterpillars moved away from this structure (Fig. 1, right). The caterpillars never resumed feeding and in every case died a few days later.

The departure of parasite larvae strongly decreased the weight of the caterpillar by about 50%: the mean weight loss was 119 mg (SD=48 mg, n=63). This loss remained constant what-

ever the developmental conditions of the caterpillar. However, parasite larvae developmental time strongly depended on rearing conditions ($F_{3,76}$=46.9; P=0.0001) and is positively related to temperature in controlled conditions ($F_{1,23}$=23.6; P=0.0001) (Table 1).

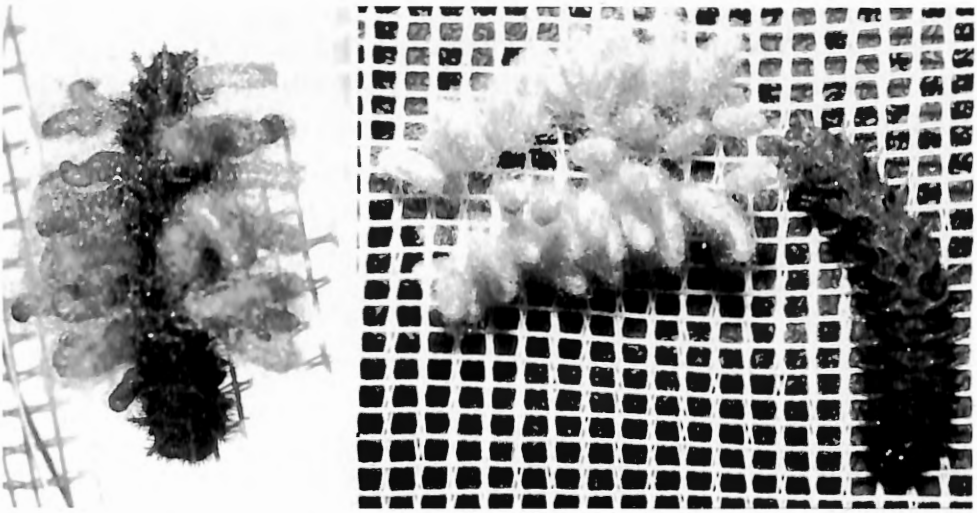

Fig. 1. – Left: emergence of *C. vestalis* larvae from the body of *P. eunomia* caterpillar. (1cm = 0.25 Lm) – Right: *P. eunomia* caterpillar escaping from the muff formed by *C. vestalis* cocoons. (1cm = 0.3 Lm).

TABLE 1

Mean duration of the development of P. eunomia *caterpillars before emergence of the parasite larvae at different temperature conditions (time between the collection in the field and the emergence of parasite larvae) Outside = at the back door of the laboratory, n = number of caterpillars.*

	Temperature conditions (°C ± SD)	*n*	*duration (days ± SD)*
controlled A	23.5 ± 1.8	11	5.9 ± 1.8
controlled B	28.8 ± 1.4	14	3.0 ± 1.2
room	15.7 ± 1.8	5	6.6 ± 4.3
outside	16.1 ± 5.2	50	17.7 ± 5.8

Parasitism affected the developmental time of the host: 4 of the 5 healthy caterpillars pupated before the first emergence of the parasite larvae in the same temperature conditions. The 5 uninfected caterpillars pupated successfully (1 in controlled conditions B and

4 outside). The 4 remaining larvae died without being dissected and were probably parasitized which can raise the parasitism rate up to 95%.

The distribution of *C. vestalis* shows a high disjunction between the Belgian and alpine/medio-European populations. This pattern results probably more from a lack of data than from a true biogeographical process. On the other hand, the host species *Proclossiana eunomia* has a boreo-montane distribution, while *Hypodryas cynthia* is an alpine species, but both host species occur in Bulgaria (4).

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