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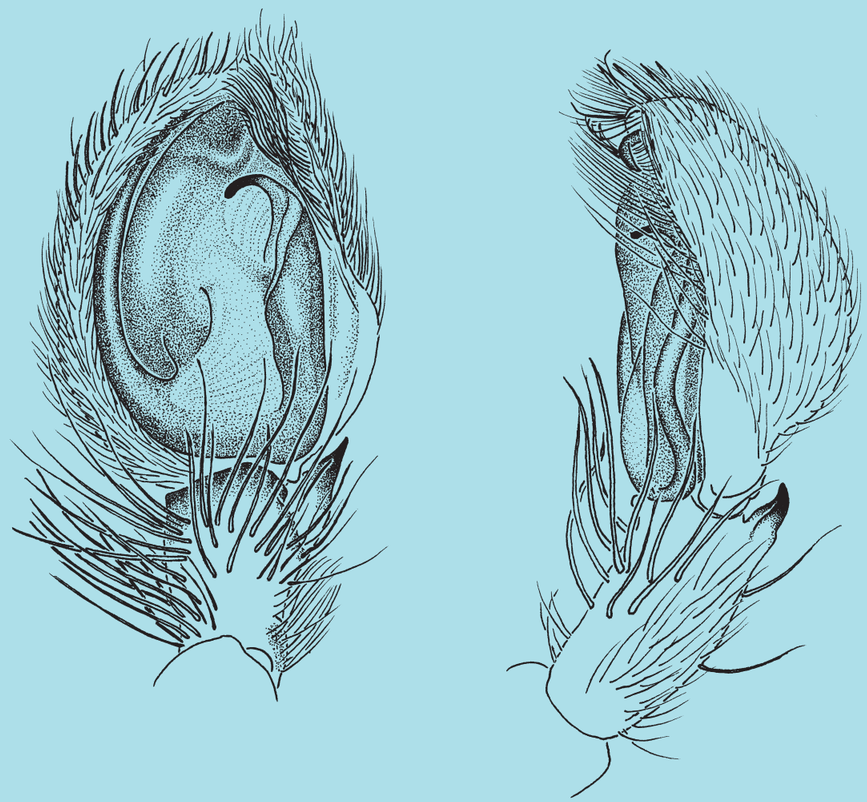

BELGIAN JOURNAL OF ZOOLOGY

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Belgian Journal of Zoology

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ERRATUM

Occurrence of a continental slope decapod crustacean community along the edge of the minimum oxygen zone in the south eastern Gulf of California, Mexico

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In this article that appeared in the Belgian Journal of Zoology, 131 (Supplement 2, Proceedings of the 8th Benelux Congress of Zoology). wrong files have been used for figures 5, 6 and 7. The right figures and captions are given below. The interested reader can receive a PDF file of the complete corrected article from the author (michel@mar.icmyl.unam.mx) or from the editor (BJZ@luc.ac.be) or can be downloaded from the journal's web page (http://kbvd-www.uia.ac.be/kbvd/bjz).

On page 105:

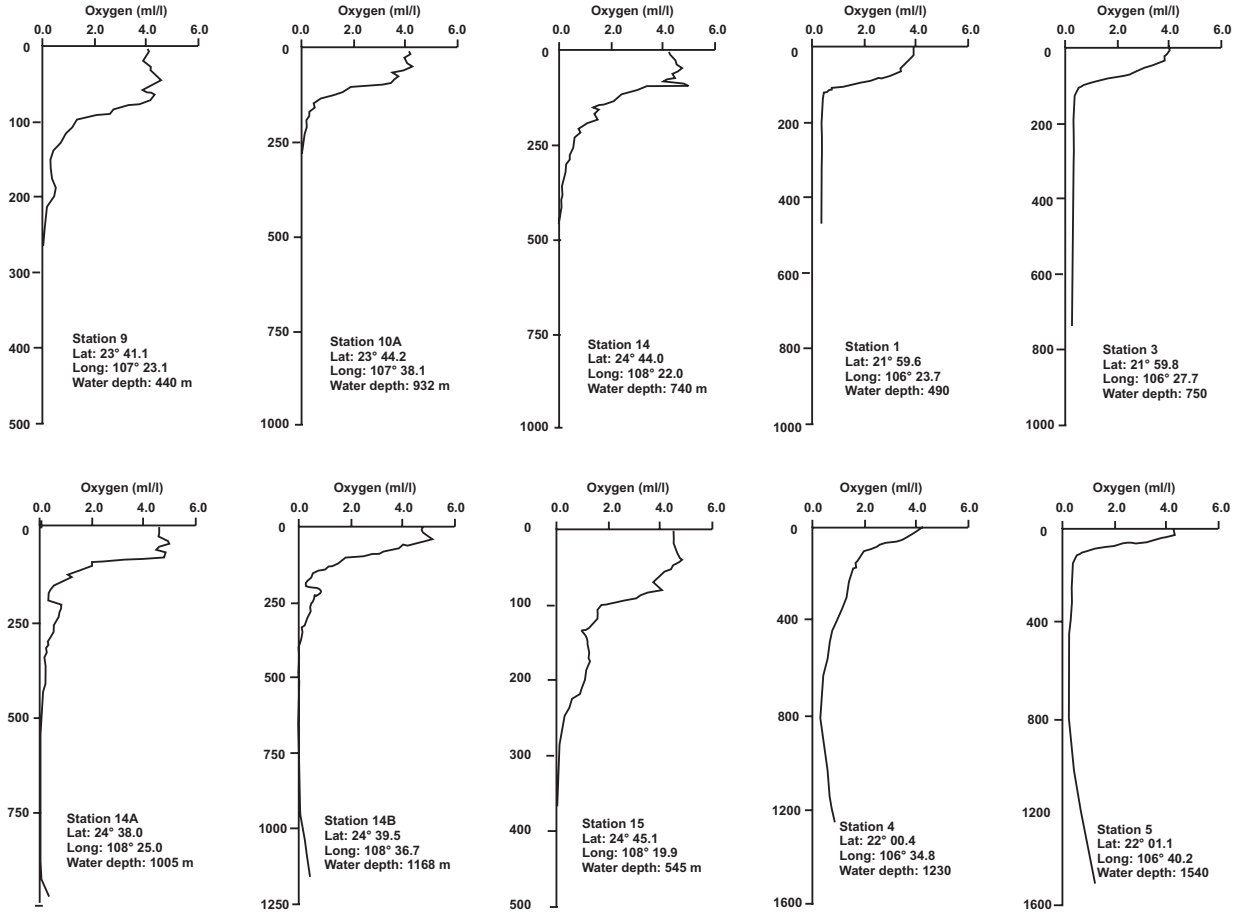

Fig. 5. – Vertical distribution of oxygen at selected stations; TALUD III (St. 9, 10A, 14, 14A, 14B and 15) and TALUD IV (St. 1, 3, 4 and 5) cruises.

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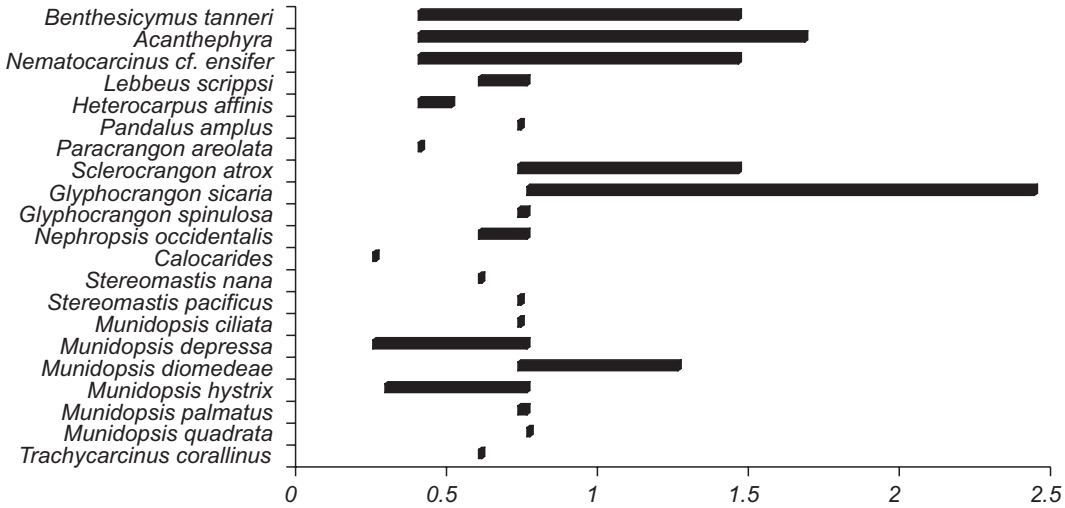

Fig. 6. – Interval of epibenthic oxygen content corresponding to species collected during the TALUD III and IV cruises.

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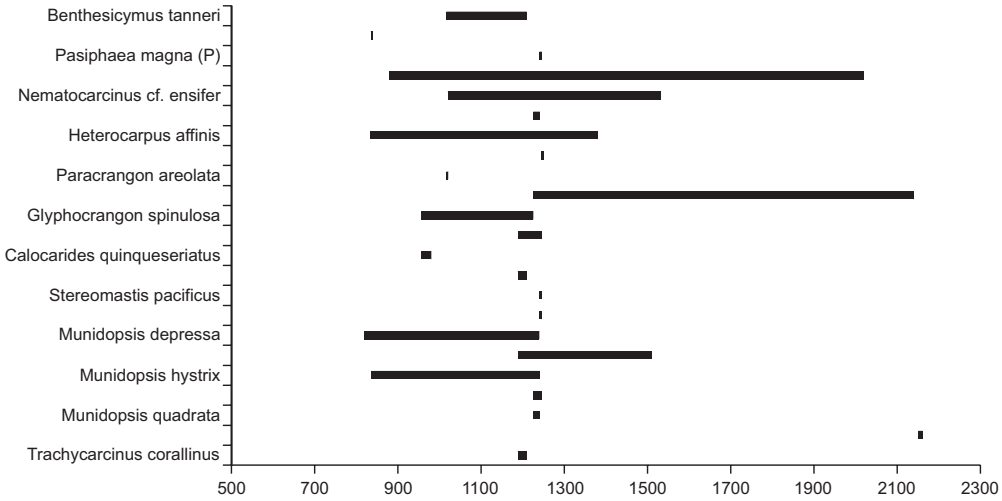

Fig. 7. – Bathymetric range observed for species collected during the TALUD III and IV cruises. (P) probably pelagic species.

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Segonzactis hartogi sp. n. (Condylanthidae) and other sea anemones of the Aegean deep water

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ABSTRACT. A new deep-water actiniarian species, *Segonzactis hartogi* sp.n. is described from the Mediterranean (Aegean Sea). This is the first record of the family Condylanthidae from the Mediterranean Sea. Morphometric and ecological differences between the new species and *S. platypus* Riemann-Zürneck, 1979, the only known species of the genus, are discussed. Another six species of the Actiniarian fauna of the deep Aegean are also presented. Information is given on their geographical distribution and habitat.

KEY WORDS: *Segonzactis*, Actiniaria, Deep water fauna, Aegean Sea.

INTRODUCTION

Forty nine valid actiniarian species belonging to 16 families were previously known from the Mediterranean and the Black Sea (ANDRES, 1884; CARLGREN, 1949; PAX & MÜLLER, 1962; SCHMIDT, 1972; DOUMENC et al., 1985; Gili, 1987; CHINTIROGLOU et al., 1997; VAFIDIS et al., 1997; WILLIAMS, 1997; etc.). Twenty nine of them belonging to 10 families have been reported from the Aegean Sea (DOUMENC et al., 1985; DOUMENC et al., 1987; CHINTIROGLOU & DEN HARTOG, 1995; LOUKMIDOU et al., 1996; VAFIDIS et al., 1997).

During the biological cruises of the RV "FILIA" in the Aegean Sea, in 1987, at depths ranging from 80 to 1200 m, and from investigations of the University of Thessaloniki in the N. Aegean Sea, since 1992, at depths from 150 to 1200 m, seven actiniarian species were collected from deep water. One of those anemones collected belongs to the genus *Segonzactis* Riemann-Zürneck, 1979. This is the first record of the family Condylanthidae Stephenson, 1922 in the Mediterranean Sea. World-wide, *Segonzactis* is represented by only one species, *S. platypus*, found on the abyssal region of the Bay of Biscay between 4237 and 4850 m by RIEMANN-ZÜRNECK (1979) and from Porcupine abyssal plain at 4845 m depth by RIEMANN-ZÜRNECK (1998).

The main goal of the present paper is to give a detailed description of the new species, to compare it with *S. platypus*, and to present and discuss the actiniarian fauna of the deep Aegean Sea.

MATERIAL AND METHODS

Specimens have been collected since 1987 from 17 sampling stations in the Aegean Sea (Fig. 1), at depths varying

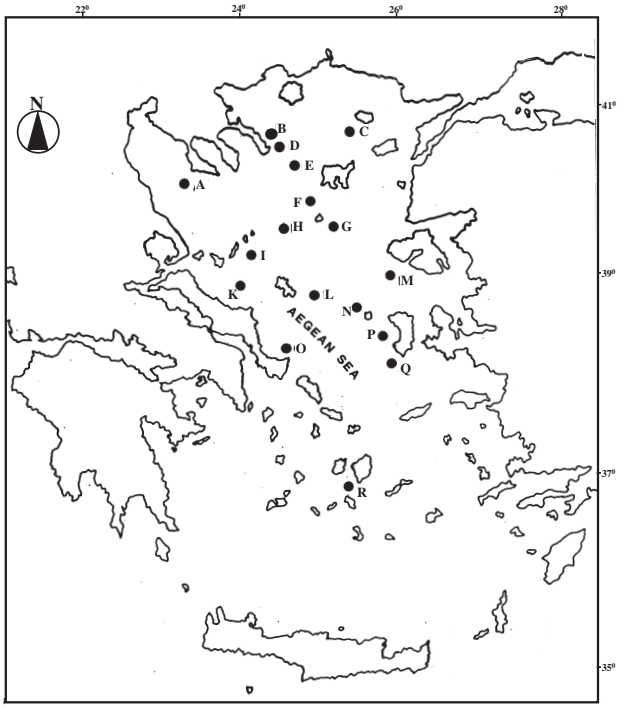

Fig. 1. – Map of the Aegean Sea, indicating the sampling stations.

Corresponding author: D. Vafidis, e-mail: dvafidis@otenet.gr

from 80 to 1200 m using Smith-McIntyre grab, fishing and Agassiz trawls. All samples were preserved in 6% formalin/sea-water. Paraffin sections, 8 μm thick and stained with hematoxylin and eosin, were prepared for histological study according to DOUMENC et al. (1985). Measurements of cnidae were taken from undischarged capsules in squash preparations (CHINTIROGLOU et al., 1997). Nematocyst nomenclature used was as proposed by ENGLAND (1991). The specimens are deposited in the Museum of the Department of Zoology in the Aristoteleion University of Thessaloniki (MZDAUT) and in the Museum of the Fisheries Research Institute of Kavala (MFRI).

RESULTS

Order ACTINIARIA R.Hertwig, 1882
Subtribe ENDOMYARIA Stephenson, 1921
Family CONDYLANTHIDAE Stephenson, 1922

Segonzactis hartogi sp.n.

(Figs 2, 3, 4, Table 1)

Type locality. Holotype, from Mount Athos, (40º17'50''N, 26º23'20''E), North Aegean Sea at 750 m depth, on silty bottom. Paratype (1) Mount Athos, (40º13'30''N, 27º26'40''E), North Aegean Sea at 800 m depth, on silty bottom. Paratypes (2) and (3) from Ios island (36º38'50''N, 25º16'40''E) Central Aegean Sea at 80 m depth, on sand-silty bottom.

Type Material. Holotype Museum of the Fisheries Research Institute of Kavala (MFRI IA_{24}, collected 2.06.91). One paratype (MFRI IA_{25}, collected 2.06.91), Museum of the Fisheries Research Institute of Kavala and two paratypes (MZDAUT B_{784}, MZDAUT B_{785} collected 29.05.91), Museum of the Department of Zoology, University of Thessaloniki.

Etymology. The specific name is dedicated in the memory of Dr. Koos den Hartog, Curator of Coelenterata et al., at the National Museum of Natural History of Leiden, The Netherlands.

Biometry of polyps. Diameter of scapus 5-7 mm; height of column 10-19 mm; diameter of base 15-27 mm; specimens weigh 0.2-2.3gr.

Colour. Pedal disc and column transparent with visible insertions of the mesenteries (Fig 2a-d).

External morphology. Column divided in scapus and scapulus (Fig. 2a). Scapus short and covered by a cuticula. Scapulus with membranous appendages of the mesogloea. When totally contracted the scapus disappears and the animal then becomes disc-shaped (Fig. 2b). There are eight tentacles, short and thick. Actinopharynx with only one ventral siphonoglyph.

Internal anatomy. Mesenteries separable into macrocnemes and microcnemes, only 8 macrocnemes. Mesenteries in five cycles, arranged hexamerously, several pairs of the fifth cycle often lacking. Mesenteries

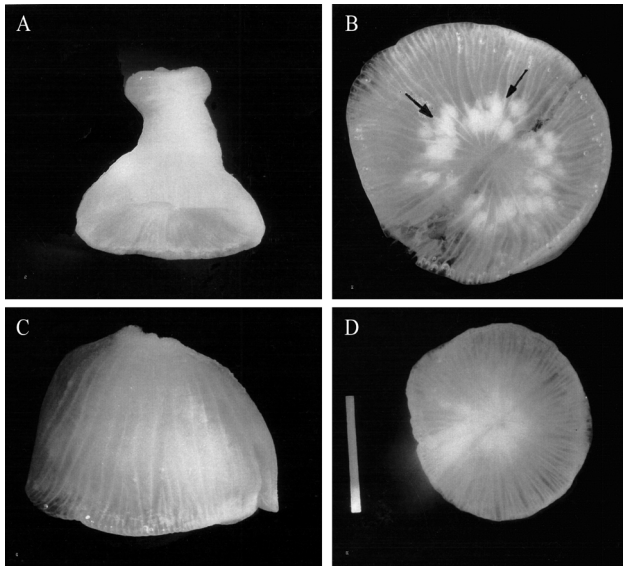

Fig. 2. – *Segonzactis hartogi* sp.n. a-b. Holotype. – a. Side view of the anemone (slightly contracted animal). – b. Lower side (pedal disc) of the anemone. – c. Side view of a completely contracted animal (paratype). – d. Lower side (pedal disc), showing the presence of gonads (paratype).

from the third up to the fifth cycle only present in the lower-most part of the body (Fig. 2c). Radial muscles of the oral disc weak, meso-ectodermal. Marginal sphincter endodermal, weak (Fig. 3a). Retractors of the eight macrocnemes circumscribed reniform and very strong (Fig. 3b). Parietobasilar muscles strong (Fig. 3c). Absence of basilar muscles. Female gonads present in three specimens occurring in the eight macrocnemes (Fig. 2d).

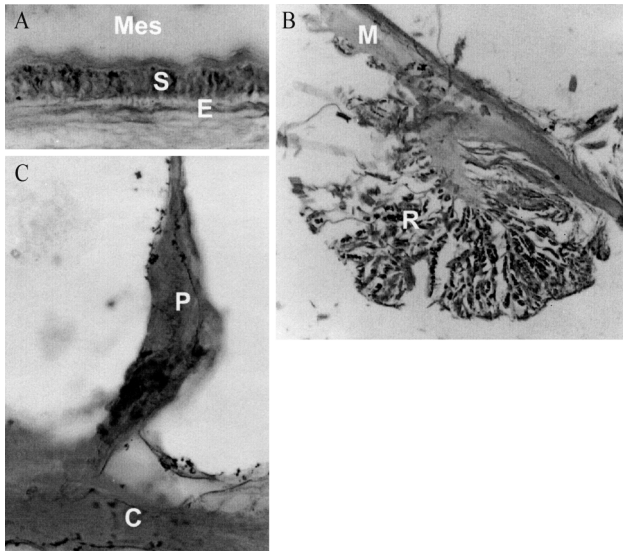

Fig. 3. – *Segonzactis hartogi* sp.n. a-c. – a. Vertical section showing diffuse sphincter muscle. – b. Transverse section of macrocneme showing retractor muscle. – c. Transverse section of macrocneme showing parietobasilar muscle. **C** column; **E** endoderm; **M** macrocneme; **Mes** mesogloea; **P** parietobasilar muscle; **R** retractor muscle; **S** sphincter muscle.

Cnidom. Cnidom* consists of spirocysts, basitrichs and microbasic p-mastigophores (Fig. 4a-e).

(a) Tentacles. basitrichs - length: 7.0-13.0 µm (mean 10.40 ± 1.7 µm, n = 60), width: 1.0-3.0 µm (mean 1.8 ± 0.47 µm, n = 60); spirocysts - length: 6.0-21.8 µm (mean 13.19 ± 3.53 µm, n = 58), width: 1.0-4.0 µm (mean 1.9 ± 0.6 µm, n = 58).

(b) Filament. basitrichs - length: 10.0-24.0 µm (mean 17.02 ± 2.69 µm, n = 20), width: 1.5-4.0 µm (mean 2.7 ± 0.73 µm, n = 20); mi-p. mastigophores (a) - length: 14.0-29.5 µm (mean 21.44 ± 4.35 µm, n = 47), width: 2.5-7.0 µm (mean 4.9 ± 1.27 µm, n = 47), shaft 8.0-24.0 µm (mean 13.16 ± 3.26 µm, n = 47); mi-p. mastigophores (b) - length: 9.0-9.5 µm (mean 9.25 ± 0.29 µm, n = 4), width: 3.0-4.0 µm (mean 3.37 ± 0.48 µm, n = 4), shaft 5.0-7.0 µm (mean 6.25 ± 0.96 µm, n = 4).

Diagnosis. Column divided in scapus and scapulus. Scapus short and covered by a cuticula. Pedal disc without basilar muscles. Sphincter endodermal, very weak. Eight tentacles, short and thick. Mesenteries in five cycles, arranged hexamerously. Eight mesenteries developed as macrocnemes, with circumscribed retractors, gonads and mesenterial filaments. Only one very strong siphonoglyph. Parietobasilar muscles strong. Cnidom: Spirocysts, basitrichs, microbasic p-mastigophores (two types).

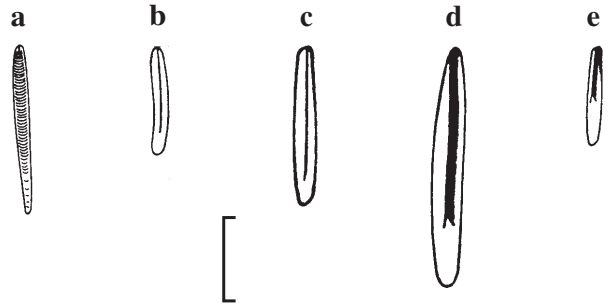

Fig. 4. – *Segonzactis hartogi* sp.n. (nematocyst signature) (see also Table 1). a-b. Tentacle. – a. Spirocyst. – b. Basitrich. c-e. Filament. – c. Basitrich. – d. Microbasic p-mastigorhore (a). – e. Microbasic p-mastigorhore (b) (scale 8 µm).

TABLE 1

Comparison between Segonzactis platypus Riemann- Zürneck, 1979 and Segonzactis hartogi sp.n.

		S. platypus	*S. hartogi*
Height of column (mm)		—	10-19
Diameter of scapus (mm)		5-10	5-7
Diameter of pedal disc (mm)		18-35	15-27
Weight (gr)		0.65-1.7	0.2-2.3
Tentacle	spirocysts (µm)	81 x 3.5-4.5	6-21.8 x 1-4
	basitrichs (µm)	27-32 x 3-4	7-13 x 1-3
Scapus	basitrichs (µm)	14.5-23 x 3-3.5	—
Filament	basitrichs 1 (µm)	32-33 x 4-4.5	—
	basitrichs 2 (µm)	14-18 x 3-3.5	10-24 x 1.5-4
	mi-p.mastigophores (a) (µm)	25-30 x 5-5.5	14-29.5 x 2.5-7
	Shaft (µm)	—	8-24
	mi-p.mastigophores (b) (µm)	—	9-9.5 x 3-4
	Shaft (µm)	—	5-7
Geographical distribution		Bay of Biscay, Porcupine abyssal plain	Mediterranean (Aegean Sea)
Vertical distribution (m)		4237-4850	80-800
References		Riemann-Zürneck (1979, 1998)	Present work

Subtribe ACONTIARIA CARLGREN (in Stephenson, 1935)

Family HORMATHIIDAE Carlgren, 1925

Hormathia coronata (Gosse,1858)

Hormathia coronata, Schmidt, 1972, p. 29, Abb. 20 a, b.- den Hartog, 1977, p. 237, fig. 5.- Manuel, 1981, p. 166, fig. 57.- Doumenc et al., 1985, p. 515.- Gili, 1987, p. 390, fig. 4.107 a, c.

Material examined. 29 specimens (stations: A, C, D, O, P) attached to various types of substrata including gastropod and bivalve shells, stones, rocks and biogenic detritus, between 150 and 170 m.

Distribution. This Atlanto-Mediterranean species is known from: – Mediterranean basin: Western Mediterranean (Carus, 1885; Schmidt, 1972; Azouz, 1973; Gili, 1987; etc.); Central Mediterranean (Parenzan, 1973; Arena & Li Greci, 1973; etc.); Adriatic Sea (Pax, 1952; Pax & Müller, 1962; Gamulin-Brida, 1974;

* Histological sections did not reveal any conspicuous nematocysts in the scapus.

etc.); Eastern Mediterranean (Doumenc et al., 1985) – Atlantic ocean: Eastern Atlantic, from all coasts of western Europe and Britain (Stephenson, 1935; Carlgren, 1949; Lafargue, 1969; den Hartog; 1977; Manuel, 1981; etc.).

Short description. Base broad and moderately adherent. Column divided into scapus and scapulus. Scapus with small solid tubercles, which tend to form 12 longitudinal rows distally, leading into 12 low, inconspicuous longitudinal ridges on the scapulus. Thin periderm is usually present on the scapus. Tentacles moderate in length, neatly and hexamerously arranged in five cycles. Sphincter perioral mesogloean. Retractor muscles diffuse and not well developed. Cnidom: Spirocysts, basitrichs, microbasic p-mastigophores, microbasic b-mastigophores.

Actinauge richardi (Marion, 1882)

Actinauge richardi, Stephenson, 1935, p. 289, text-figs. 7(D), 20, 31, 40, 94-98.- Manuel, 1981, p. 172, fig. 60. – Doumenc et al., 1985, p. 513, fig. 3. – Gili, 1987, p. 389, fig. 4.107 b, e.

Material examined. 42 specimens (stations: B, E, F, G, K, M, N, Q) were collected on silty bottoms at depths from 105 to 320 m.

Distribution. This Atlanto-Mediterranean species is known from: – Mediterranean basin: Western Mediterranean (Gravier, 1922; Stephenson, 1935; Rossi, 1958; Gili, 1987; etc.); Central Mediterranean (Arena & Li Greci, 1973); Adriatic Sea (Bombace & Froglia, 1973); Eastern Mediterranean (Doumenc et al., 1985) – Atlantic ocean: Eastern Atlantic, from Norway to Senegal (Stephenson, 1935; Carlgren, 1949; Manuel, 1981; etc.).

Short description. Basal disc forming a rounded cavity enclosing mud or sand. Column divided into scapus and scapulus. Scapus with numerous often large solid tubercles and periderm. Tentacles hexamerously arranged in five cycle. Sphincter mesogloean very strong. Retractor muscles diffuse and not well developed. Cnidom: Spirocysts, basitrichs, microbasic p-mastigophores (two types), microbasic b-mastigophores.

Calliactis parasitica (Couch, 1842)

Calliactis parasitica, Schmidt, 1972, p. 40, Abb. 20 d.- Manuel, 1981, p. 174, figs. 2C, 3, 61. – Doumenc et al., 1985, p. 516. – Gili, 1987, p. 393, fig. 4.105 e, 4.106 a, d.

Material examined. 77 specimens (stations: A, C, F, H, K, L, N, O, Q, R) attached to various types of substrata including gastropod and bivalve shells, stones, rocks and biogenic detritus, between 5 and 160 m.

Distribution. This Atlanto-Mediterranean species is known from: – Mediterranean basin: Western Mediterranean (Carus, 1885; Schmidt, 1972; Azouz, 1973; Gili, 1987; etc.); Central Mediterranean (Parenzan, 1973; Arena & Li Greci, 1973; Micallef & Evans, 1968; etc.); Adriatic Sea (Pax, 1952; Pax & Müller, 1953, 1962; Gamulin-Brida, 1974; etc); Eastern Mediterranean (Geldiay & Koçatas, 1972; Doumenc et al., 1985; Chintiroglou & Koukouras, 1991) – Atlantic ocean: Eastern Atlantic, from North Sea and around to south-west Europe (Stephenson, 1935; Carlgren, 1949; Manuel, 1981; etc.).

Short description. Cinclides fairly prominent in a zone just above the limbus. Tentacles moderate in length and very numerous, hexamerously arranged. Acontia are readily emitted from cinclides and actinopharynx. Column fairly wide. Sphincter mesogloean very strong. Retractor muscles diffuse and not well developed. Cnidom: Spirocysts, microbasic p-mastigophores, microbasic b-mastigophores.

Adamsia palliata (Bohadsch, 1761)

Adamsia palliata, Schmidt, 1972, p. 35, Abb. 19 c.- Gili, 1987, p. 394, figs. 4.101 b, 4.106 c.

Adamsia carciniopados, Manuel, 1981, p. 176, fig. 62.- Doumenc *et al*., 1985, p. 517.

Material examined. 12 specimens (stations: D, G, I, O, R) attached to gastropods cells of the species *Gibbula magus* and *Lunatia catena* inhabited by hermit crab *Pagurus alatus*, between 90 and 120 m.

Distribution. This Atlanto-Mediterranean species is known from: – Mediterranean basin: Western Mediterranean (Carus, 1885; Schmidt, 1972; Gili, 1987; etc.); Central Mediterranean (Parenzan, 1973; Arena & Li Greci, 1973; etc.); Adriatic Sea (Pax, 1952; Pax & Müller, 1953, 1962; etc.); Eastern Mediterranean (Pérès & Picard, 1958; Doumenc et al., 1985) – Atlantic ocean: Eastern Atlantic, from all coasts of western Europe and Britain (Stephenson, 1935; Carlgren, 1949; Lafargue, 1969; Manuel, 1981; etc.).

Short description. Base forming two lobes enveloping a hermit grab and its gastropod shell so that the disc is beneath the crab with the two lobes meeting on its dorsal side. Tentacles very numerous and short. Cinclides are present on low mounds on the lower part of the column. Retractor muscles diffuse and not well developed. Cnidom: Spirocysts, microbasic p-mastigophores, microbasic b-mastigophores.

Amphianthus dohrnii (Koch, 1878)

Amphianthus dohrnii, Schmidt, 1972, p. 40, Abb. 20 d. – Manuel, 1981, p. 178, fig. 63.- Gili, 1987, p. 392, fig. 4.106 g, i.

Material examined. 4 specimens (station: D) found on the gorgonian *Eunicella verrucosa* at 100 m depth.

Distribution. This Atlanto-Mediterranean species is known from: – Mediterranean basin: Western Mediterranean (ANDRES, 1884; CARUS, 1885; SCHMIDT, 1972; ROSSI, 1950; GILI, 1987); Central Mediterranean (ARENA & LI GRECI, 1973); Adriatic Sea (PAX & MÜLLER, 1962); Eastern Mediterranean (VAFIDIS et al., 1997) – Atlantic ocean: Eastern Atlantic, from Portugal, west coasts of France and southern of British Isles (NOBRE, 1931; STEPHENSON, 1935; CARLGREN, 1949; MANUEL, 1981).

Short description. Base adherent, often elongated along the axis of the substratum (usually gorgonians or hydroids). Column short and flaring out widely to the disc, not divided into regions. Cinclides are few. Tentacles short or moderate, irregularly arranged in four or five cycles. Cnidom: Spirocysts, basitrichs, atrichs, microbasic p-mastigophores (two types).

Family SAGARTIIDAE Gosse, 1858

Sagartiogeton undatus
(O.F. Müller, 1788)

Sagartiogeton undatus, SCHMIDT, 1972, p. 55. – MANUEL, 1981, p. 158, figs. 54A-C. – DOUMENC et al., 1985, p. 519, fig. 4. – GILI, 1987, p. 385, fig. 4.108 g, h.

Material examined. 19 specimens (stations: B, C, F, K, L, N, Q) attached to various types of substrata including gastropod shells, stones, rocks, between 15 and 260 m.

Distribution. This Atlanto-Mediterranean species is known from: – Mediterranean basin: Western Mediterranean (CARUS, 1885; SCHMIDT, 1972; FEBVRE, 1968; GILI, 1987; etc.); Central Mediterranean (PARENZAN, 1973); Adriatic Sea (CARUS, 1885; PAX & MÜLLER, 1962; etc); Eastern Mediterranean (DOUMENC et al., 1985); Black Sea (PASPALEFF, 1933; BACESCU et al., 1971; MÜLLER, 1971, 1973) – Atlantic ocean: Eastern Atlantic, from Scandinavia and around south-west Europe (STEPHENSON, 1935; CARLGREN, 1949; MANUEL, 1981; etc.).

Short description. Base wide, usually wider than the disc, its outline relatively smooth as basal laceration does not occur (cf. *S. laceratus*). Column very tall in full extension, cylindrical but when tightly contracted it may become remarkably flat. Disc translucent, pale grey or brown, usually with narrow opaque cream lines on the mesenteric insertions. Tentacles long or very long and gracefully displayed, hexamerously arranged. Cnidom: Spirocysts, basitrichs, microbasic p-mastigophores (two types), microbasic b-mastigophores.

DISCUSSION

Sixteen families of actiniarian fauna were previously known from the Mediterranean. Of those only the Hormathiidae and Sagartiidae have representatives in the bathyal zone. Six species – *Actinauge richardi* (Marion, 1882), *Calliactis parasitica* (Couch, 1842), *Adamsia palliata* (Bohadsch, 1761), *Hormathia coronata* (Gosse, 1858), *Amphianthus dohrnii* (Koch, 1878) and *Sagartiogeton undatus* (O.F. Müller, 1788) – have been reported in the deep Mediterranean waters. Three of them, i.e. the species *A. richardi*, *H. coronata* and *A. dohrnii* have been characterized as real residents of the lower circalittoral or the upper bathyal, because the other three have a wide bathymetrical range, from the upper infralittoral to bathyal zone (CARLGREN, 1949; SCHMIDT, 1972; PÉRÈS, 1985; DOUMENC et al., 1985).

The family Condylanthidae Stephenson, 1922, has not previously been recorded from the Mediterranean Sea. It consists of six genera – *Condylanthus* Carlgren, 1899; *Pseudormathia* Carlgren, 1928; *Macrocnema* Carlgren, 1928; *Charisea* Torrey, 1902; *Charisella* Carlgren, 1949, *Segonzactis* Riemann-Zürneck, 1979 – which differ from each other by the presence/absence of the pseudospherules, the presence of tubercles of the column, and by the number of the macrocnemes (CARLGREN, 1949; DOUMENC & VAN PRAËT, 1987). Their geographical distribution is limited to polar zones with the exception of species *Charisella elongata* (Carlgren, 1950) and *Macrocnema nicobarica* Carlgren, 1928, which are tropical, and *Segonzactis platypus* Riemann-Zürneck, 1979, which was found in the abyss of the Bay of Biscay. Only *M. nicobarica* and *S. platypus* have been found in deep waters, in the bathyal and abyssal zone respectively (CARLGREN, 1928; RIEMANN-ZÜRNECK, 1979).

S. platypus Riemann-Zürneck, 1979 and *S. hartogi* sp. n. are distinguished from each other on the cnidae biometry (Table 1). Other important characteristics, geographical and vertical distribution of the two species are also given in Table 1.

In order to explain the very low degree of endemism and a low diversity of the deep-sea fauna of the Mediterranean compared to the fauna of the Northeast Atlantic, BOUCHET & TAVIANI (1992), suggest that the larval ecology of individual species is the most important factor governing the composition of the deep Mediterranean benthos. Primarily on the basis of research on gastropods, they suggest that much of this Mediterranean deep-sea fauna consists of reproductively sterile pseudo-populations that are constantly derived through larval inflow from Atlantic mother populations. The presence of female gonads in the macrocnemes of *S. hartogi* in the Aegean Sea suggests the reproduction ability of this species and consequently the existence of a real population in this area.

The occurrence of *S. hartogi* in the Aegean Sea raises the number of the Actiniarian species known from Mediterranean to fifty and those from Aegean Sea to thirty. *S. hartogi* is considered an endemic Mediterranean species.

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Osteology and myology of the cephalic region and pectoral girdle of *Glyptothorax fukiensis* (Rendahl, 1925), comparison with other sisorids, and comments on the synapomorphies of the Sisoridae (Teleostei: Siluriformes)

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ABSTRACT. The cephalic and pectoral girdle structures of the sisorid *Glyptothorax fukiensis* (tribe Glyptothoracini) are described and compared with those of representatives of the other three sisorid tribes, namely *Glyptosternon reticulatum* (tribe Glyptosternini), *Bagarius yarreli* (tribe Bagariini) and *Gagata cenia* (tribe Sisorini), as well as with those of several other catfishes, as the foundation for a discussion on the synapomorphies and phylogenetic relationships of the Sisoridae. Our observations and comparisons support de Pinna's (1996) phylogenetic hypothesis, according to which the Asiatic Sisoridae is the sister-group of a clade formed by the Neotropical Aspredinidae and the Asiatic Erethistidae. In addition, our observations and comparisons pointed out a new, additional character to diagnose the family Sisoridae, namely: presence of a well-developed, wide, deep fossa on the neurocranial floor between the ventro-medial surface of the pterotic and the ventro-lateral surface of the exoccipital.

KEY WORDS: catfish, cephalic region, comparative morphology, *Glyptothorax*, myology, pectoral girdle, phylogeny, Sisoridae, Siluriformes.

INTRODUCTION

The Siluriformes is "one of the economically important groups of fresh and brackish water fishes in the world: in many countries, they form a significant part of inland fisheries; several species have been introduced in fish culture; numerous species are of interest to the aquarium industry where they represent a substantial portion of the world trade" (TEUGELS, 1996). Among the 35 siluriform families (FERRARIS & DE PINNA, 1999), the Sisoridae, with 14 genera and more than 97 species, is one of the largest and most diverse Asiatic families (DE PINNA, 1996). This higher-level phylogeny and systematics of the Sisoridae were recently revised by DE PINNA (1996), who concluded that six genera previously included in this family, namely *Conta* Hora, 1950, *Erethistes* Müller and Troschel, 1849, *Erethistoides* Hora, 1950, *Hara* Blyth, 1860, *Laguvia* Hora, 1921 and *Pseudolaguvia* Misra, 1976, were more closely related to the Neotropical Aspredinidae than to the remaining 14 sisorid genera. Therefore, these six genera were assigned to the family Erethistidae, which, according to DE PINNA (1996), is the sister-group of the Neotropical Aspredinidae, with the clade formed by these two families being, in turn, the sister-group of the Sisoridae sensu stricto. Still according to the phylogenetic results of DE PINNA (1996), the Sisoridae (sensu stricto) can be divided into the subfamilies Sisorinae and Glyptosterninae, with the former comprising the tribes Sisorini (including the genera *Sisor* Hamilton, 1822, *Gagata* Bleeker, 1858, *Nangra* Day, 1877) and Bagariini (*Bagarius* Bleeker, 1853), and the latter comprising the tribes Glyptothoracini (*Glyptothorax* Blyth, 1860) and Glyptosternini (*Glyptosternon* McClelland, 1842, *Glaridoglanis* Norman, 1925, *Oreoglanis* Smith, 1933, *Exostoma* Blyth, 1860, *Myersglanis* Hora & Silas, 1952, *Coraglanis* Hora & Silas, 1952, *Euchiloglanis* Regan, 1907, *Pseudexostoma* Chu, 1979, *Pseudecheneis* Blyth, 1860).

The morphology of the sisorids has been the subject of several studies, such as, e.g., BATHIA (1950), GAUBA (1962, 1966, 1968, 1969), TILAK (1963), LAL *et al.* (1966), MAHAJAN (1963, 1966ab, 1967ab), CHARDON (1968),

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SHRESTHA (1970); HE (1996, 1997). However, most of these studies are concerned exclusively with osteological structures, while some capital aspects of the morphology of this important group of catfishes, such as, for example, the configuration of both the muscles and the ligaments of their cephalic region or the configuration of the structures associated with their mandibular barbels, are practically unknown. This not only complicates the study of the functional morphology of the sisorids, but also restricts considerably the data available for inference of the phylogenetic relationships of these catfishes (see DE PINNA, 1996: 9).

The aim of this work is to describe in detail the bones, cartilages, muscles and ligaments of the cephalic region (branchial apparatus excluded) and pectoral girdle of the sisorid *Glyptothorax fukiensis* (Rendahl, 1925) (Glyptosterninae, Glyptothoracini), and to compare these structures with those of representatives of the other three sisorid tribes, namely *Glyptosternon reticulatum* McClelland, 1842 (Glyptosterninae, Glyptosternini), *Bagarius yarreli* (Sykes, 1839) (Sisorinae, Bagariini) and *Gagata cenia* (Hamilton, 1822) (Sisorinae, Sisorini), as well as with those of several other catfishes, as the foundation for a discussion on the synapomorphies and phylogenetic relationships of the Sisoridae.

MATERIAL AND METHODS

The fishes studied are from the collection of our laboratory (LFEM), from the Musée Royal de l'Afrique Centrale of Tervuren (MRAC), from the Université Nationale du Bénin (UNB), from the Muséum National D'Histoire Naturelle of Paris (MNHN), from the University of Gent (UG) and from the National Museum of Natural History of Washington (USNM). Anatomical descriptions are made after dissection of alcohol-fixed or trypsin-cleared and alizarine-stained (following TAYLOR & VAN DYKE's 1985 method) specimens. Dissections and morphological drawings were made using a Wild M5 dissecting microscope equipped with a camera lucida. The trypsine-cleared and alizarine-stained (c&s) or alcohol-fixed (alc) condition of the studied fishes is given in parentheses following the number of specimens dissected. A list of the specimens dissected is given below.

Amphilius brevis (Amphiliidae): MRAC 89-043-P-403, 3 (alc); MRAC 89-043-P-2333, 1 (c&s). *Amphilius jacknosi* (Amphiliidae): LFEM, 2 (alc). *Andersonia leptura* (Amphiliidae): MNHN 1961-0600, 2 (alc); *Arius hertzbergii* (Ariidae): LFEM, 1 (alc). *Arius heudelotii* (Ariidae): LFEM, 4 (alc). *Aspredo aspredo* (Aspredinidae): USNM 226072, 1 (alc). *Auchenoglanis biscutatus* (Claroteidae): MRAC 73-015-P-999, 2 (alc). *Bagarius yarreli* (Sisoridae): USNM 348830, 2 (alc); LFEM, 1 (c&s). *Bagre marinus* (Ariidae): LFEM, 1 (alc); LFEM, 1 (c&s). *Bagrus bayad* (Bagridae): LFEM, 1 (alc); LFEM, 1 (c&s). *Bagrus docmak* (Bagridae) : MRAC 86-07-P-512, 1 (alc); LFEM, 2 (alc); MRAC 86-07-P-516, 1 (c&s). *Belonoglanis tenuis* (Amphiliidae): MRAC P.60494, 2 (alc). *Bunocephalus knerii* (Aspredinidae): USNM 177206, 2 (alc). *Cetopsis coecutiens* (Cetopsidae): USNM 265628, 2 (alc). *Chrysichthys cranchii* (Claroteidae): LFEM, 1 (alc); LFEM, 1 (c&s). *Chrysichthys auratus* (Claroteidae): UNB, 2 (alc); UNB, 2 (c&s). *Chrysichthys nigrodigitatus* (Claroteidae): UNB, 2 (alc); UNB, 2 (c&s). *Clarias gariepinus* (Clariidae): MRAC 93-152-P-1356, 1 (alc), LFEM, 2 (alc). *Conta conta* (Erethistidae): LFEM, 2 (alc). *Cranoglanis bouderius* (Cranoglanididae): LFEM, 2 (alc). *Diplomystes chilensis* (Diplomystidae): LFEM, 2 (alc). *Doumea typica* (Amphiliidae): MRAC 93-041-P-1335, 1 (alc); MRAC 93-052-P-152, 1 (alc). *Erethistes pusillus* (Erethistidae): USNM 044759, 2 (alc). *Gagata cenia* (Sisoridae): USNM 109610, 2 (alc). *Genidens genidens* (Ariidae): LFEM, 2 (alc). *Glyptosternon reticulatum* (Sisoridae): USNM 165114, 1 (alc). *Glyptothorax fukiensis* (Sisoridae): USNM 087613, 2 (alc). *Hara filamentosa* (Erethistidae): USNM 288437, 1 (alc). *Helogenes marmuratus* (Cetopsidae): USNM 264030, 1 (alc). *Hemibagrus wycki* (Bagridae): LFEM, 1 (alc); *Hemicetopsis candiru* (Cetopsidae): USNM 167854, 1 (alc). *Heterobranchus longifilis* (Clariidae): LFEM, 2 (alc). *Heteropneustes fossilis* (Heteropneustidae): USNM 343564, 1 (alc); USNM 274063, 1 (alc). *Ictalurus punctatus* (Ictaluridae): LFEM, 5 (alc). *Leptoglanis rotundiceps* (Amphiliidae): MRAC P.186591-93, 3 (alc). *Loricaria cataphracta* (Loricariidae): LFEM, 1 (alc). *Mochokus niloticus* (Mochokidae): MRAC P.119413, 1 (alc); MRAC P.119415, 1 (alc). *Mystus gulio* (Bagridae): LFEM, 1 (alc). *Nematogenys inermis* (Nematogenyidae): USNM 084346, 1 (alc). *Nothoglanidium thomasi* (Claroteidae): LFEM, 2 (alc). *Parakysis verrucosa* (Akysidae): LFEM, 1 (alc). *Paramphilius trichomycteroides* (Amphiliidae): LFEM, 2 (alc). *Paraplotosus albilabris* (Plotosidae): USNM 173554, 2 (alc). *Phractura brevicauda* (Amphiliidae): MRAC 90-057-P-5145, 2 (alc); MRAC 92-125-P-386, 1 (c&s). *Phractura intermedia* (Amphiliidae): MRAC 73-016-P-5888, 1 (alc). *Pimelodus clarias (*Pimelodidae): LFEM, 2 (alc), LFEM, 2 (c&s). *Plotosus lineatus* (Plotosidae): USNM 200226), 2 (alc). *Pseudomystus bicolor* (Bagridae): LFEM, 1 (alc), LFEM, 1 (c&s). *Schilbe intermedius* (Shilbeidae): MRAC P.58661, 1 (alc). *Silurus glanis* (Siluridae): LFEM, 2 (alc). *Tandanus rendahli* (Plotosidae): USNM 173554, 2 (alc). *Trachyglanis ineac* (Amphiliidae): MRAC P.125552-125553, 2 (alc). *Xyliphius magdalenae* (Aspredinidae): USNM 120224, 1 (alc). *Zaireichthys zonatus* (Amphiliidae): MRAC 89-043-P-2243-2245, 3 (alc).

RESULTS

In the anatomical descriptions, the nomenclature for the osteological structures of the cephalic region follows basically that of ARRATIA (1997). The myological nomenclature is based mainly on WINTERBOTTOM (1974). However, for the different adductor mandibulae sections, DIOGO & CHARDON (2000a) is followed since recent works have pointed out that, with respect to these sections, WINTERBOTTOM's (1974) nomenclature presents serious

limitations (see, e.g., GOSLINE, 1989; DIOGO & CHARDON, 2000a). In relation to the muscles associated with the mandibular barbels, which were not studied by WINTERBOTTOM (1974), DIOGO & CHARDON (2000b) is followed. With respect to the nomenclature of the pectoral girdle bones and muscles, DIOGO *et al.* (2001a) is followed.

Glyptothorax fukiensis

Osteology

Os mesethmoideum. Situated on the antero-dorsal surface of the neurocranium (Figs 1, 2). Each of its antero-ventro-lateral margins is ligamentously connected to the premaxillary.

Os lateroethmoideum. With a well-developed, laterally-directed articulatory facet for the autopalatine (Fig. 2). Posteriorly, the lateral ethmoid presents a long, narrow lateral extension directed posteriorly alongside a significant part of the lateral margin of the frontal (Fig. 1).

Os praevomerale. Well-developed, T-shaped bone without a ventral tooth-plate.

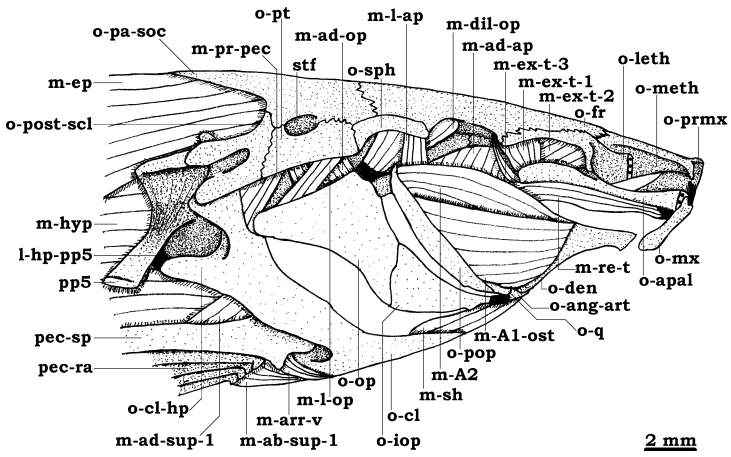

Fig. 1. – Lateral view of the cephalic musculature of *Glyptothorax fukiensis*. All the muscles are exposed; dentary and premaxillary teeth were removed. *l-hp-pp5*, ligamentum humero-vertebrale; *m-A1-ost, m-A2,* sections of musculus adductor mandibulae; *m-ab-sup-1*, musculus abductor superficialis 1; *m-ad-ap*, musculus adductor arcus palatini; *m-ad-op*, musculus adductor operculi; *m-ad-sup-1*, musculus adductor superficialis 1; *m-arr-v*, musculus arrector ventralis; *m-dil-op,* musculus dilatator operculi; *m-ep*, musculus epaxialis; *m-ex-t-1, m-ex-t-2, m-ex-t-3,* sections of musculus extensor tentaculi; *m-hyp,* musculus hypoaxialis; *m-l-ap*, musculus levator arcus palatini; *m-l-op*, musculus levator operculi; *m-pr-pec*, musculus protractor pectoralis; *m-re-t*, musculus retractor tentaculi; *m-sh*, musculus sternohyoideus; *o-ang-art* os angulo-articulare; *o-apal*, os autopalatinum; *o-cl*, os cleithrum; *o-cl-hp*, humeral process of os cleithrum; *o-den*, os dentale; *o-fr*, os frontale; *o-iop*, os interoperculare; *o-leth*, os latero-ethmoideum; *o-meth*, os mesethmoideum; *o-mx*, os maxillare; *o-op*, os operculare; *o-pa-soc*, os parieto-supraoccipitale; *o-pop*, os praeoperculare; *o-post-scl*, os posttemporo-supracleithrum; *o-prmx*, os praemaxillare; *o-pt*, os pteroticum; *o-q*, os quadratum; *o-sph*, os sphenoticum; *pec-ra*, pectoral rays; *pec-sp*, pectoral spine; *pp5*, parapophysis 5; *stf*, supratemporal fossa.

Os orbitosphenoideum. Posterior to the lateral ethmoid (Figs 1, 2). The dorsal edge of its lateral wall sutures with the ventral surface of the frontal.

Os pterosphenoideum. Posterior to the orbitosphenoid, covering, together with this bone, the gap between the frontals and the parasphenoid (Fig. 2).

Os parasphenoideum. The longest bone of the cranium (Fig. 2). It bears a pair of ascending flanges, which suture with the pterosphenoids and prootics.

Os frontale. The frontals (Figs 1, 2) are large bones that constitute a great part of the cranial roof (Fig. 1). They are largely separated by a well-developed anterior fontanel.

Os sphenoticum. Significantly smaller than the pterotic (Figs 1, 2), constituting, together with this bone, an articulatory facet for the hyomandibula (Fig. 2).

Os pteroticum. There is a well-defined, deep dorsal fossa ("supratemporal fossa": see DE PINNA, 1996) between the dorso-medial surface of the pterotic and the dorso-lateral surface of the parieto-suppraoccipital (Fig.

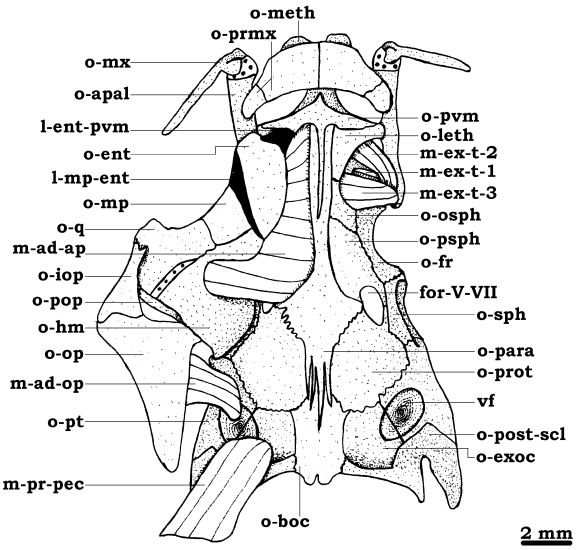

Fig. 2. – Ventral view of the neurocranium and palatine-maxillary system of *Glyptothorax fukiensis*. On the left side the suspensorium, as well as the adductor arcus palatini, adductor operculi and protractor pectoralis, are also illustrated. Premaxillary teeth were removed. *for-V-VII,* trigemino-facialis foramen; *l-ent-pvm*, ligamentum entopterygoideo-praevomerale; *l-mp-ent,* ligamentum metapterygoideo-entopterygoideum; *m-ad-ap*, musculus adductor arcus palatini; *m-ad-op*, musculus adductor operculi; *m-ex-t-1, m-ex-t-2, m-ex-t-3*, sections of musculus extensor tentaculi; *m-pr-pec*, musculus protractor pectoralis; *o-apal*, os autopalatinum; *o-boc*, os basioccipitale; *o-ent*, os entopterygoideum; *o-exoc*, os exoccipitale; *o-fr*, os frontale; *o-hm*, os hyomandibulare; *o-iop,* os interoperculare; *o-leth*, os latero-ethmoideum; *o-meth*, os mesethmoideum; *o-mp*, os metapterygoideum; *o-mx*, os maxillare; *o-op,* os operculare; *o-osph*, os orbitosphenoideum; *o-para*, os parasphenoideum; *o-pop,* os praeoperculare; *o-post-scl*, os posttemporo-supracleithrum; *o-prmx*, os praemaxillare; *o-prot*, os prooticum; *o-psph*, os pterosphenoideum; *o-pt*, os pteroticum; *o-pvm*, os praevomerale; *o-q*, os quadratum; *o-sph*, os sphenoticum; *vf*, ventral fossa.

1: stf). In addition, there is a well-defined, large, deep ventral fossa between the ventro-medial surface of the pterotic and the ventro-lateral surface of the exoccipital (Fig. 2: vf).

Os prooticum. Together with the pterosphenoid and the parasphenoid, it borders the well-developed foramen of the trigemino-facial nerve complex (Fig. 2).

Os epioccipitale. Situated on the posterior surface of the neurocranium. The extrascapulars are missing.

Os exoccipitale. Well-developed, situated laterally to the basioccipital (Fig. 2).

Os *basioccipitale.* Well-developed, unpaired bone (Fig. 2), forming the posterior-most part of the floor of the neurocranium. Its well-developed ventro-lateral arms are firmly attached to the ventro-medial limbs of the posttemporo-supracleithra.

Os parieto-supraoccipitale. Large bone constituting the postero-dorso-median surface of the cranial roof, which bears a well-developed, anteroposteriorly elongated posterior process (Fig. 1).

Os angulo-articulare. This bone (Figs 1, 3A), together with the dentary, coronomeckelian and Meckel's cartilage, constitute the mandible (Fig. 3A). Postero-dorsally, the angulo-articular has an articulatory facet for the quadrate. Postero-ventrally, it is ligamentously connected, by means of two thick ligaments, to both the interopercular (Fig. 1) and the posterior ceratohyal.

Os dentale. The postero-dorsal surface of the toothed dentary forms a dorsal process (processus coronoideus) (Fig. 3A).

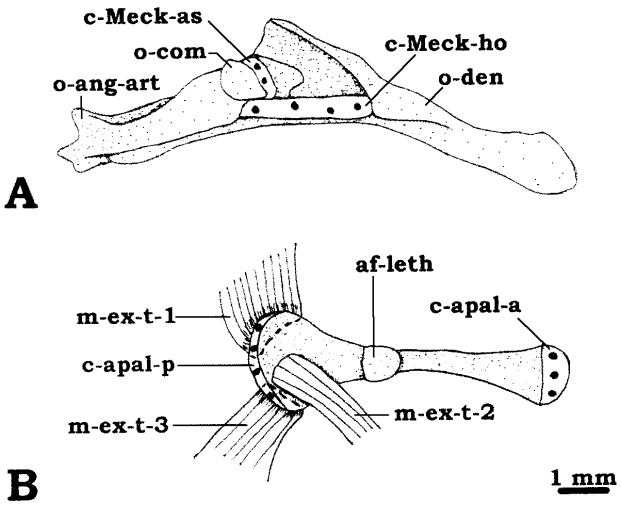

Fig. 3. – *Glyptothorax fukiensis*. (A) Medial view of the left mandible, with mandibular teeth removed. (B) Medial view of the left autopalatine and the insertions of the different sections of the extensor tentaculi on its posterior portion. *af-leth*, articulatory facet for lateral ethmoid; *c-apal-a, c-apal-p*, anterior and posterior cartilages of os autopalatinum; *c-Meck-as, c-Meck-ho*, ascending and horizontal portions of cartilago Meckeli; *m-ex-t-1, m-ex-t-2, m-ex-t-3*, sections of musculus extensor tentaculi; *o-ang-art*, os angulo-articulare; *o-com*, os coronomeckelium; *o-den*, os dentale.

Os coronomeckelium. Small bone lodged in the medial surface of the mandible. It projects to the top of the dorsal margin of the angulo-articular (Fig. 3A).

Os praemaxillare. Each premaxillary is constituted by two bony pieces (Fig. 2), which are firmly attached by connective tissue. Ventrally, the premaxillaries bear numerous small teeth (not shown in Fig. 2) having their tips slightly turned backward.

Os maxillare. The maxillary is connected to the premaxillary by means of a strong, short ligament (Fig. 1). As in most catfishes, the maxillary barbels are supported by the maxillaries.

Os autopalatinum. Rod-like, anteroposteriorly elongated bone (Figs 1, 2, 3B), with its posterior portion being markedly expanded dorsoventrally (Fig. 3B). Its posterior end is capped by a cartilage also markedly expanded dorsoventrally (Fig. 3B). Its anterior end is tipped by a well-developed cartilage with two antero-lateral concavities, which accept the two proximal heads of the maxillary (Fig. 2). Medially, the autopalatine articulates, by means of a small, circular articulatory surface (Fig. 3B), with the lateral ethmoid (Figs 1, 2).

Os hyomandibulare. Large bone presenting a poorly-developed antero-dorsal process (Fig. 4). Dorsally it articulates with both the pterotic and the sphenotic (Fig. 2), and postero-dorsally it articulates with the opercular (Figs 2, 4).

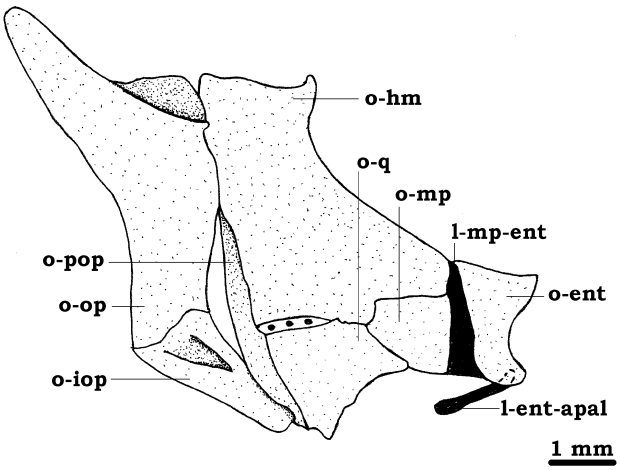

Fig. 4. – Medial view of the left suspensorium of *Glyptothorax fukiensis*. *l-ent-apal*, ligamentum entopterygoideo-autopalatinum; *l-mp-ent*, ligamentum metapterygoideo-entopterygoideum; *o-ent*, os entopterygoideum; *o-hm*, os hyomandibulare; *o-iop*, os interoperculare; *o-mp*, os metapterygoideum; *o-op*, os operculare; *o-pop*, os praeoperculare; *o-q*, os quadratum.

Os entopterygoideum. Well-developed bone attached, by means of two thick ligaments, to the metapterygoid (Figs 2, 4) and to the prevomer (Fig. 2), respectively. Its antero-dorso-lateral surface is connected, *via* a thin, somewhat long ligament (Fig. 4: l-ent-apal), to the postero-ventral surface of the autopalatinum. The ectopterygoids are absent.

Os metapterygoideum. Poorly-developed, with both its dorsal and postero-dorsal surfaces being sutured with the hyomandibular and with its postero-ventral surface being sutured with the quadrate (Fig. 4).

Os quadratum. Well-developed, triangular bone (Fig. 4). Anteriorly, it articulates with the postero-dorsal surface of the angulo-articular.

Os praeoperculare. Long and thin bone firmly sutured to both the hyomandibula and the quadrate (Fig. 4).

Os operculare. Well-developed, roughly triangular bone (Figs 1, 2, 4) ventrally attached, by means of connective tissue, to the interopercular.

Os interoperculare. Its anterior surface is ligamentously connected to the postero-ventral margin of the mandible (Figs 1, 5). Medially, the interopercular is firmly attached (Fig. 5), by connective tissue, to the lateral surface of the posterior ceratohyal.

Os ceratohyale posterior. Well-developed, somewhat triangular bone (Fig. 5) connected, by means of two long ligaments, to the postero-ventral edge of the mandible and to the medial surface of the suspensorium (the interhyal is missing), respectively.

Os ceratohyale anterior. This bone supports, together with the posterior ceratohyal, the eight branchiostegal rays (Fig. 5).

Os hypohyale ventrale. The ventral hypohyals are ligamentously connected to the antero-lateral edges of the parurohyal. The dorsal hypohyals are missing.

Os parurohyale. The parurohyal (see ARRATIA & SCHULTZE, 1990) is an irregular bone markedly compressed anteroposteriorly, which presents two well-developed postero-lateral arms and a poorly-developed postero-median process.

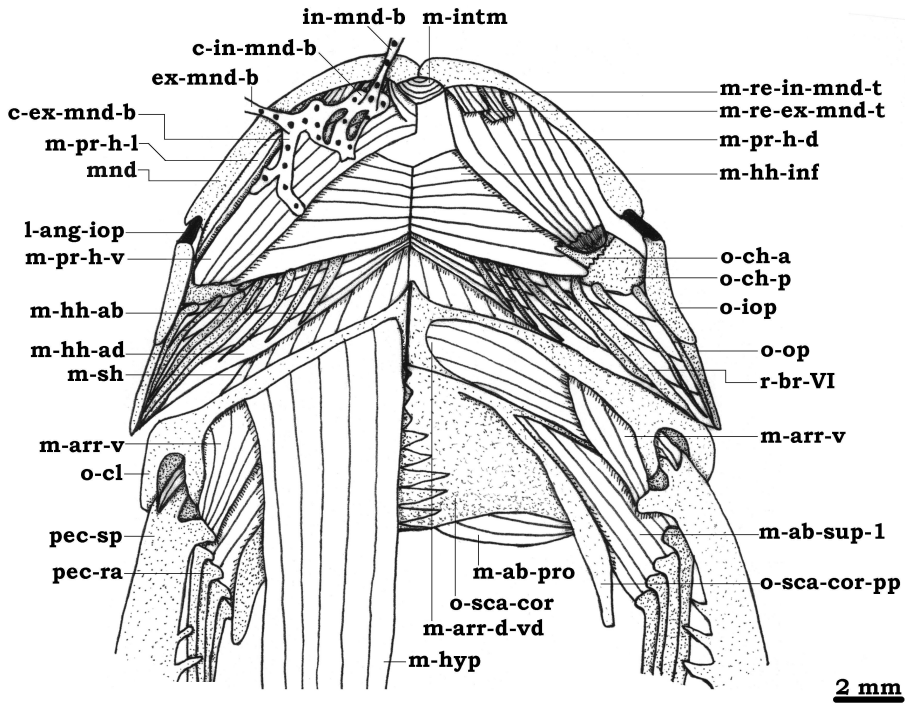

Fig. 5. – Ventral view of the cephalic region and pectoral girdle of *Glyptothorax fukiensis*. On the left side, all the muscles are exposed; on the right side, the mandibular barbels, the cartilages associated with these barbels, the hypaxialis and the ventral and lateral parts of the protractor hyoidei were removed. On both sides, the ligament between the posterior ceratohyal and the angulo-articular were removed. *c-in-mnd-b*, cartilago internus mandibularis tentaculi; *c-ex-mnd-b*, cartilago externus mandibularis tentaculi; *ex-mnd-b, in-mnd-b*; external and internal mandibular barbels; *l-ang-iop*, ligamentum angulo-interoperculare; *m-ab-pro,* musculus abductor profundus; *m-ab-sup-1*, section 1 of musculus abductor superficialis; *m-arr-d-vd*, ventral division of musculus arrector dorsalis; *m-arr-v*, musculus arrector ventralis; *m-hh-ab*, musculus hyohyoideus abductor; *m-hh-ad*, musculus hyohyoideus adductor; *m-hh-inf*, musculus hyohyoideus inferior; *m-hyp*, musculus hypoaxialis; *m-intm*, musculus intermandibularis; *mnd,* mandible; *m-pr-h-d, m-pr-h-l, m-pr-h-v*, pars dorsalis, lateralis and ventralis of musculus protactor hyoideus; *m-re-ex-mnd-t*, musculus retractor externi mandibularis tentaculi; *m-re-in-mnd-t*, musculus retractor interni mandibularis tentaculi; *m-sh*, musculus sternohyoideus; *o-ch-a*, os ceratohyale anterior; *o-ch-p*, os ceratohyale posterior; *o-cl*, os cleithrum; *o-iop*, os interoperculare; *o-op*, os operculare; *o-sca-cor*, os scapulo-coracoideum; *o-sca-cor-pp*, posterior process of os scapulo-coracoideum; *pec-ra*, pectoral rays; *pec-sp*, pectoral spine; *r-br-VI*, radius branchiostegus VI.

Os posttemporo-supracleithrum. This bone (Fig. 1), together with the cleithrum and the scapulo-coracoid, constitute the pectoral girdle. Its dorso-medial limb is firmly sutured with both the parieto-supraoccipital and the pterotic (Fig. 1). Its ventro-medial limb is firmly attached to the basiocccipital (Fig. 2). Its postero-lateral margin is deeply forked (Fig. 2), forming an articulating groove for the upper edge of the cleithrum (Fig. 1). Postero-dorsally, the posttemporo-supracleithrum has a prominent, posteriorly directed process (Fig. 1), which is firmly ankylosed with the parapophysis of the fourth vertebra.

Os cleithrum. The cleithrum (Figs 1, 5) is a large, well-ossified stout structure forming a great part of the pectoral girdle and the posterior boundary of the branchial chamber. It bears a deep crescentic, medially-faced groove that accommodates the dorsal condyle of the well-developed pectoral spine. The two cleithra are attached in the antero-medial line *via* connective tissue (Fig. 5). The well-developed humeral process of the cleithrum is connected, by means of a thick, short ligament (Fig. 1: l-hp-pp5) to the stout, strongly-flattened parapophysis of the fifth vertebra, which is highly expanded laterally (Fig. 1).

Os scapulo-coracoideum. Elongated, irregular bony plate suturing with the cleithrum along its antero-lateral edge (Fig. 5). Medially it joins

its counterpart in an interdigitation of several strong serrations (Fig. 5). Postero-laterally, the scapulo-coracoid has a prominent, posteriorly-directed posterior process (Fig. 5: o-sca-cor-pp). There is a well-developed mesocoracoid arch, which is significantly enlarged transversally.

Myology

Musculus adductor mandibulae. The adductor mandibulae A1-ost (see DIOGO & CHARDON, 2000a) originates on the preopercular and quadrate and inserts on both the angulo-articular and the dentary (Fig. 1). The A2 (Fig. 1), which lies dorso-mesially to the A1-ost but is deeply mixed with this latter, attaches posteriorly on the lateral surface of both the preopercular and the hyomandibula and anteriorly on the dorso-medial surface of both the dentary and the angulo-articular. The adductor mandibulae A3' originates on the hyomandibula and quadrate and inserts tendinously on the coronomeckelian bone. There is no A3'' nor Aω.

Musculus levator arcus palatini. Poorly-developed muscle situated medially to the adductor mandibulae A3'. It originates on the antero-dorso-lateral surface of the sphenotic (Fig. 1) and inserts on the lateral face of the hyomandibula.

Musculus adductor arcus palatini. This muscle (Figs 1, 2) runs from the lateral sides of the parasphenoid, pterosphenoid and orbitosphenoid to the medial sides of the hyomandibula and entopterygoid.

Musculus levator operculi. The levator operculi originates on the lateral margin of the pterotic and inserts on the dorsal surface of the opercular (Fig. 1).

Musculus adductor operculi. Situated medially to the levator operculi (Fig. 1). It originates on the ventral surface of the pterotic and inserts on the dorso-medial surface of the opercular (Figs 1, 2).

Musculus dilatator operculi. Well-developed, originating on the pterosphenoid, frontal, sphenotic and also on the dorso-lateral surface of the hyomandibula and inserting on the antero-dorsal margin of the opercular (Fig. 1).

Musculus extensor tentaculi. This muscle is divided into three bundles. The extensor tentaculi 1 (Figs 1, 2, 3B) runs from both the orbitosphenoid and the lateral ethmoid to the postero-dorsal surface of the autopalatine. The extensor tentaculi 2 (Figs 1, 2, 3B) originates on both the lateral ethmoid and the orbitosphenoid and inserts on the postero-medial surface of the autopalatine. The extensor tentaculi 3 (Figs 1, 2, 3B) runs from the lateral ethmoid to the postero-ventral margin of the autopalatine.

Musculus retractor tentaculi. Well-developed muscle situated medially to the adductor mandibulae (Fig. 1). It originates on the metapterygoid and inserts, by means of a thick tendon (Fig. 1), on the maxillary.

Musculus protractor hyoidei. This muscle (Fig. 4) has three parts. The pars ventralis, in which are lodged the cartilages associated with the internal and external mandibular barbels, originates on both the anterior and posterior ceratohyals and inserts on the dentary, meeting its counterpart in a well-developed median aponeurosis (Fig. 5). The pars lateralis runs from both the anterior and posterior ceratohyals to the ventro-medial face of the dentary (Fig. 5). The pars dorsalis runs from both the anterior ceratohyal to the dentary (Fig. 5).

Musculus retractor externi mandibularis tentaculi. Small muscle running from the dentary to the cartilage associated with the outer mandibular barbel, which is connected with the cartilage associated with the internal mandibular barbel and is markedly bifurcated posteriorly (Fig. 5).

Musculus retractor interni mandibularis tentaculi. Small muscle attached anteriorly to the dentary and posteriorly to the cartilage associated with the internal mandibular barbel, the posterior portion of which is pierced by a well-developed foramen (Fig. 5).

Muscle intermandibularis. Small muscle joining the two mandibles (Fig. 5).

Musculus hyohyoideus inferior. Thick muscle (Fig. 5) attaching medially on a median aponeurosis and laterally on the ventral surfaces of the ventral hypohyal, the anterior ceratohyal and the posterior ceratohyal.

Musculus hyohyoideus abductor. This muscle (Fig. 5) runs from the first (medial) branchiostegal ray to a median aponeurosis, which is associated with two long, strong tendons, attached, respectively, to the two ventral hypohyals.

Musculus hyohyoideus adductor. Each hyohyoideus adductor connects the branchiostegal rays of the respective side (Fig. 5).

Musculus sternohyoideus. Ir runs from the posterior portion of the parurohyal to the anterior portion of the cleithrum (Fig. 5).

Musculus arrector ventralis. It runs from the cleithrum to the ventral condyle of the pectoral spine (Figs 1, 5).

Musculus arrector dorsalis. This muscle is differentiated into two well-developed divisions. The ventral division (Fig. 5), situated on the ventral surface of the pectoral girdle, originates on the ventral margin of both the cleithrum and the scapulo-coracoid and inserts on the antero-lateral edge of the pectoral spine. The dorsal division, situated on the dorsal surface of the pectoral girdle, originates on the dorso-medial edge of the scapulo-coracoid and inserts on the anterior edge of the dorsal condyle of the pectoral spine.

Musculus abductor profundus. Well-developed muscle (Fig. 5) originating on the postero-medial surface of the coracoid and inserting on the medial surface of the dorsal condyle of the pectoral spine.

Musculus abductor superficialis. This muscle is differentiated into two sections. The larger section (Figs 1, 5:

m-ab-sup-1) runs from the lateral margin of the scapulo-coracoid to the antero-ventral margin of the ventral part of the pectoral fin rays. The smaller section, situated dorsally to the larger one, runs from the lateral edge of the scapulo-coracoid to the antero-dorsal margin of the ventral part of the pectoral fin rays.

Musculus adductor superficialis. This muscle is situated on the posterior margin of the pectoral girdle and is divided into two sections. The larger section (Fig. 1: m-ad-sup-1) originates on the posterior surfaces of both the cleithrum and the scapulo-coracoid and inserts on the antero-dorsal margin of the dorsal part of the pectoral fin rays. The smaller section runs from both the postero-ventro-lateral edge of the scapulo-coracoid and the dorsal surface of the proximal radials to the antero-ventral margin of the dorsal part of the pectoral fin rays.

Musculus protractor pectoralis. Well-developed muscle (Figs 1, 2) running from the ventral surfaces of both the pterotic, the posttemporo-supracleithrum and the exoccipital to the antero-dorsal surface of the cleithrum.

Glyptosternon reticulatum

The principal differences between the structures of the cephalic region and pectoral girdle of this species and those of *Glyptothorax fukiensis* are that in *Glyptosternon reticulatum*: 1) the parurohyal presents a well-developed postero-median process; 2) the anterior ceratohyal presents a well-developed antero-ventro-lateral process directed laterally; 3) the coracoid bridge (see DIOGO *et al.*, 2001a), the postero-lateral process of the scapulo-coracoid, the humeral process of the cleithrum and the ligamentous connection between this bone and the parapophysis of the fifth vertebra, the postero-dorsal process of the posttemporo-supracleithrum and the ventro-medial process of the posttemporo-supracleithrum are absent; 4) the hyomandibula articulates dorsally exclusively with the sphenotic; 9) the maxillary is markedly elongated proximo-distally; 5) the anterior portion of the autopalatine is significantly expanded transversally; 6) each premaxillary is constituted by a single bony piece; 7) the arrector ventralis is a highly-developed muscle essentially oriented transversally, and not obliquely.

Bagarius yarreli

The principal differences between *Glyptothorax fukiensis* and *Bagarius yarreli* are that in this latter species: 1) the cartilage associated with the inner mandibular barbel is not pierced, the cartilage associated with the outer mandibular barbel is not bifurcated posteriorly, and these two cartilages are not connected; 2) the ventral part of the muscle arrector ventralis is poorly developed, being confined to the ventro-lateral surface of the pectoral girdle; 3) although present, the postero-lateral process of the scapulo-coracoid is not as developed in *Glyptothorax fukiensis*; 4) the entopterygoid presents a prominent antero-lateral process, which is associated with the dorsal surface of the premaxillary by connective tissue; 5) the maxillary is markedly elongated proximo-distally; 6) the coronoid process of the mandible is poorly developed, that is, the mandible is markedly compressed ventrodorsally; 7) the mesocoracoid arch is not significantly enlarged transversally; 8) the adductor mandibulae A3'' is present, running from the lateral surface of both the hyomandibula and the quadrate to the medial surface of the angulo-articular; 9) the sphenotic bears a well-developed antero-dorso-lateral laminar projection, which extends markedly beyond the remainder of the cranial roof.

Gagata cenia

The principal differences between *Gagata cenia* and *Glyptothorax fukiensis* are that in the former species: 1) the cartilage associated with the external mandibular barbel is not bifurcated posteriorly and the cartilage associated with the internal mandibular barbel is not pierced; 2) the postero-lateral process of the scapulo-coracoid, the premaxillary teeth, and the postero-lateral extensions of the lateral ethmoid are missing; 3) the arrector ventralis and the abductor superficialis 1 are significantly more developed than in *Glyptothorax fukiensis*; 4) each premaxillary is constituted by a single bony piece; 5) the mesocoracoid arch is not enlarged transversally; 6) the maxillary is markedly elongated proximo-distally; 7) the parurohyal does not present two well-developed postero-lateral arms, but only a well-developed, wide, triangular posterior process; 8) the entopterygoid and metapterygoid are, respectively, significantly smaller and significantly wider than those of *Glyptothorax fukiensis*.

DISCUSSION

Our observations and comparisons support DE PINNA's (1996) phylogenetic hypothesis, according to which the Sisoridae is the sister-group of a clade formed by the Aspredinidae and the Erethistidae. DE PINNA's (1996) grouping of the Erethistidae, Aspredinidae and Sisoridae in a monophyletic clade was based on 10 synapomorphies (see DE PINNA, 1996: 61), of which five concern the configuration of structures examined in this work, namely: I) "posterior portion of supracleithrum (posttemporo-supracleithrum) ankylosed to margin of Weberian lamina – state 1; reversed to 0 in Glyptosternini" (see, e.g., Fig. 1); II) "parapophysis of fifth vertebra strongly flattened and expanded - reversed in Glyptosternini)" (see, e.g., Fig. 1); III) "parapophysis of fifth vertebra long, almost or quite reaching lateral surface of body wall" (see, e.g., Fig. 1); IV) "humeral process or region around it connected to anterior portion of vertebral column by well-defined ligament – state 3; reversed to 0 in Glyptosternina" (see, e.g., Fig. 1); V) "coracoid with ventral anterior (posterior) process (reversed to 0 in Glyptosternina)" (see, e.g., Fig.

5). Our observations and comparisons not only confirmed these five synapomorphies, but also pointed out an additional synapomorphy to support the clade formed by sisorids, aspredinids and erethistids:

Well-defined, long ligament attaching on the antero-dorso-lateral margin of the entopterygoid and running posteriorly to attach on the postero-ventral margin of the autopalatine.

In catfishes, the autopalatine could be ligamentously connected in several different ways to one or more elements of the pterygoid series (to the ectopterygoid in, e.g., ariids, claroteids and some "pimelodids"; to the metapterygoid in, e.g., diplomystids and nematogenyids; to the entopterygoid in, e.g., clariids, plotosids, cranoglanidids, aspredinidids, erethistidids, sisorids, some ictalurids and some schilbeids; to both the metapterygoid and the ectopterygoid in, e.g., bagrids) (this study, see also e.g. REGAN, 1911; ALEXANDER, 1965; GOSLINE, 1975; GHIOT, 1978; GHIOT et al., 1984; ARRATIA, 1987, 1990, 1992; MO, 1991; DIOGO et al., 1999, 2000, 2001b; DIOGO & CHARDON, 2000c; OLIVEIRA et al., 2001; etc.). However, a well-defined, long ligament attaching on the antero-dorso-lateral margin of the entopterygoid (see, e.g., Fig. 4) and running posteriorly to attach on the postero-ventral margin of the autopalatine is exclusively found in the aspredinids, sisorids and erethistids.

DE PINNA's (1996) proposal of a sister-group relationship between the Erethistidae and the Aspredinidae was based on five synapomorphies (DE PINNA, 1996: 64), of which three concern the configuration of structures examined in this work, namely: I) "anterior margin of pectoral spine with serrations"; II) "internal support for pectoral fin rays small in size"; III) "anterior portion of lateral line running closely in parallel to lateral margin of Weberian lamina". Our observations and comparisons not only confirmed these three synapomorphies, but also pointed out an additional synapomorphy to support the clade formed by the aspredinids and the erethistids:

Well-developed fossa between the antero-medial surface of the dorso-medial limb of the posttemporo-supracleithrum and the parieto-supraoccipital.

Plesiomorphically in catfishes there is no well-developed fossa on the dorsal surface of the posterior region of the cranium between the posttemporo-supracleitrum and the parieto-supraoccipital (see, e.g., CHARDON, 1968; MO, 1991). However, in all the aspredinids and erethistids examined, there is a well-developed, deep fossa between the antero-medial surface of the posttemporo-supraclethirum and the parieto-supraoccipital. As such a fossa is absent in all the non-erethistid and non-aspredinid catfishes examined, and particularly in the sisorids (see above), this character constitutes, very likely, an additional synapomorphy to support the clade Aspredinidae plus Erethistidae.

With respect to the synapomorphies of the Sisoridae, four characters were presented by DE PINNA (1996: 62), of which only one concerns the configuration of structures examined in this work, namely: I) "lateral ethmoid with narrow lateral extensions directed posteriorly alongside lateral margin of frontals (missing in tribe Sisorini)" (see, e.g., Fig. 1). Our observations and comparisons confirmed this synapomorphy, and also pointed out a clear, well-defined derived character that is found in the four sisorid species examined, that is, in members of all the four tribes of the family Sisoridae, and in no other catfish examined or described in the literature, which, thus, constitutes, very likely, an additional apomorphy of this taxon:

A well-developed, wide, deep fossa on the neurocranial floor between the ventro-medial surface of the pterotic and the ventro-lateral surface of the exoccipital (see, e.g., Fig. 2: vf).

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Territorial and vocal behaviour in a captive dart-poison frog, *Epipedobates tricolor* Boulenger, 1899 (Anura: Dendrobatidae)

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ABSTRACT. Territorial and vocal behaviour of captive dart-poison frogs, *Epipedobates tricolor*, were studied in captivity. Most adult males (10 out of 13) showed territorial behaviour, while there was no indication of female territoriality. Territorial males defended sites with vocal and aggressive behaviour against male intruders. The residents' aggressive behaviour consisted of chases, physical combat and vocalizations. Only male frogs were observed producing sounds. Males vocalized more often in the morning than in the evening and they preferred to call from elevated perches. Females and non-calling males mainly stayed on the ground surface, although in the afternoon females were often observed visiting male territories

KEY WORDS: Frogs, Dendrobatidae, Epipedobates, territoriality, aggression, vocalization.

INTRODUCTION

Territorial species defend areas, coincident with or included within their home range. Territoriality is best understood in the context of resource competition; the defence of space is a surrogate for the defence of resources located within that space (DONNELLY 1989a, 1989b). A "resource" can be roughly defined as any environmental factor that enhances reproductive success, and that is in limited supply, relative to the number of potential users (STAMPS, 1998). To date, territorial behaviour has been described in an enormous array of animals, including anemones, molluscs, insects, spiders, fish, amphibians, reptiles, birds and mammals. Territorial behaviour among anurans has been reported in numerous families. MARTOF (1953) described the spacing phenomenon in green frogs (*Rana clamitans* Latreille, 1801), while territorial defence has also been documented in tree frogs (e.g. MARTINS et al., 1998), dart-poison frogs (e.g. SUMMERS, 2000), glass frogs (e.g. GREER & WELLS, 1980), pipids (e.g. ÖSTERDAHL & OLSSON, 1963), Madagascan poison frogs (e.g. HEYING, 2001), leptodactylids (e.g. STEWART & RAND, 1991), and several other anuran families.

All dendrobatid species studied to date have been reported to be territorial (e.g. SEXTON, 1960; CRUMP, 1972; BUNNELL, 1973; ZIMMERMANN, 1990; ROITHMAIR, 1992, 1994; SUMMERS, 2000). Previous research has reported male and / or female territoriality in dart-poison frogs (SEXTON, 1960; WELLS, 1980a, 1980b; ROITHMAIR, 1992, 1994). Vocalizations play a fundamental role in dendrobatid behaviour. Male territorial frogs call to attract receptive females and to space territories. BUNNELL (1973) showed in the strawberry dart-poison frog (*Dendrobates pumilio* Schmidt, 1857) that the playback of a recorded call would provoke approach of a territorial male.

In this paper we studied the territorial and vocal behaviour in the Ecuadorian dart-poison frog *Epipedobates tricolor* under captive conditions. Until now little was known about the territorial behaviour of this species (but see ZIMMERMANN & RAHMANN, 1987). We addressed the following questions: (1) how is territoriality being displayed? (2) which sex is territorial? (3) do both sexes produce calls and when do they call? and (4) what places are preferred for calling?

MATERIAL AND METHODS

Study species and housing

Epipedobates tricolor is a small (males: 22.6 mm; females: 24.6 mm) diurnal dart-poison frog from south western Ecuador (SILVERSTONE, 1976). All individuals used in this study were captive-bred. Animals were fed daily

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with calcium- and vitamin-enriched Drosophilae. Colour patterns in *E. tricolor* vary between individuals, and we were able to recognise individuals based on variation in the striping patterns. The research was carried out under captive conditions. Twenty-two individuals (13 males and 9 females) were randomly divided over four standardized terraria. Two of these terraria contained two males and two females, one terrarium contained six males and two females and the fourth terrarium had three individuals of both sexes. To exclude possible influences of the number of territorial males in the same terrarium, the number of males should be kept as constant as possible. For practical reasons this was, however, not always possible. The terraria were made of glass and measured 0.5 m x 0.5 m x 0.5 m. The back and side walls were coated with rock-like brown-coloured polystyrene. Each terrarium had four elevated perches (0.02 m^2/perch). Both side walls contained one perch, while the back wall contained two perches placed at different heights. A small cave, made of brown-coloured polystyrene, was placed on top of every perch. A black film roll canister was placed in every cave as well as on the ground surface. In captivity, dart-poison frogs will use these plastic canisters as oviposition places (WALLS, 1994). Two small bromeliads were placed on the ground surface and one bromeliad was placed on top of each cave. In our results we will refer to the term 'perch' to indicate the perch with the cave and the bromeliad on top of it. Humidity was kept at a high level (75% relative air humidity) by using a centrifugal pump that pumped a constant flow of water across circa 30% of the total back wall surface area. All terraria were placed in a blacked out room with controlled lighting (12:12schedule) and temperature (circa 24°C) conditions.

Observations

Five morning and five afternoon observations were carried out for all terraria. During each observation every terrarium was observed for half an hour, and this was done for five days. This gives us a total observation time of 300 minutes per frog. The 'morning' observations started at a minimum of half an hour after lights turned on. 'Afternoon' observations started a maximum of three hours before lights turned off. For recording spacing behaviour, we used a scan sampling method (ALTMANN, 1974): every minute, we recorded the exact place of each frog in the terrarium. Call behaviour was recorded every time it occurred. Advertisement calls are loud trilling calls, making them easy to recognise and quantify. Each call consists of a series of rapidly repeated notes (see Fig. 1).

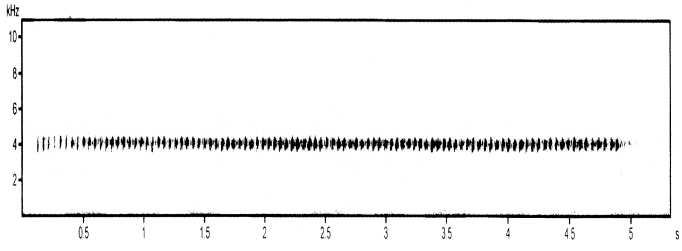

Fig. 1. – Advertisement call of a representative male frog.

Social behaviours different from space occupation and call behaviour were also continuously recorded.

Statistical analyses

Data were analysed using the statistical software programs STATISTICA (StatSoft, 1997), SPSS/PC (SPSS, 1986) and SAS (SAS INSTITUTE, 1989), following procedures outlined in SIEGEL (1956) and SOKAL & ROHLF (1981). The average proportion of the total time spent on an elevated perch, during, separately, the morning and afternoon was first calculated for each individual frog. Using SAS procedure, we carried out a two-way ANOVA (mixed ANOVA-model with period (morning and afternoon) and sex (male and female) as fixed factors, and individual as random factor, to compare proportions between all individuals. Individual was put as a random factor in the model because we used repeated measures of the same individual (morning and afternoon), because the observations are not independent. As a result, the number of degrees of freedom was adjusted by the ANOVA-model using sattertwaite formulas (see LITTELL et al., 1996). The model first tested if there was a significant interaction between period and sex. If this was the case we tested, for each separate period, if there were significant differences between the sexes, using t-statistic. Similarly we tested, for both sexes, if there were significant differences between the morning and the afternoon observations. Means and standard errors were calculated by using the statistical program SPSS. To determine if there were differences in calling activity between morning and afternoon observations, we used a paired, non-parametric Wilcoxon-test, performed in SPSS. Possible relations between the proportion of time that frogs occupied elevated areas and the call activity were tested by a Spearman Rank correlation, using STATISTICA. In all analyses, average values were calculated for each period for each individual to avoid problems with pseudo-replication. To satisfy the assumptions required for parametric statistics, proportions were subjected to arcsine square-root transformation. Two-tailed statistics were used with alpha set to 0.05.

RESULTS

Perch occupation

When comparing the proportion of time spent on a perch, a two-way ANOVA revealed a significant period x sex interaction effect, indicating that differences between the sexes depend on the observation period (two-way ANOVA, $F_{1,20}=8.20$, $p=0.0096$). During the morning observations, males spent significantly more time on elevated perches than did females (t-test, N=22, $p=0.006$; Fig. 2). During the afternoon observations there was no significant difference between the sexes (t-test, N=22, $p=0.22$; Fig. 2). The proportion of time males spent on a perch did not differ significantly between morning and

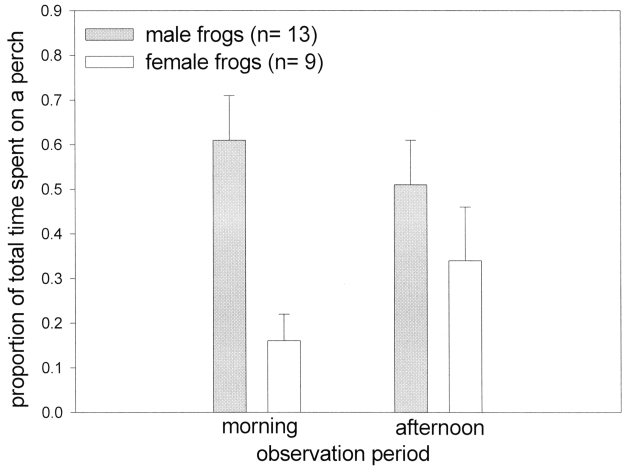

Fig. 2. – Proportion of total time (mean ± SE) spent on a perch by males and females during morning and afternoon observation periods.

afternoon observations (t-test, N=13, p=0.12; Fig.2). By contrast, females spent significantly more time on elevated perches during the afternoon than in the morning (t-test, N= 9, p= 0.029; Fig. 2). Calling males (N=10) visited 1.02 ± 0.09 (range: 0 to 4; median=1) different perches per observation period (30 minutes) whereas females visited 0.32 ± 0.08 (range: 0 to 2; median=0) different perches per observation period. Considering the entire observation period (300 minutes) calling males were observed visiting 3.10 ± 0.31 (range: 1 to 4; median=3) different perches.

Calling behaviour

Only male frogs were observed producing calls. Ten males produced a total of 2036 calls during the entire observation period. Three males were never observed calling during the entire study period.

These 'silent' males also occupied an elevated perch significantly less often than did the calling males (t-test, N=13, p=0.004; Fig. 3). Two out of three silent males were housed in the terrarium with eight individuals, the other male was housed in the terrarium with six individuals. Males produced 0 to 7 calls per minute, although one male exceeded this range and called 0 to 10 times per minute. Male frogs called significantly more during the morning observations than during the afternoon observations when they only called sporadically (Wilcoxon test, N=10, p=0.0051; Fig. 4). During the morning observations, males also called significantly more on elevated perches than on the ground surface (Wilcoxon test, N=10, p=0.025; Fig. 4). During the morning observations there was a significant positive relationship between the proportion of time spent on a perch and the average number of calls produced per hour (Spearman rank correlation coefficient=0.584, N=13, p=0.036; Fig. 5), while during the afternoon observations this relationship was not significant (Spearman rank correlation coefficient=0.377, N=13, p=0.20). Calling males were always spaced at a minimum distance of 10 cm from each other.

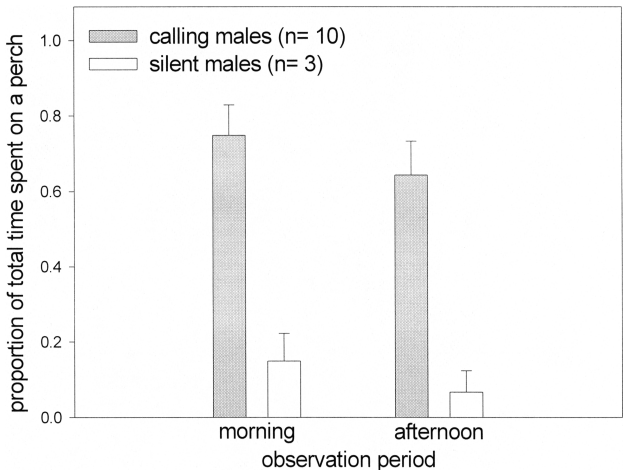

Fig. 3. – Proportion of total time (mean ± SE) spent on a perch by silent and calling males during morning and afternoon observation periods.

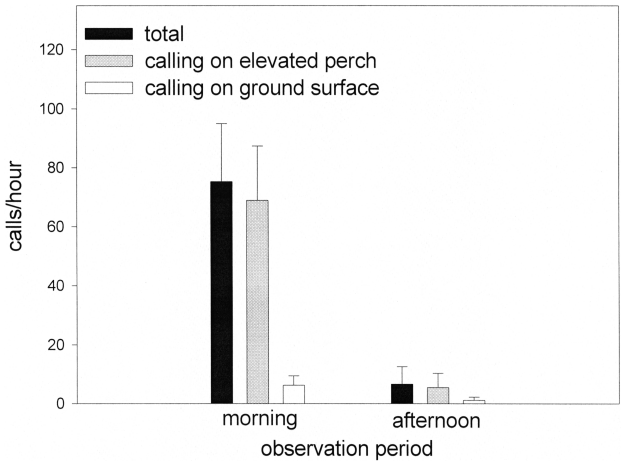

Fig. 4. – Mean number of calls produced per hour (mean ± SE) by males calling on an elevated perch and calling on the ground surface.

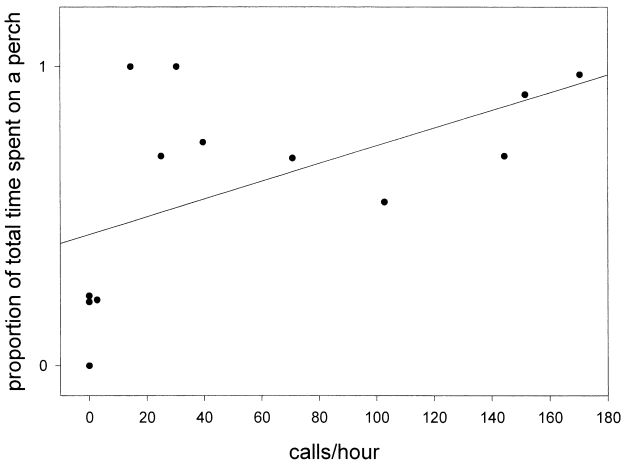

Fig. 5. – Relationship between the proportion of the total time spent on a perch and mean number of calls produced per hour by male frogs (N=13) during morning observations.

Aggressive behaviour

Only male-male aggression was observed. We observed a total of 18 aggressive conflicts, involving 10 different male – male combinations. They all occurred when a male entered the elevated perch (cave or bromeliad; see materials and methods) of a calling male. Both males then adopted an erect posture, with their front legs splayed out and rigid. The males leapt almost simultaneously at one another. They clashed, then embraced, standing upright on their hind limbs for a fraction of a second. After this encounter they circled one another, and came together again. Each frog tried to get behind its opponent so that he could jump on his back and press him downwards.

DISCUSSION

This is one of the few studies, carried out under captive conditions, explicitly designed to examine territorial behaviour in a dendrobatid species. Previous research has shown that male strawberry dart-poison frogs aggressively defend territories under captive conditions (BAUGH & FORESTER, 1994). Our data revealed that only males were territorial and produced calls. In the morning males spent significantly more time on elevated perches than did female frogs. During the afternoon, perch occupation did not differ significantly between the sexes. Field studies have shown that the occupation of elevated perches could be an indicator of territorial behaviour in many of the leaf-litter-living dendrobatid frogs (CRUMP, 1972; ROITHMAIR, 1992; GRAVES, 1999). Three males that were never observed calling spent significantly less time on elevated perches than did the other males. Moreover, we have never observed aggressiveness in or towards these 'silent' males. These observations indicate that these males were not territorial, and it is possible that they were not sexually active during the observation periods. Another explanation might be that they may act like satellite males (e.g. HOWARD, 1978; PERRILL et al., 1978; FORESTER & LYKENS, 1986), and play a 'making the best of a bad job'-strategy although we have never observed this behaviour during our study. Perhaps the presence of high quality males (e.g. males with a high call repetition rate) may influence the potential calling behaviour of these males. When we exclude the data of these three non-territorial males, we also find a significant intersexual difference in perch occupation for the afternoon observations: males spent significantly more time on elevated perches than did females (t-test, N= 19, p= 0.023). During the morning observations females mainly stayed on the ground surface, whereas during the afternoon they spent significantly more time on elevated perches. At the moment it is unclear why females behaved that way. Perhaps females mainly show mating behaviour in the afternoon (personal observations).

Most calling behaviour was observed during the morning periods. Other studies on dendrobatid calling behaviour (CRUMP, 1972; GRAVES, 1999) also found a maximum call activity during morning observations. By contrast, ZIMMERMANN & RAHMANN (1987) found in captive *E. tricolor* that sexually active males called from dawn to dusk throughout the whole day, with a peak in calling activity just before and after noon. However, their results were based on a single individual.

For the morning observations, we found a positive correlation between perch occupation and intensity of calling behaviour, indicating that males on an elevated perch call more than individuals on the ground surface. We did not find such a correlation for the afternoon observations, probably due to the low number of calls observed during this period (Fig. 3). GRAVES (1999) monitored activity patterns in two dendrobatid frogs, and found in the strawberry dart-poison frog that calling males become active earlier in the morning than females or silent males. He also found that, just after sunrise, a smaller number of individuals were active, but a larger proportion were on elevated perches, compared to later in the day (GRAVES, 1999).

Most of the calling males only visited one perch during each observation period (30 minutes). However when considering the entire observation period, most calling males were observed visiting three to four different perches, indicating that they did not occupy the same perch every day. During a night control we noticed that all frogs slept in the bromeliads on the ground surface. Perhaps, every morning males have to compete again for territories. This may also explain the intensive calling behaviour during the morning hours. In our terraria we made all elevated perches had the same surface area and had only one potential oviposition place, though they were not all at the same height (see materials and methods). As we can see in the results it might be interesting in a future experiment to include the height of an oviposition place as a quality factor, and to examine which males (call characteristics, morphological measurements)will obtain which perches. Aggressive behaviour occurred when calling males entered the elevated perch of a resident. Similar behaviour has been described in other dendrobatid species (DUELLMAN, 1966, CRUMP, 1972; SUMMERS, 2000). We have never observed aggression towards females and silent males. Furthermore we have never observed female-female aggression, as described in some other dendrobatid species (SUMMERS, 1989).

Our data about calling behaviour, perch occupation and aggressive behaviour show that in this small Ecuadorian frog, most males are probably territorial. They defend elevated areas, with potential oviposition places, by means of aggressive and vocal behaviour towards other calling males. Calling is probably used for spacing territories and attracting receptive females, although further experimental work is necessary to demonstrate this. This study further indicates that territorial behaviour in dart-poison frogs can be studied under captive conditions because males seem to behave normally in captivity.

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Long-term changes in oil pollution off the Belgian coast: evidence from beached bird monitoring

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ABSTRACT. Trends in oil pollution in the southernmost (Belgian) part of the North Sea were analysed using a dataset of 37 years (1962-99) of annual national beached bird surveys conducted in February each year. The most abundant seabird groups represented in the beached birds were auks (31%), gulls (28%), scoters (17%) and Kittiwake (9%). Oil rates of most bird species/taxa indicate a decline in oil pollution, though only *Larus* -gulls, Common Guillemot and Razorbill show significant reductions. The slope in the linear decreasing trend is steeper in inshore and midshore species, than in pelagic species. A power analysis of the results demonstrated that statistically significant trends in annual indices would be expected within 17 years for Razorbill, 29 years for *Larus*-gulls and 31 years for Common Guillemot. For other species/taxa, at least 50 years of surveying would be required. Long-term oil pollution monitoring in Belgium should be continued with a major focus on a set of abundant bird taxa, sensitive to oil-pollution and occurring in various marine habitats. Most appropriate for this purpose are grebes (inshore), Larus-gulls, Common Guillemot and Razorbill (midshore) and Kittiwake and Fulmar (offshore).

KEY WORDS: seabirds, oil pollution, trends, North Sea, beached bird surveys, temporal variation.

INTRODUCTION

Seabirds are highly vulnerable to surface pollutants. The sporadic occurrence of large numbers of seabird corpses on North Sea beaches was noted over a century ago (GRAY, 1871; ANONYMOUS, 1876). The governments of North Sea countries endeavoured to stop the contamination of the marine environment by subscribing to international agreements (OILPOL, MARPOL 73/78 and Bonn 1983), in which they agreed to take measures for prevention and surveillance. Despite the execution of the accompanying laws (MARPOL was extended with an act of enforcement in Belgium in 1995), important numbers of oiled birds continued to wash ashore on North Sea beaches during the 1980s and early 1990s (DUNNET, 1987; CAMPHUYSEN, 1989; CHRISTENSEN, 1989; SKOV et al., 1989; VAUK et al., 1990; HEUBECK et al., 1992, RAEVEL, unpublished data). As from 1 August 1999, the North Sea has been established as a Special Area under MARPOL Annex I (oil), meaning that every discharge of oil is illegal (DAHLMANN, cited in CAMPHUYSEN & VAN FRANEKER, 1992). However, current estimates of illegal oil input in the North Sea range from 15,000 tonnes per year to as much as 60,000 tonnes (PEET, 1993), with oil slicks being not uncommon in the shipping corridor between the Straits of Dover and the German Bight (OSPAR COMMISSION, 2000).

Oil pollution at sea is basically monitored in two different ways. Beached bird surveying provides cost-effective and all-weather information on the occurrence of oil, while aerial surveillance gives accurate information on the location of oil slicks and the polluter. Beached bird surveying was acknowledged at the 4th International ministerial Conference on the Protection of the North Sea (8-9 June 1995) at Esbjerg (Denmark) as a useful

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oil-monitoring tool. In Belgium, aerial surveillance started in 1991 (JACQUES et al., 1991). In this short period of monitoring no trends could be discerned so far (SCHALLIER et al., 1996; DI MARCANTONIO, 1999). Beached bird surveying has a much longer tradition in Belgium, and the first standardized data go back as far as 1962. With Belgian data obtained at the annual International Beached Bird Surveys (IBBS) conducted in February each year from 1962-1999, we will investigate whether trends in densities and oil rates of sea- and coastal birds can be demonstrated. An investigation of oil rates in various species/taxa of seabirds can shed light on the impact of oil pollution in onshore versus offshore marine waters.

MATERIAL AND METHODS

Study area

The part of the sea under Belgian jurisdiction (further referred to as the Belgian marine waters) is heavily exploited by various users (MAES et al., 2000). Situated at the entrance of the Channel, this area is characterised by a very intensive and still increasing shipping traffic (OSPAR COMMISSION, 2000).

The Belgian shoreline has a length of 65.4 km (Fig. 1), of which 3.3 km is situated in between the moles of the outer harbour of Zeebrugge (constructed in 1974-86). Narrow, sandy beaches prevail, with broad beaches restricted to the west coast near De Panne (± 3 km) and at both sides of the Zeebrugge harbour piers (± 1 km). Groins are a characteristic feature at the Belgian shoreline (at every 300-500 m on average). More than half of the entire length of the coast (34 km) is bordered with buildings and boulevards (Fig. 1) and many beaches are frequently cleaned, particularly during summer. The prevailing wind direction in Oostende (compiled from meteorological data 1941-92) is S-SW (BELL, 1994).

History

In Belgium, the first occasional counts of beached birds go back as far as the 1950s and early 1960s (KESTELOOT, 1953; HAUTEKIET, 1955, 1956, 1961, 1965; DE RIDDER, 1961; HOUWEN, 1968). Counts of the entire Belgian coastline were coordinated by Kuijken from 1962 onwards and extended to substantial parts of the Dutch and northern French coasts in 1965 (BLANKENA & KUIJKEN, 1967). This was the earliest step towards the International Beached Bird Surveys (IBBS), at first supervised by Belgian and Dutch Youth Organisations for Nature Studies (BJN and NJN). Pioneers such as KUIJKEN & ZEGERS (1968), KUIJKEN (1978a, 1978b) and VERBOVEN (1978) published early reviews of time series on Belgian data. A period of centrally governed counts was concluded with counts coordinated by MEIRE (1978a,b). The eighties were characterised by many individual actions, instigated by several large seabird strandings (DE WAELE, 1981; VAN GOMPEL, 1981, 1984, 1987; VERBOVEN, 1985; SHERIDAN & PAMART, 1988). From the winter 1991-92 onwards, beached bird surveys were centralised again, this time by the Institute of Nature Conservation (SEYS & MEIRE, 1992). Since then, annual updates are available (SEYS & MEIRE, 1993; SEYS et al., 1993; OFFRINGA & MEIRE, 1995; OFFRINGA et al., 1995, 1997; SEYS et al., 1999).

Data collection and analysis

In many countries bordering the North Sea, the Channel, the Bay of Biscaya and the Baltic, annual beached bird surveys are organised in late February (IBBS), when high water marks are searched for beached bird corpses. The results – a list of birds, containing information on the distance covered and the number of oiled and clean birds – are included in an international database. For the study-period 1962-99, the Belgian IBBS database included a surveyed overall distance of 1976 km (Table 1) with major parts of the entire coastline covered during most years. No results for 1980 were available. Although the effort in 1981 was small, the results were considered not unreasonable and retained. Throughout the entire study period the coastal villages and towns served as boundaries for different beach sections (Fig. 1). Despite the expected strong coherence among the relatively short and close sections, local differences in strandings existed (SEYS et al., 1993; OFFRINGA et al., 1995). In years with incomplete coverage, surveys were biased to specific beach stretches (1980s), so a correction factor for every

TABLE 1

Effort and total number of beached birds during IBB in Belgium, 1962-99.

Species/taxon	1960s	1970s	1980s	1990s	1962-99
divers	79	29	8	8	124
grebes	99	144	34	53	330
Fulmar	122	18	19	109	268
Northern Gannet	49	15	3	4	71
Cormorant/Shag	1	0	1	0	2
Eider	11	2	3	7	23
scoters	636	177	39	35	887
other seaducks	1	2	1	5	9
other wildfowl	41	70	5	23	139
Coot	151	190	4	3	348
waders	63	103	24	264	454
skuas	3	1	1	0	5
Larus-gulls	751	475	83	156	1465
Kittiwake	212	75	91	72	450
auks total	535	205	209	692	1641
Common Guillemot	282	69	147	588	1086
Razorbill	245	135	60	96	536
others	234	141	29	123	527
Effort (km)	502.6	644	252.5	576.4	1975.5

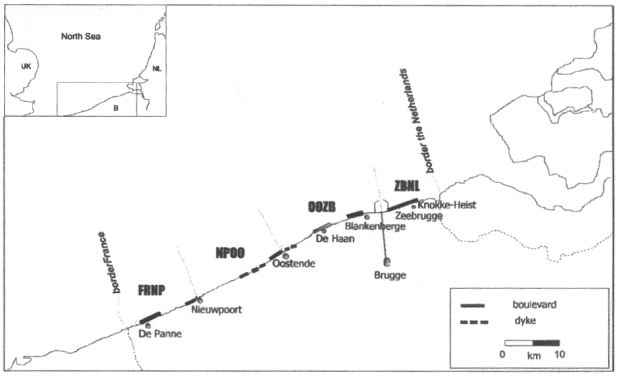

Fig. 1. – Study-area showing the boundaries of the beach sections used in Belgian beached bird surveys.

section was calculated by comparing the section density with the mean density of the entire Belgian coast ($d_{coast}/d_{transect}$). The factors are mean values of 20 years IBBS in Belgium, in which all sections were covered (Table 2). These correction-factors were applied to densities in years with incomplete coverage, when the average of the factors was taken of corresponding (combinations of) beach sections.

TABLE 2

Correction factors for Belgian beach sections, based on 20 complete IBB surveys during 1962-99.

Beach section		correction factor
French border - Nieuwpoort	(FRNP)	0.69
Nieuwpoort - Oostende	(NPOO)	1.14
Oostende - Zeebrugge	(OOZB)	0.90
Zeebrugge - Dutch border	(ZBNL)	2.14

Grouping of species in taxa roughly followed STOWE (unpublished data) and CAMPHUYSEN (1989). We focussed on birds that occupy different habitats and are unequally vulnerable for oil pollution: divers (Red-throated Diver *Gavia stellata* Pontoppidan, 1763, and Black-throated Diver *G.arctica* L., 1758), grebes (Great-crested Grebe *Podiceps cristatus* L., 1758), Fulmar (*Fulmarus glacialis* L., 1761), Northern Gannet (*Morus bassanus* L., 1758), Eider (*Somateria mollissima* L., 1758), scoters (Common Scoter *Melanitta nigra* L., 1758, and Velvet Scoter *M. fusca* L., 1758), Coot (*Fulica atra* L., 1758), waders, skuas (Great Skua *Skua skua* Brünnich, 1764, Pomarine Skua *S. pomarinus* Temminck, 1858, Arctic Skua *S.parasiticus* L., 1758), *Larus*-gulls L., Kittiwake (*Rissa tridactyla* L., 1758), Common Guillemot (*Uria aalge* Pontoppidan, 1763) and Razorbill (*Alca torda* L., 1758). Total numbers are of little value when comparing years with different effort, change in environmental conditions, etc. This was accounted for by using 'number/km' (density), and 'oil rate' (the proportion of complete bird corpses with oil: $n_{oiled}/n_{all\ birds}$ * 100%). The latter is widely accepted as a good indicator of oil pollution (SKOV, 1991; CAMPHUYSEN, 1993, 1995, 1998) and is presumably only influenced by post-mortem contamination and rejection by scavengers. Oil rates were not calculated when less than ten complete bird corpses were available (SKOV et al., 1996). Trends in oil rates were calculated after logit-transformation of the data by means of linear regression (by least-squares estimation). The probability that a trend, if present, will be detected as statistically significant, was studied by means of a power analysis (CAMPHUYSEN, 1995).

RESULTS

Overall trend in density and oil rate of beached birds

The total (non-corrected) numbers, effort and oil rates are summarized in Tables 1 and 3.

A total of 6743 birds of 80 different species have been recorded at Belgian IBB surveys during 1962-99. The Shannon-Wiener diversity index decreased significantly over the years (Kendall τ = -0.285, N = 37, P < 0.05), whilst the effort did not go down significantly (τ = -0.221, N = 37, P = 0.051). The total density of beached birds did not change during the study-period (R^2 = 0.041, *rms* = 11.7, b = -0.06, N = 37, P = 0.23). However, four discrete periods can be demonstrated, coinciding more or less with the four decades (Fig. 2). The 1960s were characterised by high overall densities (average 5.7, s.d. 1.9, birds/km) and high oil rates (average 73.2, s.d. 15.0,%). In the 1970s, the situation had changed dramatically (2.1, s.d. 2.2, birds/km and 51.0, s.d. 27.1,%), but by the end of that decade numbers increased again. The agony of the sixties was repeated in the 1980s, but now the birds came in pulses (2.8, s.d. 2.0, birds/km and 62.8, s.d. 19.2,%). The densities were slightly reduced again in the 1990s (2.6, s.d. 2.1, birds/km) and oil rates markedly declined (44.7, s.d. 20.8,%). Over the entire study-period, oil-rates showed a downward slope for all species/taxa except one

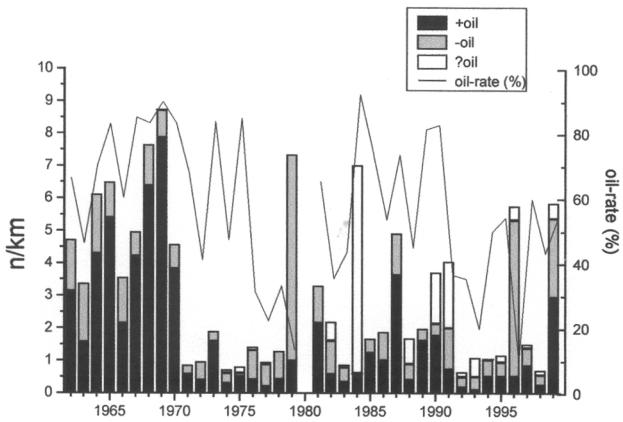

Fig. 2. – Densities (bars) and oil rates (line) of all beached birds collected during IBB surveys in Belgium during 1962-99. Densities are subdivided into 'oiled', 'unoiled' and 'not scored' specimens.

(Kittiwake). Scoters and *Larus*-gulls were most abundant as beached birds during the 1960s; Kittiwake and Common Guillemot were particularly common in the 1980s (and 1990s for the Common Guillemot).

Onshore versus offshore bird taxa

Larus-gulls, Northern Gannet, scoters and divers significantly decreased in densities at IBB surveys during 1962-99. The Common Guillemot is the only common species that became significantly more abundant during the study-period (Table 3). Taxa or species that do not show significant trends appear to be either offshore species (Fulmar, Kittiwake, skuas) or birds known to be sensitive to cold winter weather (Coot, grebes, waders).

Oil rates of a typical offshore species, the Kittiwake, did not change significantly during the 37 years of study (Table 3, Fig. 3). Two groups of birds that are very common at 10-30 km from the coast, the *Larus*-gulls and the Common Guillemot, show significant declines in oil rate. The oiling among gulls was heaviest in the 1960s (69%) and decreased to 20% in the 1990s. The average oil rate in the Common Guillemot was higher, both in the 1960s (99%) and in the 1990s (61%). The decline in oil rate in the Razorbill – a species occurring in the same wintering areas as the Common Guillemot – was less pronounced but still significant (1960s: 98%, 1990s: 64%). Inshore and coastal species all show lower oil rates now than some forty years ago, though none of the trends was significant (Table 3). That this should be attributed to the sample size, being too small to reveal trends, can be demonstrated with a power analysis. For waders, grebes and scoters we need at least 50 years of surveying to get a 90% probability to find an existing trend (Fig. 4). Only for Razorbill (17 years), *Larus*-gulls (29 years) and Common Guillemot (31 years), were the IBB surveys able to produce significant trends at this probability.

TABLE 3

Trends in (a) densities (n/km) and (b) logit-transformed oil rates of the most important sea- and coastal bird species/taxa at the Belgian coast during the winters of 1962-99. Shown are mean values of densities and non-transformed oil rates by decade and linear trends (rms = residual variance; b = slope of regression; R^2; n = number of winters; significance: n.s. = non significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). Notice that the numbers of skuas were too low to calculate trends in oil-rate.

(a)	**Density (n/km) by decade**				**trend**				
Species/taxon	60s	70s	80s	90s	*rms*	slope *b*	R^2	*n*	*P*
divers	0.19	0.05	0.05	0.03	0.008	-0.004	0.228	37	**
grebes	0.19	0.17	0.27	0.10	0.053	-0.003	0.020	37	n.s.
Fulmar	0.31	0.04	0.13	0.23	0.118	-0.001	0.002	37	n.s.
Northern Gannet	0.11	0.03	0.05	0.02	0.002	-0.002	0.276	37	***
scoters	1.23	0.22	0.21	0.08	0.411	-0.029	0.212	37	**
Coot	0.33	0.33	0.08	0.02	0.249	-0.009	0.040	37	n.s.
waders	0.12	0.17	0.21	0.57	0.312	0.011	0.045	37	n.s.
skuas	0.02	0.02	0.03	0.00	0.000	-0.000	0.075	37	n.s.
Larus-gulls	1.52	0.65	0.44	0.29	0.444	-0.035	0.268	37	**
Kittiwake	0.42	0.11	0.54	0.16	0.189	-0.004	0.009	37	n.s.
Common Guillemot	0.56	0.10	0.78	1.00	0.456	0.021	0.110	37	*
Razorbill	0.48	0.20	0.32	0.18	0.136	-0.009	0.074	37	n.s.
(b)	**Oil rate (%) by decade**				**trend**				
Species/taxon	60s	70s	80s	90s	*rms*	slope *b*	R^2	*n*	*P*
divers	99	93	50	-	-	-	-	2	-
grebes	88	55	76	42	0.755	-0.043	0.305	10	n.s.
Fulmar	53	75	40	45	-	-	-	2	-
Northern Gannet	99	75	-	-	-	-	-	2	-
scoters	89	71	83	55	0.746	-0.051	0.153	13	n.s.
Coot	47	3	-	-	0.079	-0.100	0.902	4	n.s.
waders	18	10	0	1	0.088	-0.005	0.082	5	n.s.
Larus-gulls	69	41	22	20	0.315	-0.037	0.318	24	**
Kittiwake	76	59	93	62	0.527	0.020	0.105	12	n.s.
Common Guillemot	99	84	82	61	0.474	-0.051	0.492	23	***
Razorbill	98	88	92	64	0.163	-0.034	0.521	11	*

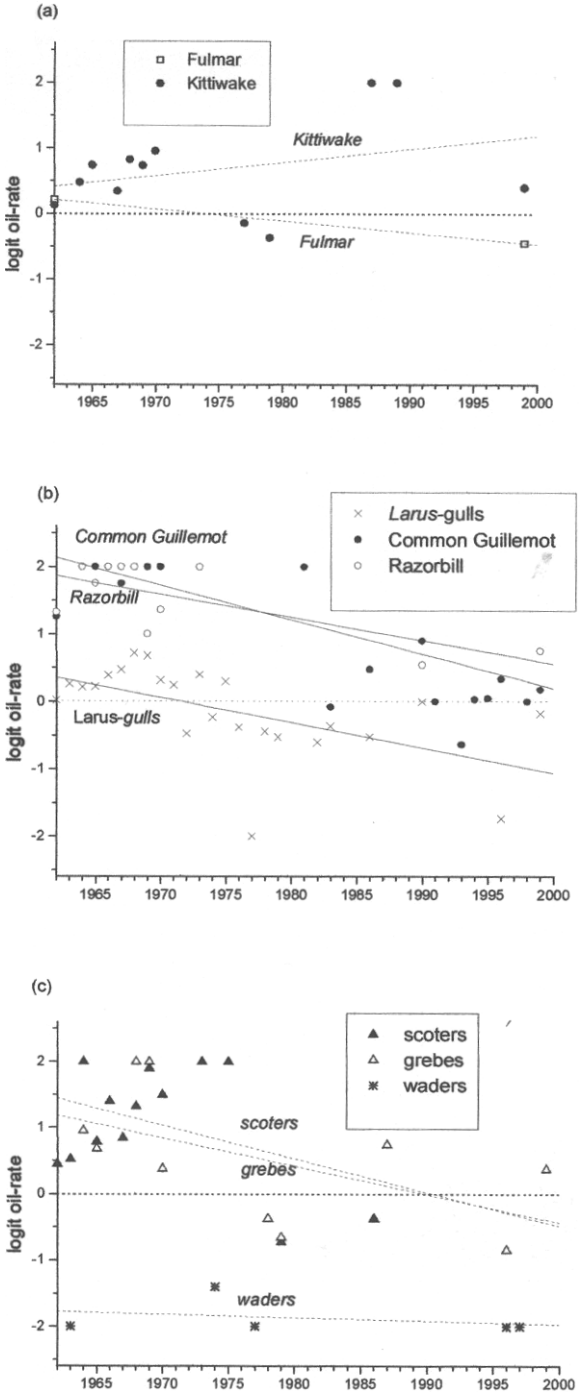

Fig. 3. – Trends in oil rate of seabird groups in Belgium based on results of February IBB surveys during 1962-99. Trends for an offshore (a), midshore (b) and inshore group of species are shown (see also Table 5). Significant trends are in solid line, others in dashed line.

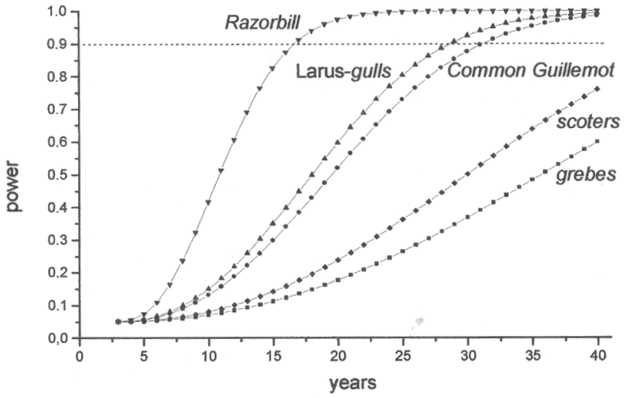

Fig. 4. – Power of trend test of oil-rates in seabird species/taxa versus number of years sampled based on IBB surveys at the Belgian coast in February during 1962-99.

DISCUSSION

Quality of the data

In IBB surveys during the 1980s the Belgian beaches were not completely covered. Hence, geographical differences may influence the outcome of the survey. RAEVEL (unpublished data) demonstrated that a minimum of 25-30% of the (Nord-Pas-de-Calais to Picardie) coastline must be surveyed to get reliable densities and oil rates for the whole coast. Accordingly SEYS et al. (2002) found oil rates of Common Guillemot to stabilize above a sample of 10-15 bird corpses, a figure corresponding to a mean surveyed distance of 25-30 km (or 40-50% of the entire Belgian coast). More than half of the coast, some 65%, (RAEVEL, unpublished data; SEYS et al., 2002) must be covered to approach the actual species richness. These figures underscore the necessity to put substantial effort into the beach surveys. This effort is normally met in Belgian BBS, but the results of years with low effort (1981, 1984, 1985, 1987) are put into question.

Oil pollution trends in the North Sea

In many countries bordering the North Sea, long-term trends in oil pollution have been described, as derived from the proportion of beached birds with oil (STOWE, unpublished data; AVERBECK et al., 1992; HEUBECK, 1995; SKOV et al., 1996; FLEET & REINEKING, 2000; CAMPHUYSEN & HEUBECK, 2001). Measurable declines have been observed in several species along the north-east English and Scottish coasts, in south-east England, in parts of Denmark (see SKOV et al., 1996), in the Netherlands (CAMPHUYSEN, 1998; CAMPHUYSEN & HEUBECK, 2001) and in Germany (AVERBECK et al., 1992; FLEET & REINEKING, 2000). The Belgium coast borders the southernmost part of the North Sea, an area heavily affected by chronic oil pollution (NORTH SEA TASK FORCE, 1993, OSPAR COMMISSION, 2000). Our data show downward trends in oil rate for most species, trends that are highly significant for only the two most abundant taxa (Common Guillemot, *Larus*-gulls).

STOWE (unpublished data) and SKOV et al. (1996) showed that in the countries around the Southern Bight, the proportions of oiled Common Guillemots – an important target species – were higher than in other West European countries. That the proportion of oiled Common Guillemots in the late 1980s and early 1990s in Belgium

is relatively low (approximately 50%) compared to surrounding countries (more than 75%; CAMPHUYSEN, 1995) can partly be attributed to a different approach: CAMPHUYSEN (1995) considered data collected on Dutch beaches during ten winters (1985/86-1994/95) and used extensive material of six monthly surveys for each winter. For Belgian beaches however, only data from IBB February surveys (1986-95) (SKOV et al., 1989; SKOV, 1991) and additional information from January 1992 up to March 1995 were used (SEYS & MEIRE, 1992, 1993; OFFRINGA & MEIRE, 1995; OFFRINGA et al., 1995). While there were several wrecks in the early 1990s that were important with regard to the Common Guillemot, low proportions of bird corpses oiled resulted in overall oil rates being lowered quite drastically. When all existing Common Guillemot data from Belgian beaches (i.e. including monthly and weekly winter surveys) are used, a mean oil rate of 65% for the period 1986-95 is found, a value intermediate between the 50% mentioned above and the 75% recorded on Dutch beaches.

In our data, offshore species such as Fulmar and Kittiwake do not show significant changes in oiling. These species occur around the offshore shipping lanes, where most oil slicks were recorded over the past eight years (DI MARCANTONIO, 1999; SCHALLIER et al., 1996;). Both species are not considered particularly vulnerable for oil since they spend much time on the wing. Nevertheless it is surprising that no downward trend can be discerned as found in the Netherlands, Germany, England and Scotland in the period 1984-95 (CAMPHUYSEN, 1995; SKOV et al., 1996). The smaller slope in the downward trend (Fig. 3) – as found in the Netherlands as well (CAMPHUYSEN, 1998) – probably explains why the trend is not (yet) significant for the Fulmar. The same applies to waders. They were often found oiled during the 1960s (indicating the beaching of oil slicks or post-mortem contamination) but now clearly have a smaller risk of becoming oil-fouled. That we do not find significant downward trends in oil rate yet, must be ascribed to the relatively slow decrease and hence the need for a higher number of sampling years (58 years to have a power of 90%). The very low numbers of seaducks beaching these days in combination with the exceedingly high number of surveying years needed to find a trend, necessitates the focus on the most common taxa (auks, *Larus*-gulls) and the collection of additional data during the rest of the winter. The slopes of the linear regression in various species confirm the general pattern of oil rates for offshore birds decreasing less quickly than for inshore and midshore species. It can be concluded that densities of most beached bird species are much smaller now than in the past, and that oil rates show consistent declines more prominent in coastal birds than in pelagic species.

Target species for oil monitoring

Future research should focus on several species and/or groups of species simultaneously to avoid problems caused by certain mortality incidents in individual species and to sample different subregions (inshore vs. offshore). For The Netherlands, CAMPHUYSEN (1995) selected Common Guillemot, Razorbill, Kittiwake, Fulmar, Northern Gannet, scoters and *Larus*-gulls as target species for beached bird surveying. A power analysis on Dutch data reveals that trends should be demonstrable – even for species showing linear trends with high residual variance and small slope – within 13-17 years. The trends we find by using only IBB surveys have a much lower power, meaning that at least some 30 years are needed before an existing trend will be detected with a chance of 90%. Considering the small length of the Belgian coastline and hence the comparatively small total number of beached birds that can be collected at each February IBB survey, oil impact monitoring should: (1) collect as many corpses and species as possible, and group species typical for each of the different marine habitats (inshore, midshore, offshore) for further analysis; (2) always be organised synchronic with counts in neighbouring countries; (3) be complemented with monthly surveys at least during winter.

In designing future beached bird surveys, one of the main considerations in relation to analysing trends in the proportion of oiled corpses, should be the limited number of beached birds (SKOV et al., 1996). Due to decreasing densities of beaching corpses, it might become more and more difficult to collect large enough samples (and keep the numerous volunteers motivated). Assuming that a sample of at least ten complete corpses is required to calculate reliable oil rates, only the Common Guillemot (as species) and auks (as taxon) can provide the necessary data in Belgium these days. The beach environment and adjacent surf zone can probably be monitored much better during winter by scoring oil rates on live birds, such as Sanderlings *Calidris alba* Pallas, 1764 (a small wader, pale in colour and constantly foraging near the water mark). For the inshore zone, the most suitable potential target species is the Great-crested Grebe (common in February in inshore waters), for the midshore area the Common Guillemot, Razorbill and several *Larus*-gull species can fulfil the role of oil indicators. For the offshore zone probably only Kittiwake and Fulmar would be suitable. Although the IBB surveys may have some drawbacks compared to repeated monthly or weekly beached bird surveys (SEYS et al., 2002) they provide invaluable information, and can build on large historical datasets collected in a standardized way over a vast area and hence should be continued and strengthened in the future.

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Lipopolysaccharide stimulates the expression of pro-opiomelanocortin mRNA in chicken macrophages, as demonstrated with a competitive polymerase chain reaction

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ABSTRACT. The goal of the present study was to develop a competitive PCR assay to measure changes in the expression of pro-opiomelanocortin (POMC) mRNA in myelomonocytic HD11 cells after *Salmonella typhimurium* lipopolysaccharide (LPS) stimulation. Pro-opiomelanocortin mRNA could be detected in HD11 cells by reverse transcription – polymerase chain reaction (RT-PCR). To our knowledge, this is the first observation of a POMC mRNA transcript in avian macrophages. Based on this observation, the effect of stimulation with bacterial LPS on the POMC expression in HD11 cells was investigated by means of a competitive PCR assay. For this purpose, the HD11 cells received a one-hour LPS challenge with LPS concentrations ranging from 10 to 100 ng/ml. A PCR MIMIC (consisting of a heterologous DNA fragment flanked by templates for the gene-specific primers) was used as an internal control in the competitive PCR assay. A ten-fold dilution series of the MIMIC was co-amplified with a constant amount of experimental cDNA. While HD11 POMC mRNA expression showed an increase of one order of magnitude following treatment with 100 ng/ml LPS as compared with untreated controls, no significant differences could be observed after treatment with 50 and 10 ng/ml LPS. Quantitative measurement of POMC mRNA levels is a first step towards a better understanding of the physiological role of non-hypophysial POMC-derived peptides in the response to immune stress in birds.

KEY WORDS: chicken, POMC, HD11 cells, lipopolysaccharide, competitive PCR.

INTRODUCTION

Pro-opiomelanocortin (POMC) is the polypeptide precursor of adrenocorticotropic hormone (ACTH), the opioid hormone β-endorphin (β-END), β-lipotropin (β-LPH) and α-, β- and γ-melanotropin (α-, β-, γ-MSH) (BERTAGNA, 1994). These molecules play a pivotal role in the stress response: ACTH has steroidogenic activity (OTTAVIANI et al., 1999), β-endorphin has analgetic properties (HIRSCH AND MILLINGTON, 1991) and MSH is an interleukin-1 antagonist (STAR et al., 1995; LUGER et al., 1997).

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Although pro-opiomelanocortin (POMC) is a well-established hormone precursor in many species, molecular information about chicken POMC has only relatively recently been revealed (BERGHMAN et al., 1998; TAKEUCHI et al.,1999; GERETS et al., 2000). In our laboratory, POMC has been intensively investigated in the pituitary and the brain of the chicken. The chicken POMC coding region appears to consist of 678 base pairs in the pituitary and also in the hypothalamus. The distribution of the POMC mRNA in the pituitary is restricted to the cephalic lobe, whereas in the brain, the signal is limited to the hypothalamic region.

Unlike the situation in mammals, information about peripheral, non-endocrine or neuroendocrine POMC is still relatively scarce in birds. Using the reverse transcription – polymerase chain reaction (RT-PCR) technique, we

were able to detect a POMC mRNA fragment in non-pituitary tissues including the bursa, thymus, liver, kidney etc. (H. GERETS, manuscript in preparation). The existence of POMC mRNA in different non-pituitary tissues of the chicken has also been demonstrated by TAKEUCHI et al. (1999). These reports are in line with the earlier work by OTTAVIANI and co-workers (1992), describing ir-ACTH peptides in chicken phagocytic leucocytes and lymphocytes. To our knowledge, POMC gene expression has not been demonstrated in a permanent avian *in vitro* model, such as HD11 myelomonocytic cells. HD11 cells are chicken macrophages transformed by the *v-myc*-encoding MC29 virus (BEUG et al., 1979). This study will focus on the expression and regulation of POMC mRNA in HD11 cells in the presence or absence of *Salmonella typhimurium* lipopolysaccharide (LPS).

MATERIAL AND METHODS

Cell culture

The MC29 virus-transformed chicken macrophage cell line HD11 (BEUG et al., 1979), was cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco BRL Life Technologies; Merelbeke, Belgium) supplemented with 5% (w/v) fetal bovine serum (FBS) (Gibco BRL Life Technologies), 2 mM L-glutamine (Sigma; Bornem Belgium), 1 mM sodium pyruvate (Sigma) and 50 ng/ml gentamycine (Gibco BRL Life Technologies). HD11 cells were maintained at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

LPS stimulation and total RNA extraction

Cells were maintained in culture and plated at a density of 1.4 10^6 cells per well in 24-well plates (Elscolab; Kruibeke, Belgium). They were treated with different concentrations of lipopolysaccharide (LPS) (Sigma) at 100, 50, 10 and 0 ng/ml, respectively, for one hour. After stimulation, total RNA was extracted from the cells with the RNeasy Mini Kit (Westburg; Leusden, The Netherlands) according to the instructions of the kit.

Reverse Transcription – Polymerase Chain Reaction (RT-PCR)

Reverse transcription – polymerase chain reaction (RT-PCR) experiments were carried out to determine whether HD11 cells express the POMC gene. For this purpose, a RT-PCR reaction was carried out according to the instructions of the GeneAmp RNA PCR kit (Perkin Elmer; Nieuwerkerk a/d IJssel, The Netherlands). To perform the reverse transcription of total RNA into cDNA, an oligo-dT primer (Perkin Elmer) was used. The sequences of the upstream and downstream primers (supplied by Gibco BRL Life Technologies) used in the PCR reactions, were based on the POMC cDNA sequence as determined previously (BERGHMAN et al., 1998; GERETS et al., 2000). The PCR-primers were designed as follows:

→ 5'-AAGCGCTCCTACTCCATGGAGCATTTCC-3' and

← 5'-GGCGTTTTTGAACAGAGTCATCAGCGGGGTCTGGCTGAGCTCC-3';

→ 5'-AGCGGCCCATGCTGGGAGAAC-3' and

→ 5'-CTGACCCTTCTTGTAGGCGC-3';

→ 5'-CTCTCGGAGAGCATCCGCAAG-3' and

← 5'-GGCGTTTTTGAACAGAGTCATCAGCGGGGTCTGGCTGAGCTCC-3'

amplifying a fragment of 330, 678 and 500 bp, respectively. The PCR cycles consisted of a denaturation (95°C, 60s), an annealing (65°C/50°C/50°C, 60s) and an elongation (72°C, 60s) step. After 36 cycles, the fragments were analyzed by horizontal agarose electrophoresis and visualized by means of ethidium bromide fluorescence. Control experiments involved omission of the reverse transcriptase enzyme and of total RNA, respectively, in order to prove that no genomic DNA contamination accounted for the observed signal.

Construction of the MIMIC

For quantification of POMC mRNA levels, an internal standard (MIMIC) with competing flanking sequences was constructed, using the PCR MIMIC Construction Kit (Clontech – Westburg; Leusden, The Netherlands) with minor modifications from the manufacturer's instructions. In the primary reaction, the cDNA was transcribed using two composite primers (5'-AAGCGCTCCTACTCCATGGAGCATTTCCCAAGTTTCGTGAGCTGATTG-3' as the sense primer and 5'-GGCGTTTTTGAACAGAGTCATCAGCGGGGTCTGGCTGAGCTCCGGGACAAGATACTCATCTGC-3' as the antisense primer). These composite primers were composed of two sections: the 3' portion annealing to the heterologous DNA fragment (supplemented with the kit) and the 5' portion annealing to the specific target gene. In the second PCR reaction only the gene-specific primers (underlined above) were used. This yielded a PCR MIMIC of 251 nucleotides (nt) consisting of a heterologous DNA fragment with templates that were recognized by a pair of gene-specific primers. The yield of the PCR MIMIC was determined by measuring the intensity of the electrophoretic bands generated by PCR MIMICs against those generated by various dilutions of known quantities of a molecular weight marker (ϕX174/*Hae* III digest) on a horizontal agarose electrophoresis. The intensities of the bands were measured after ethidium bromide staining with the Image Master (Image Master VDS; Amersham Pharmacia Biotech, The Netherlands). Subsequently, the MIMIC was diluted to a concentration of 100 attomoles/µl and used as such in the competitive PCR experiments. MIMIC stocks were stored at –20°C until use.

Competitive PCR assay

The entire pool of total RNA from one condition was simultaneously reverse transcribed to cDNA, with the use of the reverse transcriptase enzyme and the oligo(dT) primer. In the competitive PCR (cPCR) reaction, a ten-fold dilution series of the MIMIC was amplified together with a constant amount of experimental cDNA. The cPCR reactions were performed as described above. The resulting fragments were separated by horizontal agarose gel electrophoresis, stained with ethidium bromide and analyzed with the Image Master. Variations in fluorescence due to the molecular weight differences between target and MIMIC were corrected using the following formula: (Target / MIMIC fluorescence) x (MIMIC / target size (bp)) = corrected fluorescence ratio. For each individual PCR reaction the corrected fluorescence ratio (y-axis) was plotted against the initial MIMIC concentration on logarithmic scales. A line was drawn from a linear regression analysis of the data points. The amount of target POMC molecules was calculated by determining the x-intercept for the points of the curve where the ratio target / MIMIC equals 1 (when y = 0).

Statistical Analysis

Data are represented as means ± SE (n = 8). Results from the four groups of HD11 cells (controls, 10, 50 and 100 ng/ml LPS-stimulated) were compared using the non-parametric Wilcoxon test found on the website *http://fonsg3.let.uva.nl:8001/Service/Statistics/Signed_Rank_Test.html*. Differences with a $*P < 0.05$ were considered significant.

RESULTS

Reverse Transcription – Polymerase Chain Reaction (RT-PCR)

RT-PCR reactions showed that HD11 cells indeed express POMC mRNA: fragments of 330 nt (Fig. 1A) and 500 nt (Fig. 1C) were detected. Also the entire coding region of POMC (678 bp) was amplified in these cells (Fig. 1B), although seemingly in a very low concentration when compared with the 330 nt fragment. When this fragment was amplified, two other bands of approximately 350 and 400 bp also appeared. Control experiments, which did not receive any total RNA input in the reverse transcription reaction, were all negative (data not shown).

Competitive PCR assay

As an internal standard, a PCR MIMIC consisting of a heterologous DNA fragment flanked with templates for the gene-specific primers was used. Due to the different sizes of the MIMIC and the target DNA (330 nt *vs*. 251 nt), these can be distinguished by horizontal agarose gel electrophoresis. Fig. 2 (p. 122) shows the competitive PCR reaction on

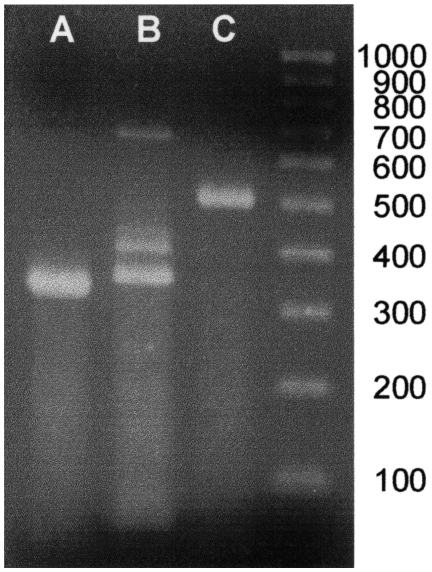

Fig. 1. – Polymerase chain reaction amplification of pro-opiomelanocortin cDNA of HD11 cells. Three different primer sets were tested to determine the length of the POMC transcript in HD11 cells. **A**. A 330 bp fragment of POMC used as the target in the competitive PCR reaction. **B**. The entire POMC coding region (~ 678 bp). **C**. An intermediate 500 bp fragment. These primer combinations indicate that the entire coding region of the POMC transcript is being transcribed in HD11 cells.

mRNA from HD11 cells stimulated with different concentrations of LPS (100 - 50 - 10 and 0 ng/ml). POMC mRNA expression in cells stimulated with 100 ng/ml LPS increased with as much as an order of magnitude as compared with untreated controls. On the other hand, no statistically significant differences could be observed between the 50 and 10 ng/ml LPS-stimulated cells as compared with untreated controls (Fig. 3). Densitometric analysis of the ethidium bromide stained gels is demonstrated in Fig. 2.

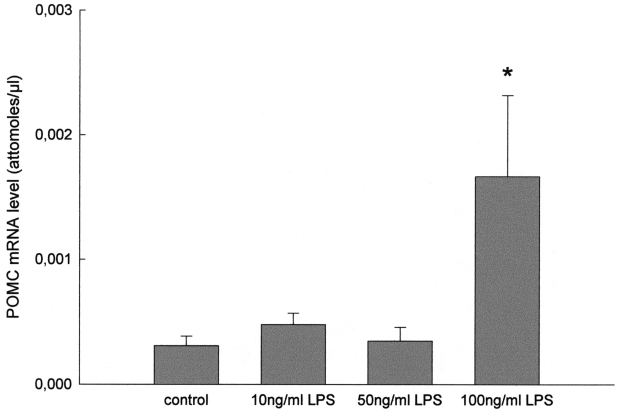

Fig. 3. – Histogram of untreated cells (controls) vs. 10 – 50 – 100 ng/ml LPS-stimulated HD11 cells. The bars represent the mean ± SE of the POMC RNA levels. The asterisk indicates a statistically significant difference between control cells and 100 ng/ml LPS stimulated cells in the Wilcoxon test (*: $P<0.05$); control and 100 ng/ml LPS (n=8); 50 and 10 ng/ml LPS (n=7) (n = number of independent experiments).

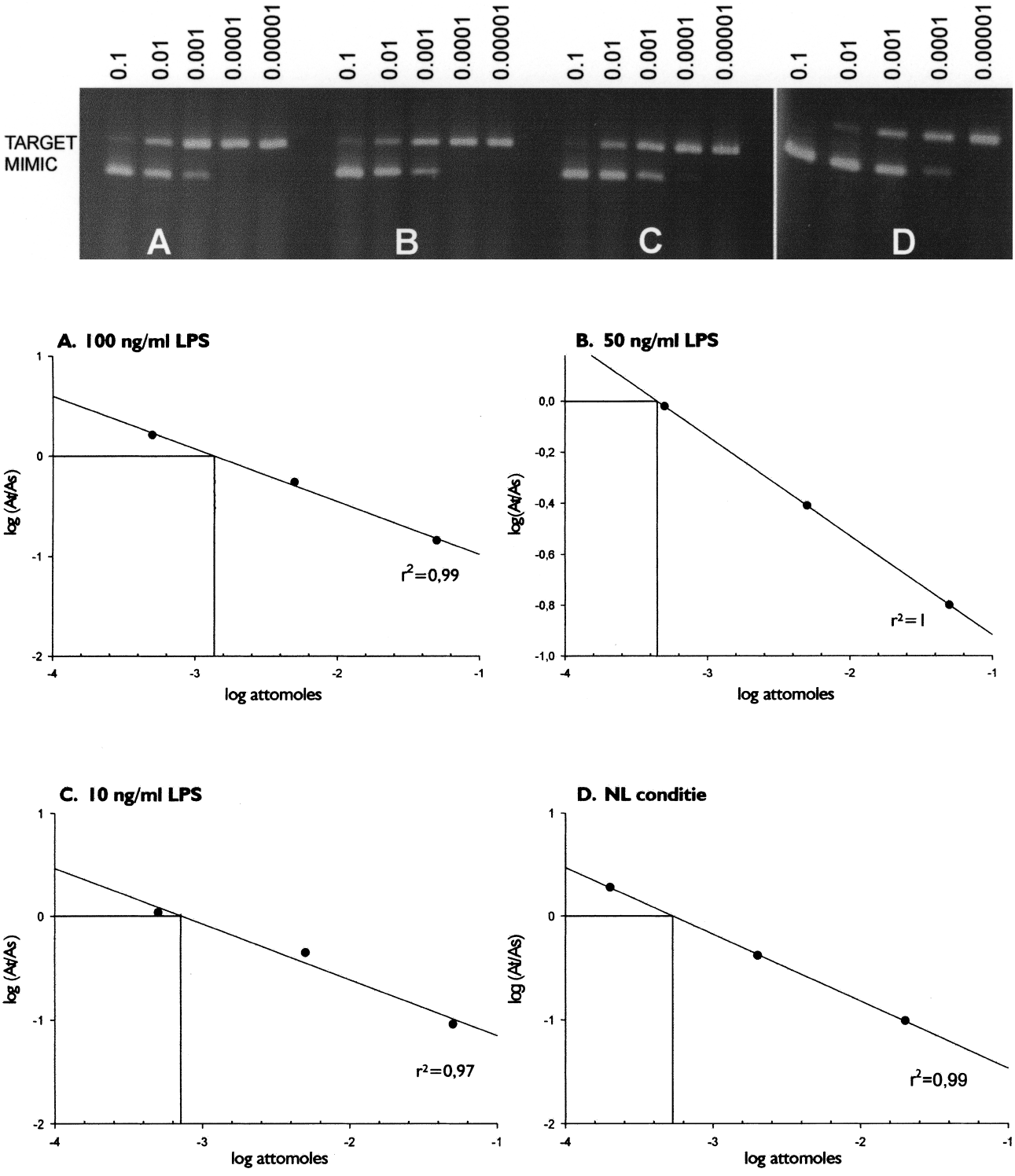

Fig. 2. – (Upper part) Competitive PCR analysis of the POMC mRNA expression in HD11 cells upon treatment with LPS. In this cPCR reaction, a ten-fold dilution series of the PCR MIMIC was co-amplified with a constant amount of experimental cDNA. The size of the target is 330 nucleotides (nt) and that of the PCR MIMIC is 251 nt.
(Lower part) Representative plot of the ratio of target DNA intensity (At) to MIMIC intensity (As) plotted against the initial concentration of MIMIC DNA in attomoles on logarithmic scales. A line was drawn from a linear regression analysis of the data points, and the amount of target POMC was calculated by determining the x-intercept for the point of the curve where the ratio target to MIMIC equals 1 (when y = 0). **A.** HD11 cells stimulated with 100 ng/ml LPS. **B.** HD11 cells stimulated with 50 ng/ml LPS. **C.** HD11 cells stimulated with 10 ng/ml LPS. **D.** Untreated HD11 cells (controls). The ten-fold dilution series of the MIMIC starts at 0.1 attomoles/µl and goes down to 0.0001 attomoles/µl indicated above the gel.

DISCUSSION

So far, information about non-pituitary pro-opiomelanocortin (POMC) in the chicken has been relatively scarce. Using the RT-PCR technique, we were able to detect a POMC fragment in the bursa, thymus, liver, kidney, etc. (H. GERETS, manuscript in preparation). The expression of the chicken POMC gene in extra-hypophysial tissues, including adrenal gland, gonads, kidney and a host of other tissues has also been reported by TAKEUCHI et al. (1999). These observations were among the first to demonstrate that POMC mRNA is indeed present outside the pituitary and the brain of the chicken. In mammals, by contrast, non-pituitary POMC gene expression has already been described in detail a number of years ago (DEBOLD et al., 1988a,b).

Using the RT-PCR technique on HD11 cells, we presently report the expression of the POMC gene in a myelomonocytic cell line. Elaborating on these results, the regulation of these cells by lipopolysaccharide (LPS) was investigated. For this purpose, a competitive PCR assay was developed. Lipopolysaccharide, an integral component of the outer membrane of gram negative bacteria, activates important cellular mechanisms in the acute phase of a bacterial infection (GOETHE et al., 1998).

HD11 cells belong to the myelomonocytic lineage and our present results show a low level of POMC mRNA expression under normal conditions. Upon treatment with LPS, increased levels of POMC mRNA in HD11 cells were rapidly induced, with a minimal effective dose of 100 ng/ml. Lower doses of 10 and 50 ng/ml LPS had no effect compared with control cells; possibly these concentrations are too low to be effective. GOETHE & PHI-VAN (1998) used concentrations up to 5 µg/ml LPS to stimulate HD11 cells.

An important conclusion from these findings is that the non-pituitary POMC transcript in chicken macrophages seems to be subject to physiological regulation at the transcriptional level. This corroborates the hypothesis that also in birds POMC-derived peptides of non-hypophysial origin play a biological role in the immune response, similar to what has been shown before in mammals (OTTAVIANI et al., 1999). This is perfectly in line with experiments done by HENDRICKS & MASHALY (1998) and by HENDRICKS et al. (1995) showing that only a particular subset of avian splenic leucocytes, the macrophages, produce ACTH in response to corticotropin releasing hormone (CRH) stimulation and that this effect is inhibited by corticosterone.

Earlier experiments in mice also demonstrated that macrophage-like cells and other immune cells produce ACTH and β-END when stimulated with different stimuli such as LPS, Newcastle Disease Virus (NDV) and CRH (HARBOUR et al., 1991). Interestingly, different stimuli seem to result in differential processing of the POMC-derived peptides: Newcastle Disease Virus and CRH induce the production of POMC peptides with the molecular weight of $ACTH_{1\text{-}39}$ and β-END, whereas LPS leads to the production of a truncated form of ACTH ($ACTH_{1\text{-}24\ to\ 26}$) and β-END (α- or γ-END) (HARBOUR-MCMENAMIN et al., 1985; HARBOUR et al., 1987). These data seem to point towards the existence of distinct regulation mechanisms for the respective processing enzymes involved in the post-translational processing of the POMC precursor protein (HARBOUR et al., 1987).

Another important question that remains to be answered in the presently described avian in vitro model, is whether the observed POMC message will be translated and if the precursor protein will be properly processed into biologically active, secreted peptides. RT-PCR experiments using the primer set that spans the entire POMC coding region yielded a fragment of 678 bp, corresponding to the size of the pituitary POMC transcript as determined previously (GERETS et al., 2000), but since none of the primer sets used in this study included the message for the signal peptide, our present data do not allow us to answer the question.

However, since the entire coding region of POMC is synthesized in the HD11 cells, it is theoretically possible that the POMC-derived peptides are being translated and have a biological function in the cells of the macrophage lineage. It has been shown that POMC-derived peptides are important mediators in the overall response to endotoxin in immune cells (HARBOUR et al., 1991). MECHANICK et al. (1992) demonstrated that both POMC mRNA and ir-β-END exist in rat spleen and lung macrophages. Because immune cells possess receptors for POMC-derived peptides, it is possible that these peptides have autocrine and/or paracrine roles within these immune tissues (MECHANICK et al., 1992). β-Endorphin for example has been found in macrophages where it acts on opioid receptors to lessen pain (BLALOCK, 1994). GALIN et al. (1991) did not detect a full length POMC transcript in murine lymphocytes upon CRH treatment despite the inherent sensitivity of the PCR technique. Instead, two truncated POMC transcripts were observed that were quite different in structure in that one lacked the extreme 5' end of exon 3 while the other contained all of exon 3.

Finally, proteolytic cleavage of the POMC precursor is another requirement for the production of biologically active, secreted peptides. This implies expression of the prohormone convertases enzymes PC1 and/or PC2, which have been shown to be indispensable for cleavage of the POMC precursor (SEIDAH & CHRÉTIEN, 1992). The particular enzyme profile present within the cell indeed determines the nature of the peptides synthesized and secreted. Therefore, future studies will focus on the question whether the chicken equivalents of PC1 and/or PC2 are being expressed in chicken HD11 cells, in particular, and in avian immune tissues, in general.

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Particularities of the bucco-pharyngeal apparatus in *Zenarchopterus kampeni* (Pisces: Hemiramphidae) and their probable significance in feeding

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ABSTRACT. The present study shows several new anatomical particularities of the buccal and pharyngeal parts of the halfbeak *Zenarchopterus kampeni*. The upper buccal jaw consists of premaxillaries and maxillaries tightly joined by ligaments. A 10° lowering of the mandible leads to a 30° elevation of the upper jaw. The adductor mandibulae is reduced to bundles A^2 and Aω. As in the Labridae, the lower pharyngeal jaw articulates with the scapular girdle. The upper pharyngeal jaw consists of distinct second pharyngobranchials followed by the third pharyngobranchials fused into a powerful posterior component. This part fits into and slides along a longitudinal ventral gutter of the neuroranium, thanks not only to the dorsal retractor muscles but also to specific retractors of the second pharyngobranchials. The power and dentition of the pharyngeal parts contrasts with the fragility of the buccal elements.

KEY WORDS: Pisces, Hemiramphidae, cephalic morphology, osteology, myology.

INTRODUCTION

"Halfbeak" is the common name for fish of the Hemiramphidae family containing approximately 80 species (ROSEN, 1964; COLETTE & SU, 1986; ALLEN, 1991; NELSON, 1994). It comes from the peculiar morphology of their buccal jaws: the upper jaw is short and the lower one is very long. Halfbeaks are long fish. Most species are marine and epipelagic, but some live in fresh or brackish water (ALLEN, 1991; NELSON, 1994).

Several authors have tried to establish a relationship, in fish, between the skeletal and muscular structures of the bucco-pharygeal apparatus on the one hand and feeding behaviour on the other (LAUDER, 1982; LIEM & OSSE, 1975; VANDEWALLE et al., 1995). From this point of view, the external morphology of the buccal parts of hemiramphids appears exceptional among teleosts. According to ALEXANDER (1967b), a slight lowering of the halfbeak mandible raises slightly the small upper jaw (almost) without changing the general shape of the body. This could represent an advantage for feeding at the water surface and as a means of misleading a predator by maintaining a "twig-like" appearance. The pharyngeal jaws are also original: the 5th ceratobranchials can be fused together to form a single lower jaw with a large bony wing ventrally for insertion of fibres from the sternohyoid muscles (ROSEN, 1964), and the upper pharyngeal jaws consist of independent second pharyngobranchials and often fused third pharyngobranchials (ROSEN & PARENTI, 1981).

Data on the bucco-pharyngeal system, presented by ROSEN (1964), ALEXANDER (1967b), and ROSEN & PARENTI (1981), are not complete enough to explain several functional originalities. The aim of the present work was to complement the existing knowledge of bucco-pharyngeal morphology in hemiramphids with a study of this system in *Zenarchopterus kampeni* (Weber, 1913) (species determination according to ALLEN, 1991).

MATERIAL AND METHODS

The *Z. kampeni* specimens came from the little estuaries opening into Hansa Bay, north of Papua New Guinea.

Observation of the skeleton and musculature was done on 20 specimens (total length between 16 and 18 cm) that had either been preserved in 70% alcohol (15) or frozen (5). Seven specimens were trypsin-cleared and stained with alizarin according to TAYLOR & VAN DIJK (1985) in order to observe certain bony structures in greater detail.

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The dentition was observed with a JEOL JSM 840A scanning electron microscope.

LIST OF ABBREVIATIONS

AA: articulo-angular
Aω: adductor mandibulae ω
$A_2\alpha$: second adductor mandibulae α
$A_2\beta$: second adductor mandibulae β
ADARC: adductor arcus palatini
AD 1-5: adductores branchiales 1 to 5
BOC: basioccipital
DC: dorsal crest
DE: dentary
DIOP: dilatator operculi
EBR1: first epibranchial
EPOT: epiotic
HM: hyomandibular
IO: interopercular
IGH: insertion of the protractor hyoidei
IIM: insertion of the intermandibular muscle
IRD: insertion of the retractor dorsalis
IRPBR2: insertion of the retractor muscle of the 2nd pharyngobranchial
K: keel
LEAP: levator arcus palatini
LEPO: levator posterior
LETH: lateral ethmoid
LEXT 1-4: levatores externi 1 to 4
Li. 1-10: ligaments 1 to 10
LINT 2-3: levatores interni 2 and 3
LPJ: lower pharyngeal jaw
MX: maxillary
O: opercular
PAL: palatine
PASPH: parasphenoid
PBR2-3: pharyngobranchials 2 and 3
PCDE: dentary coronoid process
PHCLE: pharyngoclavicularis externus
PHCLI: pharyngoclavicularis internus
PMX: premaxillary
PO: preopercular
PROT: prootic
PTOT: pterotic
Q: quadrate
RA: retro-articular
RD: retractor dorsalis
RPBR2: retractor of the second pharyngobranchial
SO: subopercular
SOC: supraoccipital
STH: sternohyoideus
SUSP: suspensorium
TAω: tendon of the adductor mandibulae ω
$TA_2\alpha$: tendon of the second adductor mandibulae α
$TA_2\beta$: tendon of the second adductor mandibulae β
TRPBR2: tendon of the retactor of the second pharyngobranchial
TRV: transversus ventralis
UH: urohyal
UPJ: upper pharyngeal jaw
VO: vomer
V2-3: second and third vertebrae

RESULTS

Buccal apparatus

Skeleton

The left and right premaxillaries are flattened, bent, and tightly joined mesially over their entire length by very short fibres, thus constituting a triangular plate. They bear three to four rows of very small conical teeth. The maxillaries partly cover the premaxillaries ventrally and externally and are dorsally covered by the premaxillaries (Fig. 1B). The maxillaries and premaxillaries are attached to

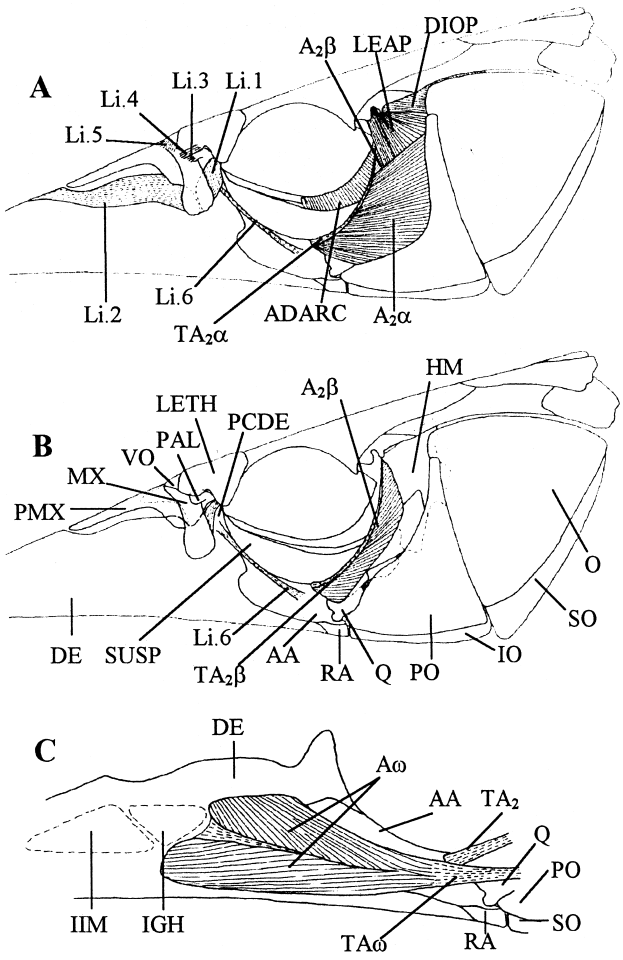

Fig. 1. – *Zenarchopterus kampeni.* A, left lateral view of the head, showing the musculature covering the suspensorium and several ligaments; interrupted lines show the limits of the ligaments. B, left lateral view of the head showing the positions of the $A_2\beta$ muscle and two ligaments; interrupted lines indicate the limits of certain bony structures. C, internal lateral view of the right half-mandible, showing the Aω muscle; interrupted lines indicate muscle insertion sites. On all three drawings, the anterior part of the mandible is not represented.

each other by very short fibres so as to form a single, rigid upper jaw. The maxillaries articulate with the anterior processes of the palatines and with the lateral ethmoids (Fig. 1B). A ligament (Li.3) connects the maxillaries to the front of the palatines and another (Li.4) connects them to the antero-dorsal face of the lateral ethmoids (Fig. 1A). Each palatine is firmly attached to the lateral ethmoid by very short fibres. Furthermore, a ligament (Li.5) connects the maxillaries and premaxillaries to the vomer (Fig. 1A). It consists of fibres increasing in length from the outer part to the middle of the vomer.

The mandible consists of the dentaries and articulo-angulars, fused to the retroarticulars (Fig. 1B). The dentaries are very elongated (Fig. 2), being very thin in front and broadening toward the rear. Their dorsal side is flat and their ventral side is rounded. Dorsally they are joined by short fibres up to the level of the upper jaw. From this level onward the distance between them increases. They bear three to four rows of little pointed teeth, located anteriorly and externally with respect to the premaxillaries. The coronoid processes are well developed (Fig. 1C).

The articulo-angulars, ensuring the articulation between the mandible and the quadrate, extend at the inner face of the dentaries, and their anterior tips penetrate a postero-mesial cavity of the dentaries. The dentaries and articulo-angulars are fused, making the posterior portion of each half-mandible very rigid.

The retroarticulars lean against the interoperculars and are connected with them by very short fibres. The interoperculars are joined by short fibres to the subopercular.

Two large ligaments connect the lower jaw to the inner ventral face of the maxillaries: Li.1, attached to the coronoid process, and in front Li.2, which contributes to the lower lip. A long ligament (Li.6) links the outer posterior edge of the maxillary to the articulo-angular.

Musculature

The intermandibular muscle is wide and thick (Fig. 1C). The protractor hyoidei muscles insert to the front on the inner face of the dentaries (Fig. 1C) and to the rear on the hyoid bars. Posteriorly, the sternohyoid muscle extends between the scapular girdle and a long, fine urohyal (Fig. 5A).

The cheek is occupied principally by the adductor mandibulae. According to Winterbottom's nomenclature (1974), this is bundle A_2 divided into $A_2\alpha$ and $A_2\beta$ (Fig. 1A,B). $A_2\alpha$, the larger, more external bundle (Fig. 1A), is attached posteriorly to the preopercular, the metapterygoid, the symplectic, and the quadrate. In front it is attached via a tendon to a spur on the articulo-angular (Fig. 1A,C). $A_2\beta$ is attached on the one hand to the hyomandibular and pterygoid and on the other hand it is extended by a tendon that fuses with the $A_2\alpha$ tendon (Fig. 1B,C).

On the inner face of the mandible lies a bipennate $A\omega$ adductor bundle. It is attached to the inner faces of the dentary and articulo-angular and continued by a tendon, which passes above the quadrato-mandibular joint and attaches to the inner face of the quadrate (Fig. 1C).

There is no levator operculi. The rest of the cephalic musculature shows no special features.

Movements

A 10° lowering of the mandible (which seems to be a maximum) causes a 30° rotation of the upper jaw and makes the interopercular move backward, causing the operculum to rotate around its articulation with the hyomandibular (Fig. 2).

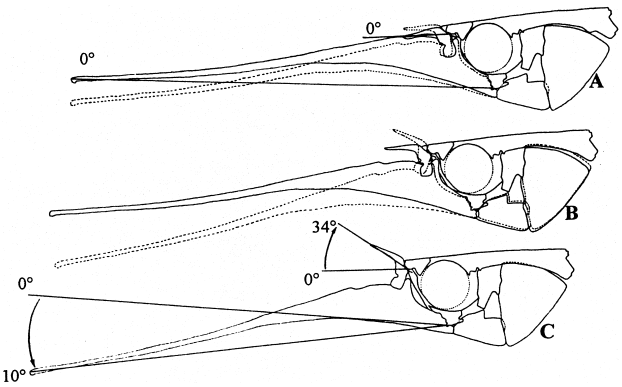

Fig. 2. – *Zenarchopterus kampeni*. Lateral view of the head. The preopercular is not represented. A, mouth closed (full lines) and mouth half-open (interrupted lines); B, mouth half-open (full lines) and mouth wide open (interrupted lines); C, mouth wide open.

Pharyngeal apparatus

Skeleton

The first branchial arch has no pharyngobranchials. The first epibranchials are tapered and point inward. Their cartilaginous extremities are bound by a short ligament to the parasphenoid (Fig. 5A) and their posterior parts articulate with the pharyngobranchials of the second arch.

The second and third branchial arches are complete. The second pharyngobranchials are independent (Fig. 5B). They are narrow and pointed in front and are wider on the back side (Fig. 5B). In front they hang from the parasphenoid by loose fibres and each bears a tooth plate limited to the wider part (Fig. 3B). The teeth are numerous, small, and conical and they curve slightly backward. The second pharyngobranchials articulate laterally with the second epibranchials. Caudally, they are connected to the third pharyngobranchials by the pharyngeal epithelium and connective tissue. These pharyngobranchials are fused, forming a single tooth-bearing pharyngeal bone (Fig. 3B,C,D) with two anterior points. A straight suture is visible between the two halves, although the two bones

cannot be separated. The teeth are large and tricuspid, but the lateral cusps can be rather slight. (Fig. 4B). The median cusp of the longest teeth extends backward in a kind of ridge. This pharyngeal bone has two long, straight dorsal ridges running from front to back. These ridges fit into two grooves on the posterior base of the neurocranium (Fig. 3D), at the level of the prootic, parasphenoid, and basioccipital. The third and fourth epibranchials articulate with the third pharyngobranchials. The second and third pharyngobranchials together constitute the upper pharyngeal jaws (Fig. 3A,B,C,D).

The fifth ceratobranchials are fused into a triangular tooth-bearing lower pharyngeal jaw (Fig. 3A). Tooth size decreases from front to rear (Fig. 4A) and from without to within. The dorsal faces of the largest teeth have a medial groove and a rather sharp tip pointing backward (Fig. 4A). In the back, the pharyngeal jaw bears two latero-posterior processes by which it abuts against the anterior face of the cleithra (Fig. 3C). The inner part of these processes is linked to the scapular girdle by a large ligament (Li.9) (Fig. 5A). Ventrally, the lower jaw bears an wing shaped like a keel (Fig. 3A).

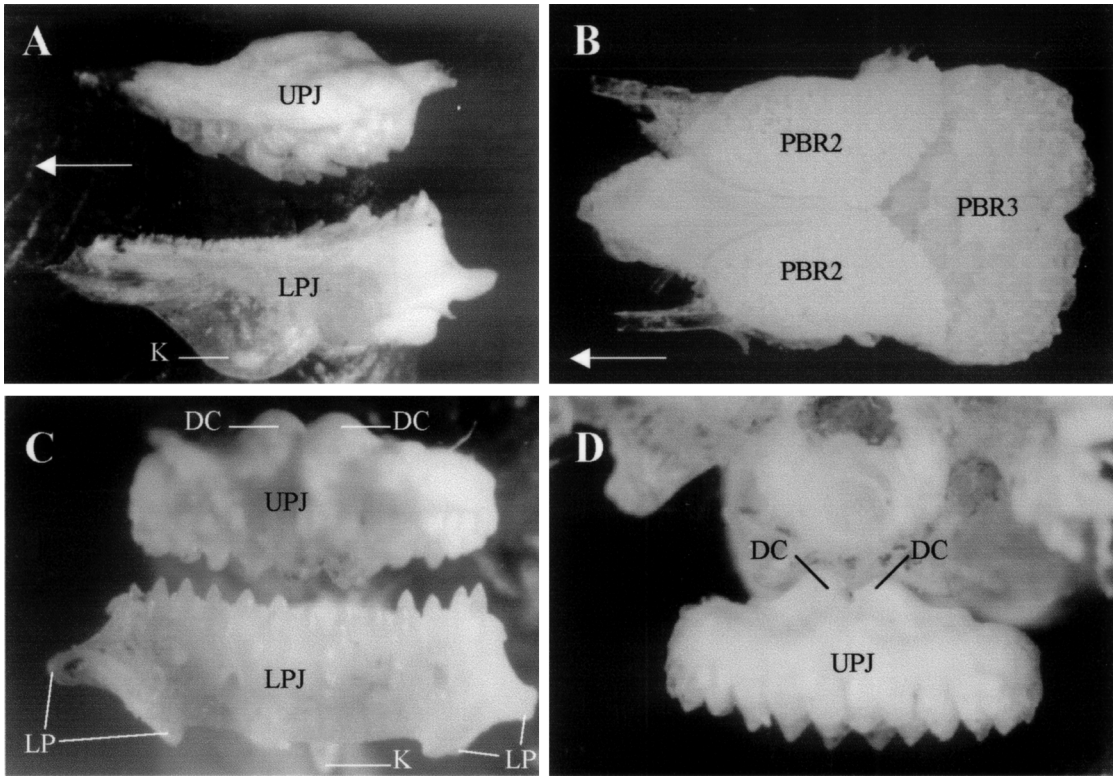

Fig. 3. – *Zenarchopterus kampeni*. Photos of the pharyngeal jaws. A, lateral view of the pharyngeal jaws; B, ventral views of the upper pharyngeal elements; C, posterior view of the pharyngeal jaws; D, posterior view of the third pharyngobranchials and lower part of the neurocranium. Arrows indicate the front.

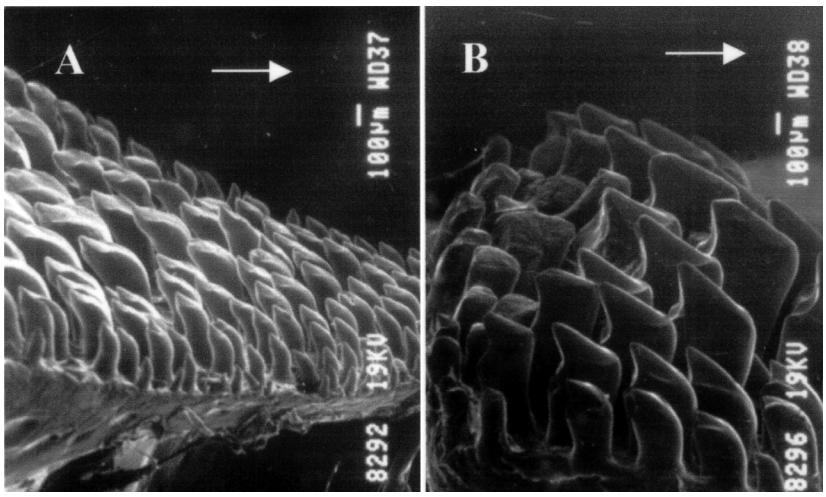

Fig. 4. – *Zenarchopterus kampeni*. Photos taken with a scanning electron microscope, showing part of the dentition of the lower jaw in A and of the third pharyngobranchials in B. Arrows indicate the front.

Musculature

The branchial musculature is original in several respects. The pharyngoclaviculares interni and externi are highly developed and connect the lower pharyngeal jaw to the scapular girdle (Fig. 5A). The outer muscles are attached to the ventral face of the pharyngeal jaw, the inner ones to the keel. The levatores interni 3 and externi 4 tilt markedly forward. The adductores branchiales 5 are very thick and attached on the one hand to the lower jaw and on the other hand to the ceratobranchials and epibranchials of the fourth arch (Fig. 5A). The levatores posteriores present a double ventral insertion on the ceratobranchials 4 and on the latero-posterior processes of the lower pharyngeal jaw. The retractores dorsales are particularly developed and insert on one side on the third pharyngobranchials and beneath the parapophyses and bodies of the third and fourth vertebrae (Fig. 5A).

Lastly, in addition to these dorsal retractores there are other retractores, inserting in front by a tendon onto the narrow anterior part of each second pharyngobranchial and caudally, on the parasphenoid end (Fig. 5B).

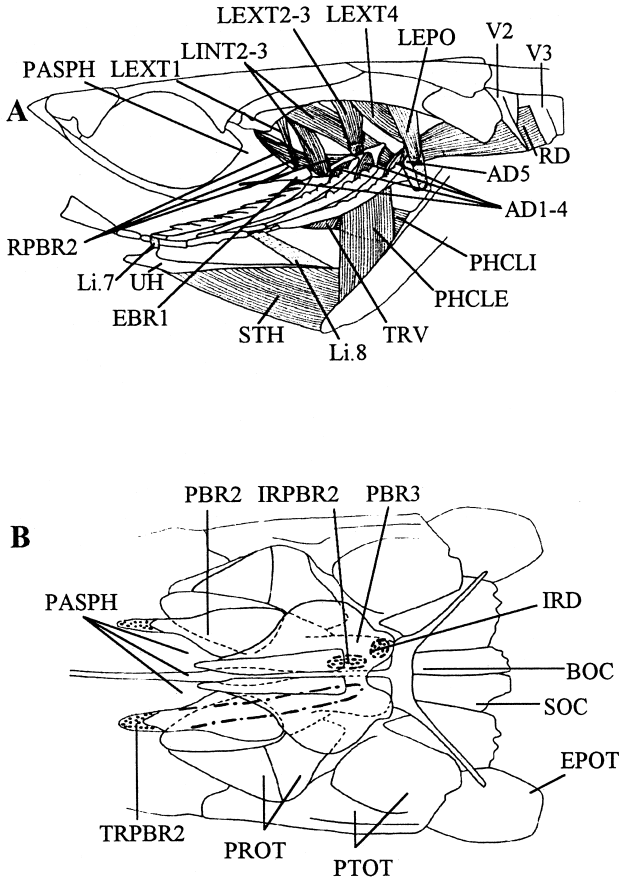

Fig. 5. – *Zenarchopterus kampeni*. A, lateral view of the neurocranium and branchial basket, showing much of the branchial musculature; B, ventral view of the back of the neurocranium, showing the pharyngobranchials and the second retractor dorsalis (thick interrupted line).

DISCUSSION

Buccal parts

Nearly all highly evolved fish have superimposed premaxillaries and maxillaries (GREENWOOD et al., 1966; OSSE, 1969; LIEM, 1970, 1991; VANDEWALLE, 1972; LAUDER, 1982, 1983). Laterally and posteriorly, the maxillaries cover the premaxillaries on the outside; mesially, they have an anterior process covering the premaxillaries and they articulate posteriorly with the palatines and ethmoid region (ALEXANDER, 1967a; LIEM, 1970; VANDEWALLE et al., 1995). The premaxillaries consist of a horizontal process (which usually bears teeth) bordering the mouth opening and a processus ascendens associated with a rostral cartilage surmounting the front of the neurocranium (GREENWOOD et al., 1966; NELSON, 1994). Ligaments hold the whole structure together (OSSE, 1969; LIEM, 1970; BENMOUNA et al., 1984; VANDEWALLE et al., 1995). When the mouth opens, the mandible is lowered, possibly by contraction of the levator operculi, sternohyoideus, protractor hyoidei, epaxials, and/or hypaxials (OSSE, 1969; LIEM, 1970; VANDEWALLE, 1978). It sets in motion the upper jaw: the premaxillaries move away from the maxillaries mesially by sliding over the front of the neurocranium, while the maxillaries remain in contact with the palatines and front of the neurocranium (ALEXANDER, 1967a; ELSHOUD-OLDENHAVE & OSSE, 1976; VAN HASSELT, 1978; LIEM, 1979; MOTTA, 1984; WESTNEAT & WAINWRIGHT, 1989). The mouth is closed by the adductor mandibulae bundles inserted on the maxillary (A_1) and lower jaw (A_2, A_3). Insertion of A_1 on the maxillary notably allows modulation of protrusion and mouth opening (ALEXANDER, 1967a; LIEM, 1991, 1993). The intermandibular is very small and probably slightly modifies the distance between the two half-mandibles (VANDEWALLE, 1972). This type of protrusible upper jaw is found in some Atherinomorpha species (sensu ROSEN & PARENTI, 1981) (ALEXANDER, 1967b) but the organisation of adductor mandibulae bundles seems variable: according to ALEXANDER (1967b), *Atherina presbyter* has an organisation like that of the Perciformes, with A_1 located dorsally with respect to A_2, whereas ROSEN (1964) describes in several Atherinomorpha species a crossing of the (outer) A_1 and (inner) A_2 bundles as in the Cypriniformes (ALEXANDER, 1966; BALLINTIJN et al., 1972; VANDEWALLE, 1975). ALEXANDER (1967b) does not describe the adductor mandibulae in *Dermogenys* sp.

In *Z. kampeni* as in other hemiramphids (ALEXANDER, 1967b) and also in belonids (BOUGHTON et al., 1991), the superposed maxillaries and premaxillaries are closely bound together over their entire length by very short fibres. Contrary to what ALEXANDER (1967b) describes in *Dermogenys* sp., there is no true processus ascendens or rostral cartilage in *Z. kampeni*.

In *Z. kampeni*, the mandible can only be lowered by the ventral and epiaxial musculature, since the levator oper-

culi is absent. The movements of the mandible cause in fact the operculum elevation and lowering. When the mandible is lowered, the upper jaw behaves like a single element. The mandible, acting via ligament Li.2, pulls on the upper jaw, which rotates upwards around a transversal axis running between the front of the left and right palatines and lateral ethmoids. With respect to the vomer, this movement is possible because ligament Li.5 has longer median fibres than outer fibres. No protrusion is possible. A 10° lowering of the mandible causes a considerable rotation of the upper jaw. This rotation is greater in *Z. kampeni* (over 30°) than in *Dermogenys* sp. (20°) (ALEXANDER, 1967b). A slight lowering of the mandible causing a greater rotation of the upper jaw is explainable only by the fact that the distance between (1) the articulation of the maxillary with the palatine and the front of the neurocranium and (2) the point where ligament Li.2 exerts its traction , is markedly shorter than the distance between the quadrato-mandibular joint and the coronoid process of the dentaries.

The mouth is closed by contraction of the A_2 and Aω bundles. Contraction of the latter raises the mandible, which pulls on the upper jaw via ligaments Li.1 and Li.5. The upper jaw moves backward and downward. The absence of the A1 bundle is probably related to the fact that the bones of the upper jaw cannot move with respect to each other. Given this rigidity of the upper jaw, this fish is probably unable to modulate the opening of its mouth as do other highly evolved teleosts (ALEXANDER, 1967a; LIEM, 1991). Only the intermandibular muscle might exert some modulation, being particularly large. Its contraction might bring closer together the ventral edges of the mandible, and thus move the coronoid processes apart. This in turn could widen the mouth and/or favour rotation of the upper jaw. It should also bring the anterior parts of the suspensoria closer together, somewhat reducing the volume of the buccal cavity.

Pharyngeal jaws

ROSEN (1964) described in hemiramphids a single lower pharyngeal jaw very similar to that of the Cichlidae and Embiotocidae, but with an additional ventral wing. This description is incomplete. In *Z. kampeni*, this jaw has an additional feature: it articulates with the scapular girdle like those of the most evolved Pharyngognathi, the Labridae and Scaridae (LIEM & GREENWOOD, 1981; LIEM & SANDERSON, 1986; MONOD et al., 1994). As in these fish, there is no pharyngohyoideus muscle. The ventral wing is the insertion site of the pharyngoclaviculares interni and not of the sternohyoid muscle as described by ROSEN (1964).

In Scaridae species, NELSON (1969), GOBALET (1989) and MONOD et al. (1994) described upper pharygeal jaws closely bound together by connective fibres. This, according to LIEM & GREENWOOD (1981), constitutes the final stage in the transformation of the upper pharyngeal jaws in Pharyngognathi. Fused third pharyngobranchials have been described only in four Exocoetoidei species (ROSEN & PATTERSON, 1969; ROSEN & PARENTI, 1981). *Z. kampeni* is an addition to this list. Yet this species seems to be the only one with two dorsal ridges fitting into gutters at the base of the neurocranium. This arrangement seems unique. In Pharyngognathi, the upper pharyngeal jaws have always been described as articulating with the neurocranium and animated by swinging movements (AERTS et al., 1986; LIEM, 1986; LIEM & SANDERSON, 1986; CLAES & DE VREE, 1989, 1991). In *Z. kampeni*, the upper jaw is divided in two parts: the posterior part constituted by the third blended pharyngobranchials, can only slide back and forth in the neurocranial gutters, movements initiated by all the retractores muscles; the second pharygobranchials are free from one another and loosely fixed to the third ones by small fibers, and their position and orientation can be modified by the contraction of their second retractor bundles during the antero-posterior displacements of all the upper pharyngeal jaws.

The shapes of the largest upper and lower pharyngeal teeth show clearly that they can coapt: the ridges of the upper ones fit into the concave parts of the lower ones.

Among the Pharyngognathi, scarids possess the most powerful pharyngeal system (LIEM & GREENWOOD, 1981; GOBALET, 1989; MONOD et al., 1994; BULLOCK & MONOD, 1997). *Z. kampeni*'s is even more remarkable. Opposite to a single triangular lower jaw articulating with the girdle are the second pharyngobranchials, bearing teeth as in most acanthopterygians, followed by a single large, broad pharyngeal bone. We propose the following hypothesis regarding the functional participation of this system in feeding. The upper parts protrude and the front of the lower jaw tilts downward. Upon arrival of a prey between the pharyngeal elements, the lower jaw would be raised by contraction of adductors 5, possibly associated with that of the levatores posteriores and externi 4 and with a forward rotation of the scapular girdle. The prey would be seized between the lower jaw and the second phryngobranchials which can be relieved by the contraction of their retractor muscles. It would then be crushed between the jaws by successive lowering movements due to contraction of the pharyngoclaviculares muscles, followed by elevation of the lower pharyngeal jaws. Then the retractores dorsales associated with the retractors of the second pharyngobranchials would pull the dorsal elements backward, the upper pharyngeal bone sliding in the neurocranial gutters, only the relative position and orientation of the second pharyngobranchial can be modulated. This movement would move the food backward while shearing it. Lastly, the pharyngocleithrales would depress the lower jaw (SIBBING, 1982) and the levatores interni 3 and externi 4 (principally) would protrude the upper jaws, guided by the neurocranial gutters. This pharyngeal system seems rigid, allowing only amplitude variations in the movements of its different components, contrary to what has been observed in Pharyngognathi (AERTS et al., 1986;

LIEM, 1986; LIEM & SANDERSON, 1986; CLAES & DE VREE, 1989, 1991). In the latter the mastication cycles, notably, differ from the transport and swallowing cycles. The upper pharyngeal jaw movements in the Pharyngognathi appear to follow several motor patterns or even a single pattern that can be modulated. There is probably no modulation in the third pharyngobranchial movements in *Z. kampeni*. By contrast, a variability in the second pharyngobranchial movements is possible because these elements are loosely connected to the third pharyngobranchials.

Comment on feeding behaviour

During the fishing expedition, some of the *Z. kampeni* specimens were caught near the water surface. Once in the aquarium, specimens often stayed horizontal near the surface (personal observation). This supports ALEXANDER'S (1967b) hypothesis (see introduction), further supported by the observation of ALLEN (1991) and ALLEN & SWAINSTON (1992) that halfbeaks eat floating insects. Yet these same authors report that captured halfbeaks also eat aquatic insect larvae, prawns, or fishes. These prey can be either pelagic or benthic. Feeding on benthic animals could be related to foraging behaviour: the lower jaw could rummage through the sediment and send particles and organisms into temporary suspension. Whether the prey is an insect or a crustacean, the buccal jaws should only be able to seize the prey. The teeth of these jaws are very small and the upper ones are behind the lower ones. The upper jaw does not appear to have the size and solidity that would make it a good tool for crushing. This would be the task of the pharyngeal jaws, with powerful musculature and bearing many teeth. The prey is probably seized between the lower pharyngeal jaw and the second pharyngobranchials, and reduced principally by the third pharyngobranchials and the lower jaw before transporting it to the oesophagus.

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Some noteworthy free-living copepods from surface freshwater in Belgium

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ABSTRACT. *Cyclops singularis* Einsle, 1996 has only recently been separated from *Cyclops strenuus* Fisher, 1851. It was described from temporary waters in South Germany and has now been rediscovered in a similar environment in Flanders. Of the confused genus *Acanthocyclops*, we find that there is at least three species living in Belgium. One of these, here named *Acanthocyclops americanus* (Marsh, 1892) is related to but different from *A. robustus* (Sars, 1863) and *A. vernalis* (Fischer, 1853), both of which were previously known from Belgium. These three species are all subject to nomenclatorial uncertainty, which we suggest should be solved by the designation of neotypes by a college of representative copepodologists. *Paracyclops affinis* (Sars, 1863) and *Elaphoidella gracilis* (Sars, 1863), both from a smallest artificial pond, are second records from Belgium.

KEY WORDS: Copepod fauna, fresh water, Belgium, cyclopoids, harpacticoids, biogeography.

INTRODUCTION

Until recently, the list of free-living fresh- and brackish-water copepods of Belgium stood at 71 species and subspecies (DUMONT, 1989). A recent paper on the fauna of leaf litter copepods increased that number to 80 species (FIERS & GHENNE, 2000). However, since over 500 species of copepods are known from the continental waters of Europe (KIEFER, 1978), it should not come as a surprise that each sampling effort adds one of more species to the Belgian list.

MATERIAL AND METHODS

Most of our material was collected in May-October 1999 in a series of water-bodies in the vicinity of Ghent and in some city ponds: seven ponds, four temporary pools, four drainage canals and two roadside ditches in all. Plankton and near-shore communities were sampled with a plankton net with a mesh size of 100 um. For each sample about 100 l. of water were filtered. Samples were preserved in 70% ethanol and analysed few days after collection. Animals were placed in glycerol, dissected and drawn with a camera lucida, using a Leitz-Wetzlar microscope under oil immersion.

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For comparison we also collected *A. vernalis* from its type locality, a pond in the vicinity of St. Petersburg, Russia (spring 2001), and in lake Shohsee, Ploen, Germany (spring, 2002). All material used is kept in the collection of the Zoological Institute, Academy of Sciences, St Petersburg.

RESULTS

Thirty species of copepods were found. Two of these are new to Belgium, while two are second records for the country. Here, we give a brief description of these species, with illustrations of distinctive characters, as well as information on their ecology and status.

Cyclops singularis Einsle, 1996
(Fig 1, Fig. 5 B-D)

Material examined: 10 females and 4 males from the central park of Ghent and 3 females and 3 males from the botanical garden of Ghent University.

Female. Full body length without caudal setae 1.97-2.23 mm. Cephalosome 1.1 times as long as wide, with maximum width in the middle. Genital double somite conical, as long as wide, with small, round seminal receptacle. Distal part of the segment covered with pustules (Fig. 1A). Male spermatophores on ventral surface of the seg-

ment, shaped as in Fig 1A. Other segments of abdomen also with pustules on their surface. Caudal rami 6.1-6.3 times as long as wide, with long, dense hairs on their internal surface. Inner caudal seta bent distally. Relative lengths of distal setae, beginning from outer seta 1/3.6/4.1/2. Dorsal seta as long as outer seta. Antennule of 17 segments, reaching the middle of the first free somite. Basipod of second antenna at frontal side (Fig. 1C) with long line of robust spinules and group of small spinules mixed with small dots near insertion of two apical setae. At caudal side (Fig. 1D) this segment with three groups of large and three groups of tiny spinules. The above- mentioned pattern of small spinules mixed with small dots also present on the caudal side.

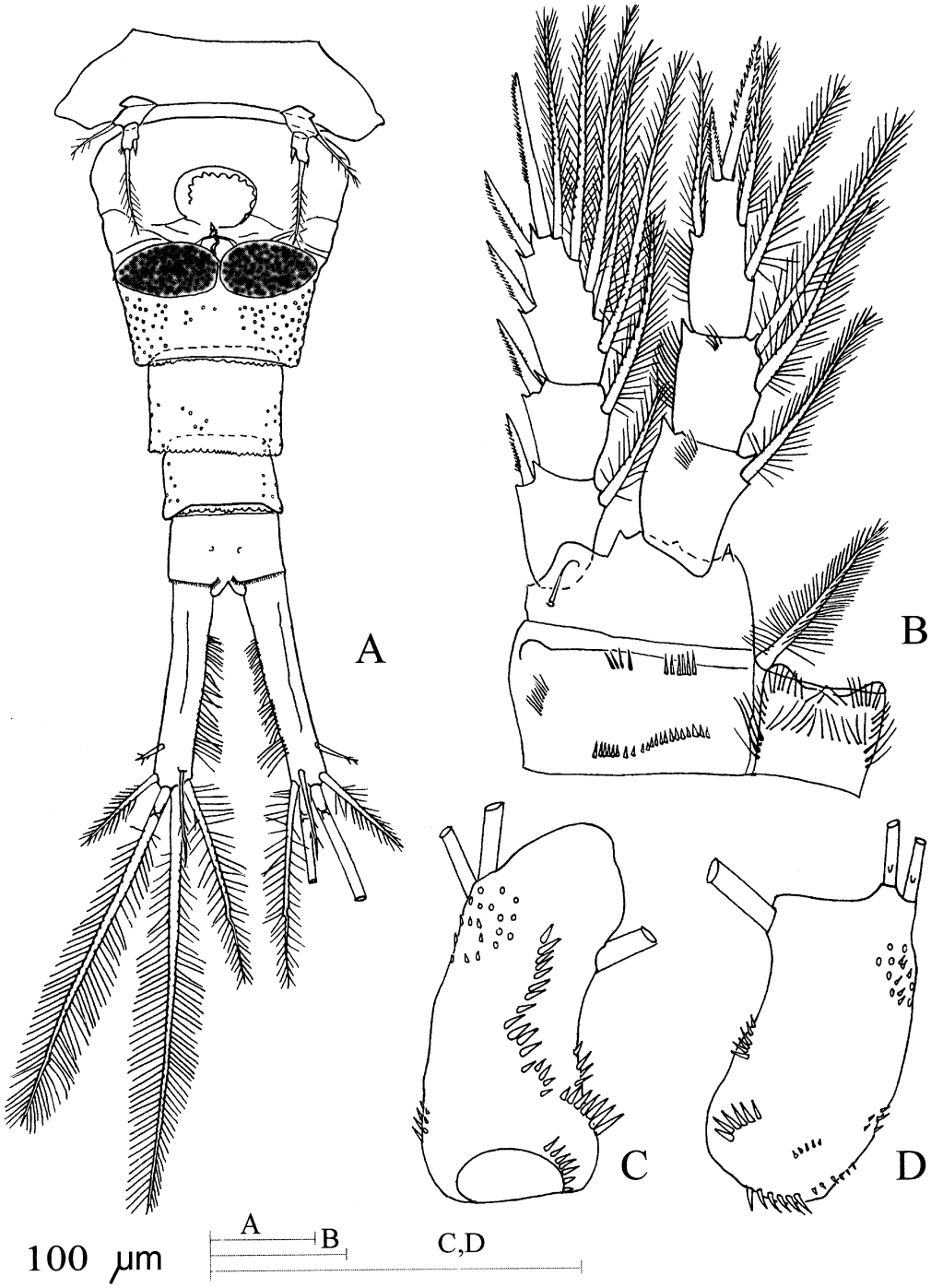

Fig. 1. – *Cyclops singularis* Einsle, female, Ghent, Belgium. A - abdomen, ventral view; B - Leg 4, caudal side; C,D -antennal basipod, frontal and caudal sides.

Swimming legs 3-segmented, with spine formula of distal exopod segments 3/4/3/3. Distal segment of endopod leg 4 about 2.5 times as long as wide. Distal inner spine of the segment about twice the length of outer spine. Coxal segment of leg 4 with three groups of spinules and a group of hair-spinules as shown in Figure IB. Intercoxal sclerite of leg 4 with two lines of hair-spinules and rather high hillocks on its free edge. Rudimentary fifth leg of 2-segments with rather weak inner spine at distal segment. Both setae of leg 5 relatively short.

Male. Full body length 1.65 mm. Genital somite without pustules and inner setae of caudal rami straight or only slightly bent in its last third. Coxal segment of leg 4, intercoxal sclerite of leg 4, and antennal basipod similar to that in female.

Differential diagnosis

C. singularis is easily separated from other *Cyclops* species by the segmentation of the antennula, the shape of the genital somite, the presence of pustules on its distal part, the inner caudal seta bent in its distal sector, the relative length of the distal spines of endopod P4, and the armament of the coxal segment of leg 4.

Distribution and ecology

This species was found in June and October in a small pond collecting water from a fountain in the central parc of Ghent, and in two micro reservoirs for water plant cultivation in the botanical garden of Ghent University. All these artificial biotopes exist in summer and dry up in winter. The maximum abundance of the species was about 500 ind. m^{-3}.

Acanthocyclops americanus (Marsh, 1892)

(Figs 2, 4, 5)

Material examined: 40 females and 15 males from a land-locked, slightly brackish-water creek in Jan-in-Eremo (Belgium) and 12 females and 5 males from the central park of Ghent.

Female. Full body length without caudal setae 1.00-1.2. mm. Cephalosome as long as wide, with maximum width in last third of its length. Genital double somite 1.3 times as long as wide, with relatively big, ellipsoid seminal receptacle. Seminal receptacle in its frontal part with wide transparent zone, regarded as a specific feature separating it from *A. vernalis* (LOWNDES, 1926; RYLOV, 1948). Caudal rami 4.7-5.3 times as long as *wide.* Inner caudal seta twice as long as outer seta. Relative lengths of distal seta, starting from outer seta, 1/5/7.3/2. Dorsal seta 1.5 times as long as outer setae.

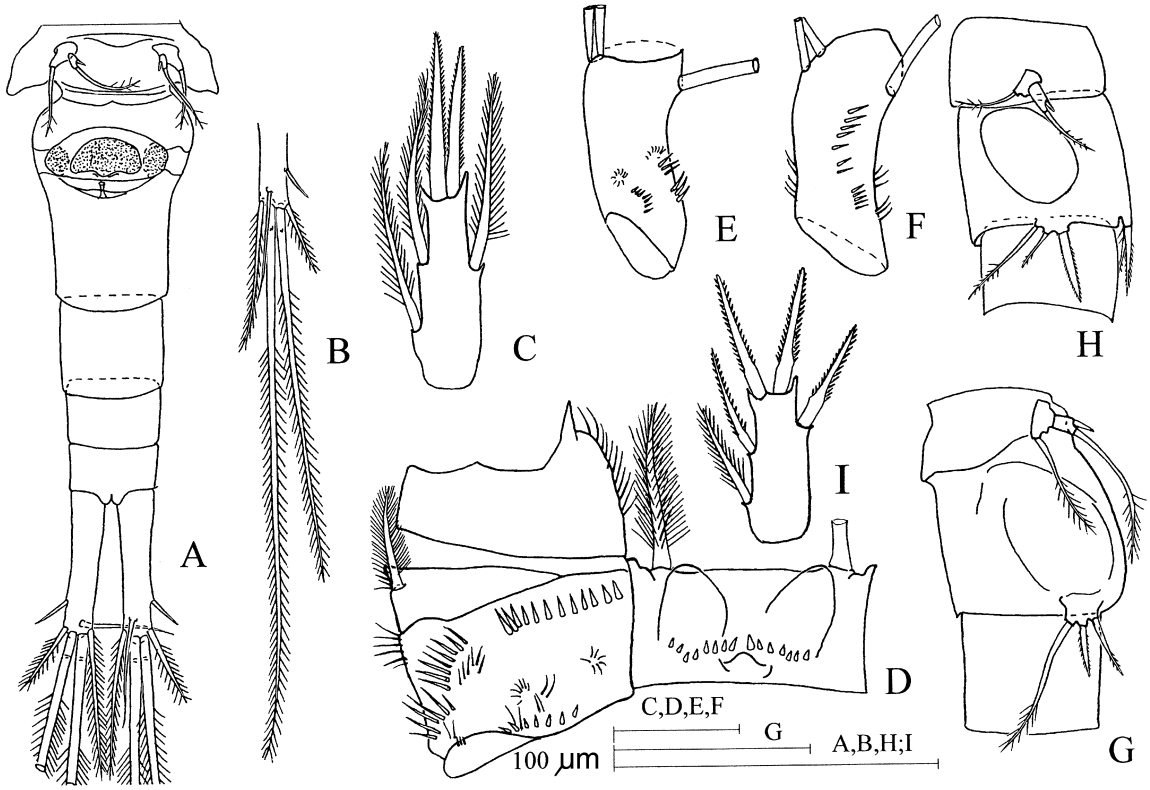

Fig. 2. – *Acanthocyclops americanus* (Marsh) (A-G) and *A. robustus* (Sars) (H-I) from Belgium. A-F -female; G-I - male. A - abdomen, ventrally; B - caudal ramus, dorsally; C - distal segment of endopod, leg4; D - coxa and intercoxal sclerite, leg 4 caudal side; E,F - antennal basipod, caudal and frontal sides; G,H - rudimentary legs 5 and 6, laterally; I - distal segment of endopod, leg4

Antennule of 17 segments, reaching first free somite. Basipod of second antenna at frontal side with three groups of spinules. Caudal side of this segment with three groups of strong spinules and two groups of hair-spinules.

Distal segment of endopod leg 4 about 2.6 times as long as wide. Distal inner spine 1.15 times longer than outer spine. Inner setae of this segment not transformed into spines.

Coxal segment of leg 4 with several groups of spinules and hair-spinules on caudal side. Intercoxal sclerite of leg 4 with line of spinules.

Rudimentary fifth leg 2-segmented, with rather strong inner spine at distal segment and two long setae, practically equal in length.

Male. Full body length 0.9-1.0 mm, rudimentary leg 6 (Fig. 2G) narrow plate with outer seta 2-2.5 times as long as inner seta or spine. Coxal segment of leg 4 with two groups of spinules and two groups of hair-spinules on caudal side. Intercoxal sclerite of leg 4 with line of spinules on top and line of spinules at the bottom.

Differential diagnosis

The discrimination of *A. americanus* from two closely related congeners is discussed below.

Distribution and ecology

A. americanus was found in abundance in the plankton of several pond-like water bodies in Belgium, e.g. the pond in the central park of Ghent, the pond of the botanical garden of Ghent University, and the brackish-water creeks of St. Jan-in-Eremo. In all of these, it was the dominant copepod species from May till October, reaching a density of 40,000 ind m^{-3} in June. The most likely explanation for the numerous records and rapid expansion of *A. americanus* in Europe and in Asia in the course of the 20th century is an involuntary man-mediated introduction from North America via Great Britain in the 19th century (Alekseev, 1998). Among other things, this may explain why Kiefer (1976) erroneously selected specimens of *A. americanus* as lectotypes of *A. robustus* from lake Mjosa in Norway (see further).

Elaphoidella gracilis
(Sars, 1863)

Material examined: 20 females from a small artificial pond in a private garden in St. Laureins (Belgium).

Female. Full body length without caudal setae 0.7-0.8 mm. Leg 1 is 3-segmented, legs 2-4 with 3-segmented exopods and 2-segmented endopods. Endopod of leg 4 with a seta at its first segment. Anal plate rounded, with numerous small spinules at free margin. Rudimentary leg 5 with elongated exopod, about twice as long as wide, bearing two short, spine-like setae, two long terminal setae and a short inner seta. Baso-endopod of leg 5 with two long medial setae and two short setae placed at both sides of the medial setae. Caudal rami conical, about two times as long as wide.

Differential diagnosis

The only congener of *Elaphoidella gracilis* currently known for Belgium is *E. leruthi* Chappuis, 1937, described from underground water (Leruth, 1939). Both are easily separated because leg 5 in *E. leruthi* has only three spiniform setae on its short exopod and three strong setae on its baso-endopod.

Distribution and ecology

This species was first found in May 2000, in a smallest artificial pond with brown-coloured water in the polder village of St. Laureins, 35 km north of Ghent, when it was the only species of copepod in the pond. Its density was about 2 l^{-1}. A second sample, collected in May 2001 again contained the species, this time accompanied by *Canthocamptus staphylinus* (Jurine, 1820) and *Paracyclops affinis* (Sars, 1863).

DISCUSSION

There is one previous record of *E. gracilis* from Belgium, at two sites, in leaf litter (Fiers & Ghenne, 2000), but it may have been missed by previous investigators because of its small size and low density in open water. Its recent discovery in a semi-terrestrial biotope suggests that it may be rather widespread (Fiers & Ghenne, 2000). *Paracyclops affinis* is listed in Lindberg's (1950) list of cyclopoids from Belgium, stating A. Capart as the collector, but without locality or other data. Dr Frank Fiers kindly checked the late André Capart's records at the Royal Institute of Natural Sciences, Brussels, and found one record but no voucher specimens: "Baudour, puddle in forest, 17 Oct. 1945". The present record of this highly distinctive species, the only European *Paracyclops* with an antennula of 11 segments (Karaytug, 1999), is therefore a welcome confirmation of its presence in Belgium.

In contrast, both first records represent previous confusions with related taxa. *C. singularis* has only recently been separated from some closely related congeners (Einsle, l996a), and the present record is only the second for this species, the first outside of Germany. As in the type locality, it was found in a temporary water body. Obviously, in many previous studies of this kind of environment, *C. singularis* may have been mistaken for the widespread *C. strenuus* Fischer, 1863.

A. americanus poses a more difficult problem. It was originally described by Marsh (1892) from the United States of America, but has been on record from Europe

since the early 20th century (LOWNDES, 1926). KIEFER (1976), in trying to work out the morphological differences between the related *A. robustus* and *A. vernalis,* sunk it into the synonymy of both. Its name was subsequently omitted from the world list of cyclopoids of DUSSART & DEFAYE (1985) while in EINSLE's (1996b) treatment of *Acanthocyclops*, KIEFER's view was accepted. The question, however, is whether this position can be maintained. As we will show hereunder, three species exist in Europe, one of which we here designate by the binomen *A. americanus* (Marsh, 1892). No type material of any of the three species has survived, and none of the three original descriptions or illustrations are adequate according to present-day standards. MARSH (loc. cit.) may not have had one, but two and possibly three species before him when describing *A. americanus*. However, the same applies to *A. robustus* and *A. vernalis* and, more broadly, to virtually all cyclopoids described in the nineteenth century. There is thus no ground for discriminating the name *americanus*. We also found *A. americanus* to occupy a specific niche in pond-like water bodies in Belgium, different from that of *A. robustus* and *A. vernalis*. We suggest several arguments in support of the existence of three species, and apply the name *americanus* to the "third species".

Morphological differences

Two morphological differences between *A. americanus* and *A. vernalis/robustus* were recognised by RYLOV (1948) and confirmed by MONCHENKO (1961). In female *A. americanus,* the frontal part of the genital double somite is rounded, while in *A. vernalis/robustus* it is triangular (Fig. 2). This character is clearly recognisable in Marsh's original description, and is our main argument upon which to found the specific nature of *A. americanus*. Unfortunately, KIEFER's (1976) revision of the "robustus-group" only added to the confusion, because at the outset, the specimens from Norway selected by him as lectotypes of *A. robustus* (KIEFER's figures 1-20) represented specimens of *A. americanus*, sensu the present paper. We have little doubt that these invaded Norway, as they did the rest of Europe, after Sars' time.

Males can be separated by the difference in length of the seta of rudimentary leg 6. In *A. americanus* the outer seta of leg 6 is 2-2.5 times the length of the seta-like inner spine.

In male *A. vernalis* the outer seta P6 is not more than 1.3-1.8 times the length of the strong inner spine (Fig. 2G-H, 3G).

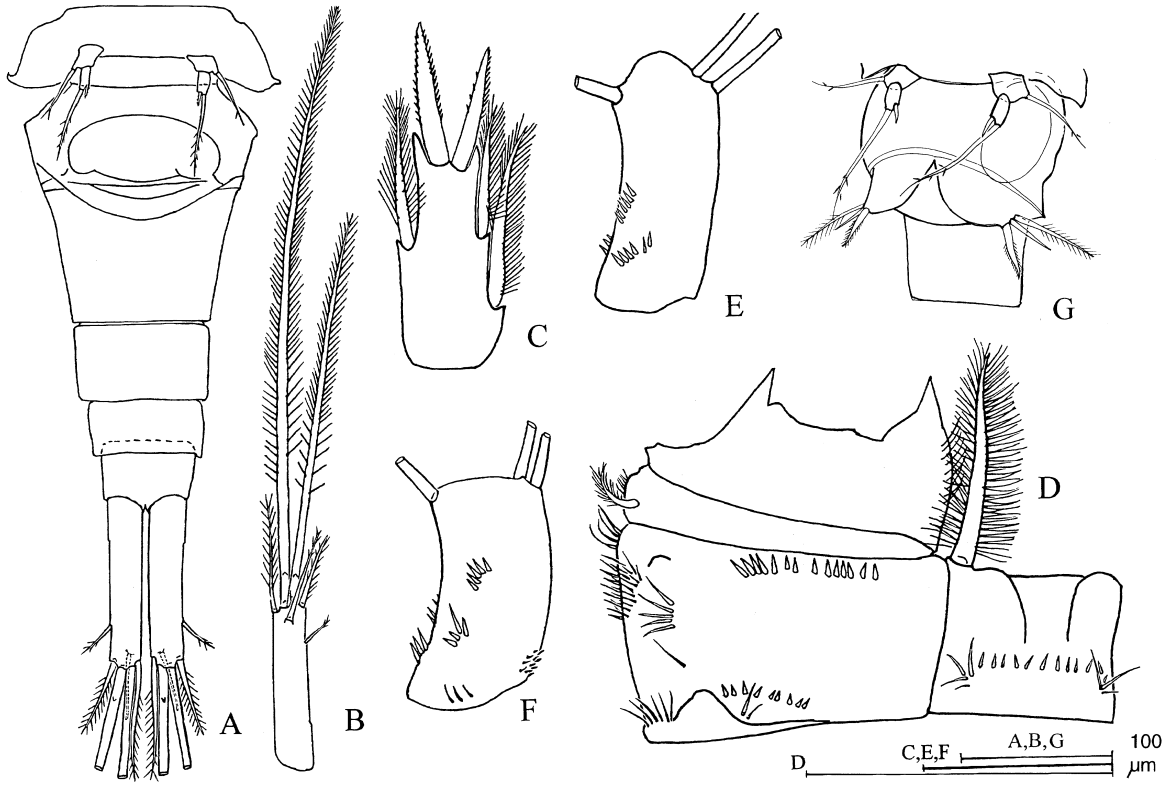

Fig. 3. – *Acanthocyclops vernalis* (Fischer), female, St. Petersburg, Russia (A-D) and male, Plön, Germany (G). A - abdomen, ventral view; B- caudal ramus, dorsally; C - distal segment of endopod, leg4; D - coxa and intercoxal sclerite, leg 4, caudal side; E,F - antennal basipod, frontal and caudal sides; G. P5 and P6.

A. robustus was discriminated from *A. vernalis* by SARS (1863) on evidence of the transformation of the inner seta of the distal segment of endopod leg 4 into a spine. Other taxonomic signatures of *A. robustus-vernalis* include the shape of the genital somite in females, while leg 6 in males is similar in both (GURNEY, 1933; RYLOV, 1948).

We confirm that *A. robustus* sensu Sars (1863) really exists in nature, by finding 5 females and 3 males of the species in a near-road ditch in the vicinity of Gent (see Fig. 2H-I, 4A,C,E). Some morphological features of *A. vernalis,* a species described by S. FISCHER from Russia, can also be seen in Fig. 4. All these differences were well exemplified by specimens of *A. americanus* from Belgium. We could easily separate it from *A. vernalis* as well as from *A. robustus* by the shape of the genital somite in females and the armament of P6 in male. There are also differences between the three species in the armament of the intercoxal sclerite of leg 4. In *A. americanus,* we found a curved row of strong denticles on the caudal surface of the sclerite. In *A. vernalis* these denticles were slender and never produced a wavy row, and two groups of hair-like spinules occurred on both sides of the line of denticles. In *A. robustus,* the denticles were strong and formed a straight line.

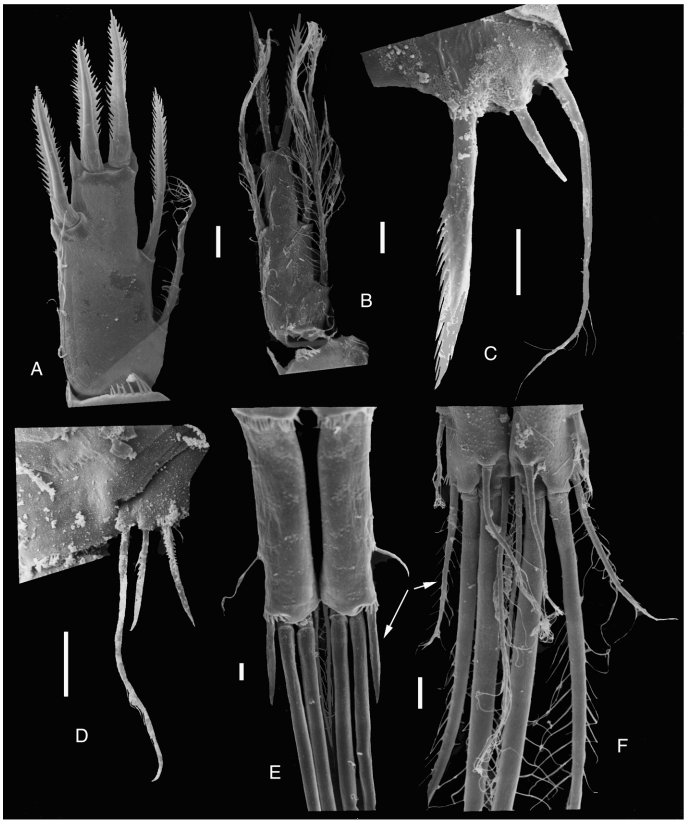

Fig. 4. – SEM's of A. *Acanthocyclops robustus*, endopod 3 of P4; B. *A. americanus*, endopod 3 of P4; C. *A. robustus*, P6; D. *A. americanus*, P6; E. *A. robustus*, furca, F. *A. americanus*, furca (terminal external seta arrowed). All scales represent 10 micrometers.

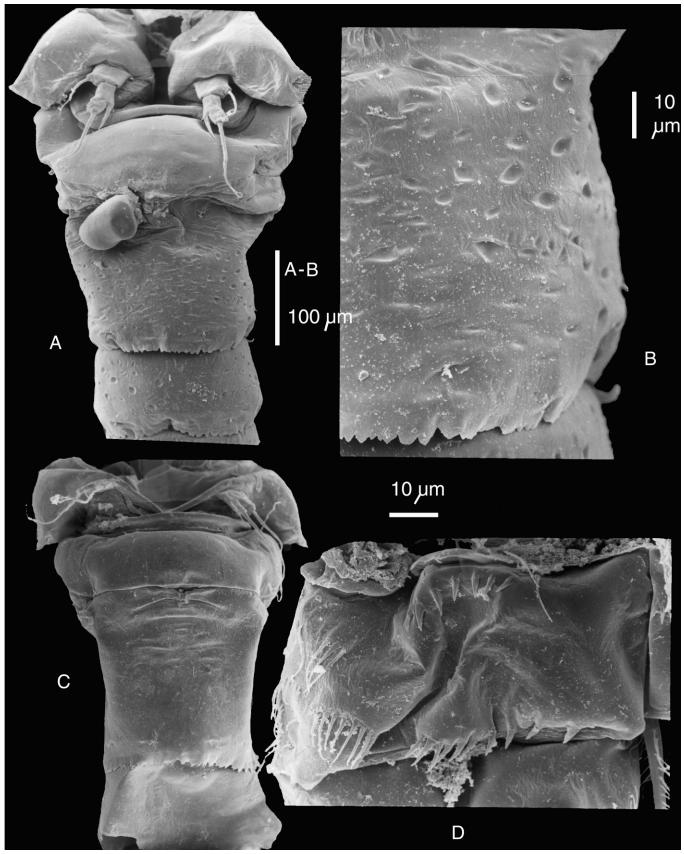

Fig. 5. – SEM's. A. *Acanthocyclops americanus*, P5 and genital segment in ventral view; B-D. *Cyclops singularis*. B. P5 and genital segment, ventral view, C. Pitted surface of the genital segment, D. Coxa of P4.

Ecological differences

A. americanus is a pelagic animal, inhabiting eutrophic water bodies. In recent decades, it became the dominant form of the summer limnetic plankton in reservoirs on the River Volga and the Dniepr (MONCHENKO, 1961, 1974; ALEKSEEV & KOSOVA, 1977). Its abundant representation in eutrophic lakes and lakelets in Belgium may, likewise, be of relatively recent date.

A. vernalis and *A. robustus* inhabit the near-shore area and/or near-bottom zone in lakes. They are rarely collected mixed with truly planktonic species.

Some differences in behaviour between nauplii of *A. vernalis* and *A. americanus* have also been documented (ALEKSEEV, 1983). These reflect the ecological preferences of each species. Nauplii of *A. vernalis* live a benthic life. In samples, they often attach to glass walls. When shaking a jar, they seek refuge on the bottom. Nauplii of *A. americanus*, conversely, behave like planktonic animals. They never attach to surfaces and, when stressed, swim around in the water column.

Finally, in all cases where we found *A. vernalis* and/or *A. robustus* together with *A. americanus,* no hybrids were detected (ALEKSEEV & KOSOVA, 1986).

CONCLUSION

Rather than creating a new name, we maintain *A. americanus* (Marsh, 1892) as a valid species, morphologically and ecologically separated from *A. vernalis* (Fischer, 1853) and *A. robustus* (Sars, 1863). This species is currently an important element of eutrophic, limnetic plankton communities in Europe but is likely an early invader from North America. We realise that the objection "original description insufficient" may be raised, but at least one diagnostic female character can be seen on the original illustrations. Stability may thus be served best by preserving the available name *A. americanus* beside those of *A. robustus* and *A. vernalis*, after – *inter alia* – selecting a neotype for each. We refrain from doing this here, because we would rather see a representative group of todays' copepodologists reach a consensus on this question.

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A cladistic analysis of Zoropsidae (Araneae), with the description of a new genus

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ABSTRACT. A cladistic analysis of the spider family Zoropsidae has been performed. The ingroup of the analysis consisted of eight species traditionally classified in Zoropsidae, including *Zoropsis cyprogenia* Bosselaers, 1997, of which the hitherto unknown male has been recently discovered, and *Takeoa nishimurai* (Yaginuma, 1963). The outgroup contained four species, belonging to the genera *Ctenus* Walckenaer, 1805, *Acanthoctenus* Keyserling, 1876 and *Griswoldia* Dippenaar-Schoeman & Jocqué, 1997. Three different weighting schemes allowed selection of one preferred, most parsimonious tree. This tree implies that *Zoropsis cyprogenia* has to be placed in a new genus within Zoropsidae. A description of *Akamasia* n. gen. is given, as well as a redescription of *Acanthoctenus gaujoni* Simon, 1906.

KEY WORDS: Araneae, Zoropsidae, Ctenidae, *Zoropsis*, *Takeoa*, *Akamasia*, *Acanthoctenus*, cladistics, parsimony, weighting.

INTRODUCTION

According to SIMON (1892: 227-230), the spider family Zoropsidae Bertkau, 1882, consisted of two subfamilies: Acanthocteninae, with only one genus, *Acanthoctenus* Keyserling, 1876, and Zoropsinae, composed of three genera: *Raecius* Simon, 1892, *Zorocrates* Simon, 1888, and *Zoropsis* Simon, 1878. SIMON (1903: 974-975) later added *Uduba* Simon, 1880 to Zoropsinae. PETRUNKEVITCH (1923: 170; 1928: 146) raised Simon's Acanthocteninae and Zoropsinae to family rank and included *Zorodictyna* Strand, 1907, in the family Zoropsidae. ROEWER (1954: 1283-1288) also listed both groups as families.

LEHTINEN (1967: 374-378) dispensed with Zoropsidae and Acanthoctenidae altogether: he transferred *Raecius*, *Uduba*, *Zorocrates* and *Zorodictyna* to Miturgidae and placed *Zoropsis* in the family Zoridae. He also described a new genus, *Takeoa* Lehtinen, 1967, which was attributed to Zoridae as well and to which he transferred *Zoropsis nishimurai* Yaginuma, 1963. Furthermore, LEHTINEN (1967) redelimited *Acanthoctenus* and placed it in Ctenidae, Acanthocteninae, a subfamily to which he added a large number of other genera: *Acantheis* Thorell, 1891, *Africactenus* Hyatt, 1954, *Anahita* Karsch, 1879, *Asthenoctenus* Simon, 1897, *Caloctenus* Keyserling, 1876, *Centroctenus* Mello-Leitão, 1929, *Enoploctenus* Simon, 1896, *Gephyroctenus* Mello-Leitão, 1936, *Leptoctenus* Koch, 1878, *Nothroctenus* Badcock, 1932, *Phymatoctenus* Simon, 1896, *Trujillina* Bryant, 1948, and *Viracucha* Lehtinen, 1967.

Several authors (WUNDERLICH, 1986; LEVY, 1990; CODDINGTON & LEVI, 1991; GRISWOLD, 1993; GRISWOLD et al., 1999) have since revalidated the family Zoropsidae, because the eye arrangement and the presence of scopulae in *Zoropsis* does not fit well in Lehtinen's Zoridae. The family Zoropsidae is limited nowadays to the genera *Zoropsis* and *Takeoa* (PLATNICK, 1993, 2002). However, cladograms presented by CODDINGTON & LEVI (1991), GRISWOLD (1993), and GRISWOLD et al. (1999) group *Acanthoctenus* together with Zoropsidae in one clade.

The family Zoropsidae, as presently delimited, has the following somatic characters (LEVY, 1990: 139): presence of a calamistrum and a narrow, bipartite cribellum; carapace with a longitudinal thoracic groove and eight eyes in two rows, arranged in a pattern similar to that of the family Lycosidae; toothed chelicerae; all legs with strong spines, tibiae and metatarsi I and II equipped with several pairs of ventral spines; metatarsi bearing scopulae and an apical, soft membranous rim on dorsum, tarsi bearing scopulae, trichobothria, claw tufts and two tarsal claws. The family is palearctic, but *Zoropsis spinimana* (Dufour, 1820) has recently been introduced into North America (GRISWOLD & UBICK, 2001).

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ROEWER (1954) listed 12 species and two subspecies in the genus *Zoropsis*. BRIGNOLI (1983) catalogued five more species and PLATNICK (1993) yet another species. BOSSELAERS (1997) described an additional species based on a female specimen from Cyprus. Many of the species described, e.g. in DAHL (1901a, 1901b), have later been synonymised (LEHTINEN, 1967; WUNDERLICH, 1994). On the other hand, WUNDERLICH (1994: 724) and THALER & KNOFLACH (1998: 179) removed *Zoropsis quedenfeldti* Dahl, 1901 and *Zoropsis oertzeni* Dahl, 1901 from synonymy with *Zoropsis spinimana*. In the absence of a complete revision of the family Zoropsidae, this leaves us with 14 Zoropsid species: *Zoropsis beccarii* Caporiacco, 1935, a doubtful species described from a juvenile from Turkey; *Zoropsis bilineata* Dahl, 1901 from Morocco, Algeria and Mallorca; *Zoropsis coreana* Paik, 1978 from Korea; *Zoropsis cyprogenia* Bosselaers, 1997 from Cyprus; *Zoropsis libanica* Simon, 1884, a doubtful species from the Middle East; *Zoropsis lutea* (Thorell, 1875) from the eastern Mediterranean; *Zoropsis markamensis* Hu & Li, 1987 from China; *Zoropsis media* Simon, 1878 from the western Mediterranean; *Zoropsis oertzeni* from Italy and the Balkan; *Zoropsis pekingensis* Schenkel, 1953 from China; *Z. quedenfeldti* from Morocco; *Zoropsis rufipes* (Lucas, 1838) from the Canary isles and Madeira; *Zoropsis spinimana* (Dufour, 1820) from southern Europe and northern Africa and *Takeoa nishimurai* (Yaginuma, 1963) from Japan and China (CHEN & ZHANG, 1991).

The most recent treatments of seven Mediterranean and Atlantic *Zoropsis* species can be found in WUNDERLICH (1994) and THALER & KNOFLACH (1998).

Recently, the male of *Zoropsis cyprogenia* has been collected. Upon study of the specimen, it became obvious that this species does not belong in *Zoropsis* and has to be placed in a genus of its own. A cladistic analysis has been performed to study the matter in more detail.

MATERIAL AND METHODS

Specimens were studied by means of incident light under a stereomicroscope equipped with an eyepiece grid. Internal female genitalia were observed after clearing the vulva in methyl salicylate. Cleared vulvae were observed with a compound microscope using transmitted light. The vulva illustrated was photographed in several focal planes under a compound microscope and the photographs of these optical sections were subsequently used for the execution of the drawing. All measurements are in mm.

Cladistic analyses were performed using the computer programmes Pee-Wee 2.6 (GOLOBOFF 1997a), NONA 2.0 (GOLOBOFF 1997b), PAUP 4.0 beta 4a (SWOFFORD 1999) and SEPAL 1.1 (SALISBURY 2000a). Optimisation of character states and printing of the preferred tree was performed using Winclada 1.0 (NIXON 2002). All analyses were run on a 1 Ghz pentium III machine with 256 Mb RAM.

ABBREVIATIONS USED

APS:	apparent phylogenetic signal
ci:	consistency index
do:	dorsal
fe:	femur
MA:	median apophysis of the male palp
MOQ:	median ocular quadrangle
mt:	metatarsus
MTP:	membranous tegular process
pa:	patella
pl:	prolateral
rc:	rescaled consistency index
ri:	retention index
rl:	retrolateral
RAM:	random access memory
RTA:	retrolateral tibial apophysis
ta:	tarsus
TBR:	tree bisection and reconnection
ti:	tibia
ve:	ventral
vsp:	ventral spine pairs

Abbreviations of personal and institutional collections (curators in parentheses):

ACJ:	Arachnological Society of Japan, Otemon Gakuin University, Osaka (Y. Nishikawa)
AMNH:	American Museum of Natural History, New York (N. Platnick)
CCD:	Collection Christa Deeleman
CJB:	Collection Jan Bosselaers
CJK:	Collection Johan Van Keer
CKT:	Collection Konrad Thaler
CMA:	Collection Martin Askins
CPC:	Collection Pedro Cardoso
CRB:	Collection Rop Bosmans
MNHN:	Muséum National d'Histoire Naturelle, Paris (C. Rollard)
NCA:	National Collection of Arachnida, Pretoria (A. Dippenaar-Schoeman)
RBINS:	Royal Belgian Institute of Natural Sciences, Brussels (L. Baert)
USMN:	United States National Museum of Natural History (D. Furth)

RESULTS AND DISCUSSION

PHYLOGENETIC ANALYSIS

Taxon choice

The ingroup of the analysis consists of eight species belonging to the two genera presently recognised within Zoropsidae: on the one hand the enigmatic eastern Asian *Takeoa nishimurai* and on the other hand *Zoropsis bilineata*, *Zoropsis lutea*, *Zoropsis media*, *Zoropsis oertzeni*, *Zoropsis rufipes*, *Zoropsis spinimana*, and the species recently described as *Zoropsis cyprogenia*. Four species constitute the outgroup. Two of these belong to the genus *Acanthoctenus*, the sister group of Zoropsidae in the cladograms published by CODDINGTON & LEVI (1991),

GRISWOLD (1993), and GRISWOLD et al. (1999): *Acanthoctenus gaujoni* Simon, 1906 and an undescribed *Acanthoctenus* species from Peru. The other two outgroup species belong to the "ctenoid complex" which GRISWOLD (1993) considers to be a sister group to the Zoropsidae-*Acanthoctenus* clade: *Griswoldia urbensis* (Lawrence, 1942) and an undescribed *Ctenus* species from South Africa that is closely related to *Ctenus gulosus* Arts, 1912. Collection details of the specimens studied can be found in Table 1.

TABLE 1

Specimens examined to provide character data, with collection data and deposition.

Acanthoctenus gaujoni Simon, 1906; 1m (holotypus): Ecuador, Loja, Zamora, Gaujon leg. (MNHN AR5168); 1f (paratypus): Ecuador, Loja, Zamora, Gaujon leg. (MNHN AR5168).

Acanthoctenus sp.; 1m, 1f: Peru, Madre de Dios, Zona Reservada Tambopata, S 12° 50' W 69° 17', elev. 290 m, 8 and 6 Jun. 1988, J. Coddington leg. (USNM 2009677).

Akamasia cyprogenia (Bosselaers, 1997); 1m: Cyprus, Akamas peninsula, Neo Chorion, March 1998, M. Askins leg. (CMA); 1f (holotypus): Cyprus, Akamas peninsula, Avakas Gorge, in leaf litter on the bank of Avgas river, elev. 20 m, 5 April 1997, J. Bosselaers leg. (RBINS 28515).

Ctenus sp.; 2m, 2f: South Africa, Kwazulu-Natal, Ngome State Forest, E 31° 26' S 27° 49', in pitfall trap in pine forest, Jan. 1993, M. v.d. Merwe leg. (NCA 94/719).

Griswoldia urbensis (Lawrence, 1942); 5m, 5f: South Africa, Kwazulu-Natal, Ngome State Forest, E 31° 26' S 27° 49', in pitfall trap in grass, Jan. 1993, M. v.d. Merwe leg. (NCA 94/712).

Takeoa nishimurai (Yaginuma, 1963); 1m (paratypus): Japan, Kyoto prefecture, Kyoto City, Fushimi-ku, Momoyama, 22 Mar. 1962, T. Nishimura leg. (ACJ); 1f (holotypus): Japan, Yamaguchi pref., Hikari City, Murozumi-cho, 7 Jul. 1962, K. Nakagawa leg. (ACJ).

Zoropsis bilineata Dahl, 1901; 1m, 1f: Algeria, W. Laghouat, Laghouat, Oued M' Zi, elev. 750 m, litter under reed, 22 Dec. 1987, R. Bosmans leg. (CRB), 2f: Morocco, Vallée du Drâa, 8 km South of Tansikht, under stones in palm tree plantation, 5 Feb. 1996, J. Van Keer leg. (CJK 1564).

Zoropsis lutea (Thorell, 1875); 2m: Greece, South Peloponnesos, Girion Sirio camping, 1 Oct. 1991, B. Knoflach leg. (CKT); 1f: Greece, Rhodos, N.E. of Laerma, in field under stones, 15 Apr. 1984 (CCD); 1f: Greece, Attika, Athens, Mt. Parnes, elev. 500 m., under stones, 5 Jan. 1985 (CCD); 1f: Crete, Ano Zaros, elev. 450 m, in olive grove under stones, 10 Apr. 1995 J. Bosselaers leg. (CJB 1190); 1f: Crete, Akrotiri, Moni Gouverneto, elev. 200 m, near entrance of Bear Cave under stones, 9 Apr. 1996 J. Bosselaers leg. (CJB 1093); 1f: Cyprus, Limassol, Akrotiri Salt Lake, salt march, under stones, 2 Apr. 1997 J. Bosselaers leg. (CJB 1274); 1f: Crete, Aghia Galini, at beach under stones, 3 May 1997, J. Van Keer leg. (CJK 1743).

Zoropsis media Simon, 1878; 1m: France, Banyuls, 2 Feb. 1912 (MNHN AR211); 3m, 8f: Algeria and Southern France (MNHN AR208); 1f: Spain, Palamós, Cap Gros, elev. 70 m, in litter in pine wood near sea, 21 Jul. 1995, J. Bosselaers leg. (CJB 1197).

Zoropsis oertzeni Dahl, 1901; 1m: Slovenia, Sezana Blazeva, Kustor leg. (CCD); 1m: Croatia, N. Dalmatia, Isle of Cres, Dragozetici, elev. 400 m, in oak litter (CCD); 1f: Croatia, N. Dalmatia, Isle of Pag, Diniste, 7 Apr. 1971 (CCD); 1f: Greece, Attiki, Piraeus, Alephari, shaded camping, 22 May 1998, R. Bosmans leg. (CRB); 1f: Greece, Peloponnesos, Achaia, Kalogria, in salt marsh, 30 May 1998, J. Van Keer leg. (CJK 1844).

Zoropsis rufipes (Lucas, 1838); 1m, 1f: Spain, Canary Islands, Tenerife, Nov. 1975, P. Oromi leg. (AMNH, Griswold's Lycosoid study exemplars).

Zoropsis spinimana (Dufour, 1820); 2m: Portugal, Baixa da Banheira, 16 Dec. 1997, P. Cardoso leg. (CPC); 1f: Spain, Gerona, Empuria Brava, under stones near house, 7 Jul. 1995, J. Van Keer leg. (CJK 1584).

Character coding and description

A series of 56 characters (43 binary and 13 multistate) was coded for the 12 taxa chosen. Character state numbering does not imply plesiomorphy or apomorphy, because character polarity is derived during cladogram search by outgroup comparison (WATROUS & WHEELER 1981; MADDISON et al. 1984; KITCHING et al. 1998). Where necessary (Characters 21, 22, 23, 24, 29, 30, 47, 48, 50, 51, 54, 55), characters were scored with character states hierarchically related, as advocated by HAWKINS et al. (1997), even though this necessitated coding missing entries due to character inapplicability in some instances (MADDISON 1993). A few of the characters used (Characters 9, 13, 14, 23, 24, 32, 40, 47) are autapomorphies and as a result phylogenetically uninformative, but they were included in the matrix nevertheless since these characters are potential synapomorphies that might be of interest to future workers preparing analyses on larger numbers of taxa. Because most of the species studied in the analysis have heavily spined legs, 12 characters refer to leg spination. Establishing homology between individual spider leg spines is not always straightforward. Fortunately, in a number of cases such as the do, pl and rl tibial spines (Characters 2, 3, 4, 9, 10, 11, 12), there can be little doubt about homology and the presence of indi-

vidual spines could be scored. However, in the case of tibial and metatarsal vsp, establishing homology of individual spines was problematic (Characters 5, 6, 7, 8). Rather than dismissing this probably important information altogether, it was decided to score these characters as multistate characters describing numbers of spine pairs.

The characters used in the analysis

Legs

1. *Male tibial crack*: (0) absent; (1) present. This character was first described by Griswold (1993: 1, 23, Figs. 3, 4)) as "a conspicuous suture line visible through the cuticle at the base of the leg tibiae of males just distal to the basal pair of ventral spines; it is visible on the surface as a shallow, depressed ring". When present, the male tibial crack is clearly visible.
2. *Male ti I basal do spine*: (0) absent; (1) present.
3. *Male ti I median do spine*: (0) absent; (1) present.
4. *Male ti I terminal do spine*: (0) absent; (1) present.
5. *Number of male ti I vsp*: (0) 5; (1) 6; (2) 8; (3) 9.
6. *Number of male mt I vsp*: (0) 2; (1) 3; (2) 4; (3) 5.
7. *Number of female ti I vsp*: (0) 5 or less; (1) 6; (2) 8; (3) 9.
8. *Number of female mt I vsp*: (0) 3; (1) 4; (2) 5.
9. *Female ti I basal pl spine*: (0) absent; (1) present. Apart from several vsp, tibiae I and II of most of the species studied also carry spines on the pl and rl faces.
10. *Female ti I basal rl spine*: (0) absent; (1) present.
11. *Female ti I median pl and rl spine*: (0) absent; (1) present. When present, both spines always occurred together in the specimens studied.
12. *Female ti I terminal pl and rl spine*: (0) absent; (1) present.
13. *Female ti I additional pl and rl spines*: (0) absent; (1) present.
14. *Dense claw tufts*: (0) absent; (1) present.
15. *Ve scopulae on ta*: (0) absent; (1) present.
16. *Patellar indentation*: (0) wide; (1) narrow. The patellar indentation is a slit-like membranous indentation on the rl side of the pa. May be very narrow ("closed") or rather wide (Simon, 1892: 22; Ledoux & Canard, 1991: Fig. 15A-15B).
17. *Trochanter notch depth*: (0) shallow; (1) deep.

Cephalothorax

18. *Ctenid eye pattern*: (0) absent; (1) present. The classic ctenid eye pattern consists of three rows (2:4:2) . Because the anterior eye row is strongly recurved, the anterior lateral eyes are situated just in front of the posterior lateral eyes (Dippenaar-Schoeman & Jocqué, 1997: 135-136).
19. *Number of male retromarginal cheliceral teeth*: (0) 2; (1) 3; (2) 4.
20. *Number of female retromarginal cheliceral teeth*: (0) 2; (1) 3; (2) 4.

Abdomen

21. *Cribellum*: (0) present; (1) absent.
22. *Cribellum shape*: (0) linear; (1) oval.

Male palp

The homology of the various tegular apophyses of the male palp (Fig. 1c) is still not fully elucidated for most spiders. A hyaline or sclerotised appendage, immovably attached to the tegulum and facing the embolus tip is considered to be a conductor in this analysis, while a sclerotised appendage that is flexibly attached to the tegulum via a thin membrane is considered to be a MA (Sierwald 1990: 21; Griswold 1993: 10). In addition to conductor and MA, many of the species studied also have a hyaline membranous flap attached to the tegulum near the embolus base: the MTP (Fig. 1c). Unfortunately, the terminology used to designate the three appendages mentioned differs significantly between authors. The MA of Griswold (1993: 10), Lehtinen (1967: 377) and Sierwald (1990: 21) is called "hooked tegular apophysis" by Levy (1990: 140) and "Tegularapophyse" by Thaler & Knoflach (1998: 175). The MTP of Griswold (1993: 15) and Levy (1990: 140) is called "distal apophysis" by Sierwald (1990: 22) and "Begleitfortsatz des Embolus" by Thaler & Knoflach (1998: 175). For unspecified reasons, and in contrast to the other authors mentioned here, Lehtinen (1967: 377) calls the conductor "secondary conductor" and considers the embolus to be a complex of embolus and "primary conductor".

23. *Conductor*: (0) present; (1) absent.
24. *Conductor texture*: (0) hyaline; (1) sclerotised.
25. *Embolus insertion*: (0) basal; (1) median; (2) apical. The embolus base can be inserted in the basal (Fig. 4a), median (Figs 5a) or apical third (Fig. 1c) of the tegulum.
26. *Embolus cross section*: (0) flattened; (1) cylindrical; (2) obtusely triangular.
27. *Embolar lamella*: (0) absent; (1) present. In many of the species studied, the embolus has a uni- or bilaterally flattened "wing", referred to as the embolar lamella in this publication (Figs 1c, 5c).
28. *MA insertion*: (0) basal; (1) median; (2) apical. The MA base can be inserted in the basal (Fig. 5c), median (Fig. 5a) or apical third (Fig. 4a) of the tegulum.
29. *MA shape*: (0) cup-shaped; (1) convex, with tip.
30. *MA tip*: (0) simple; (1) bifid; (2) mushroom-shaped.
31. *MTP*: (0) absent; (1) present.
32. *Proximal rim of tegulum*: (0) unmodified; (1) with lamella; (2) with bump. The proximal tegular rim bears a lamellar outgrowth in *Zoropsis bilineata* (Thaler & Knoflach, 1998: Fig. 10) and a conspicuous bump in *Griswoldia urbensis* (Griswold, 1991: Fig. 65).
33. *Proximal pl part of tegulum*: (0) not protruding; (1) protruding. The proximal pl part of the tegulum is protruding in most of the species studied (Fig. 1c).
34. *Proximal rl part of tegulum*: (0) not protruding; (1) protruding. The proximal rl part of the tegulum is protruding in *Takeoa nishimurai* and *Akamasia cyprogenia* (Figs 1a, 4a).

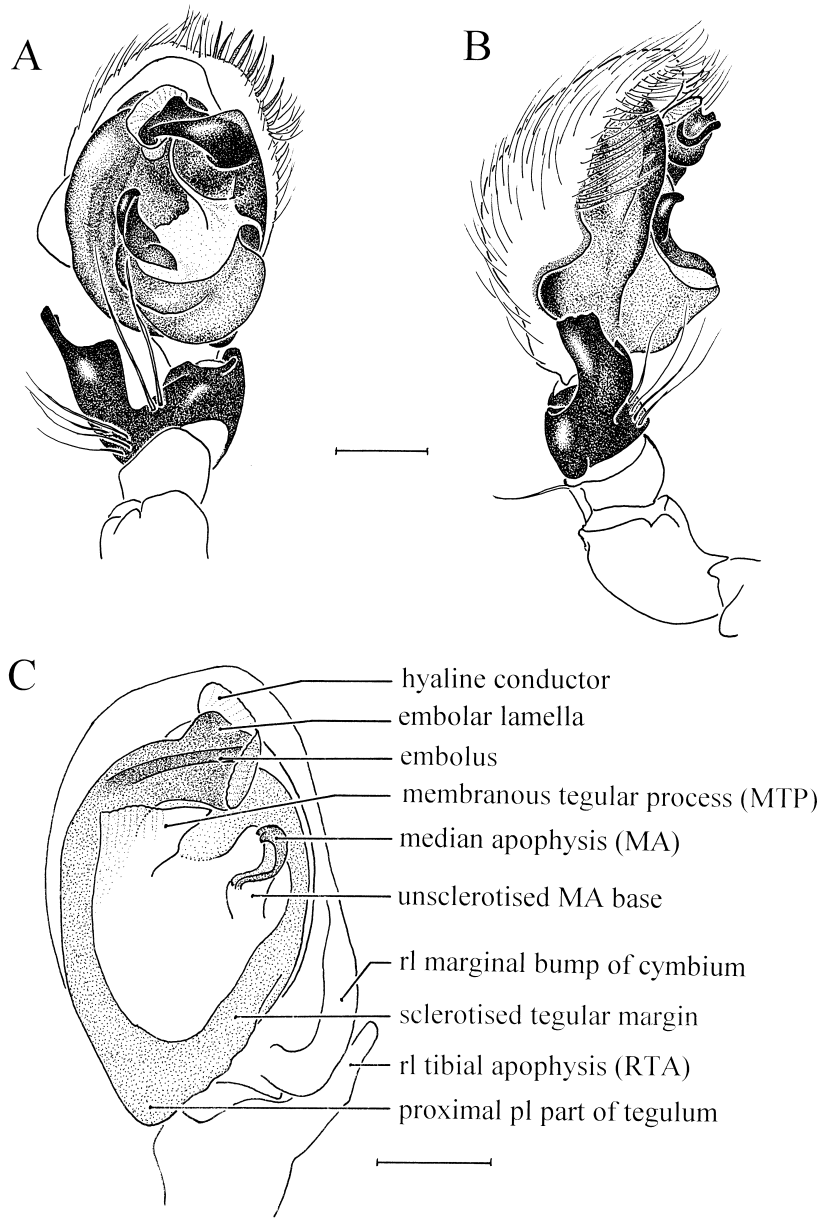

Fig. 1. – *Takeoa nishimurai*, right male palp. – A. Ventral view. – B. Retrolateral view. – *Zoropsis spinimana*. – C. Schematic ventral view of left male palp. Scale bars = 0.5 mm.

35. *Protruding basal part of tegulum*: (0) absent; (1) present. In some species, e.g. *Takeoa nishimurai*, the basal half of the tegulum, when viewed laterally, is protruding and making an angle with the distal half (Fig. 1b).
36. *Unsclerotised basal part of MA*: (0) absent; (1) present. In *Zoropsis*, the basal part of the MA is a white, unsclerotised stalk (Figs 1c, 5c).
37. *Additional sclerite next to embolus*: (0) present; (1) absent. The tegulum of *Ctenus* and *Griswoldia* has an additional, isolated sclerite, surrounded by white, membranous tissue, near the embolus base.
38. *Bristle mat on cymbium*: (0) absent; (1) present. Most of the species studied have a dense mat of short bristles on the do side of the cymbium (Levy, 1990: Fig. 7).
39. *Retrolateral marginal bump on cymbium* (Fig. 1c): (0) absent; (1) present.
40. *Basal do apophysis on cymbium*: (0) absent; (1) present.
41. *Cymbium tip shape*: (0) blunt; (1) narrowed.
42. *RTA*: (0) mesal; (1) distal. The RTA can be inserted mesally (Fig. 1a) or distally (Figs 4b, 5b, 5d) on the palpal ti.
43. *Palpal patella*: (0) unmodified; (1) swollen.

Female genitalia

44. *Epigyne lateral lobe extension*: (0) leaving central depression (Fig. 3c-d); (1) tightly enclosing scape (Fig. 3a).
45. *Epigyne lateral tooth*: (0) absent; (1) present.
46. *Epigyne lateral lobe pocket*: (0) absent; (1) present.
47. *Epigynal scape*: (0) present; (1) absent. The epigynes of most zoropsids and their allies have a conspicuous, sclerotised median scape, which often reaches beyond the poste-

rior rim of the lateral lobes of the epigyne (Fig. 3a). The scape is lacking in *Takeoa nishimurai*.

48. *Epigynal scape attachment*: (0) broad; (1) narrow. The scape can be broadly attached to the epigyne (Fig. 3b) or connected to it by a narrow stalk (Figs 3a, 3c).

49. *Sclerotised base plate in epigynal central depression*: (0) absent; (1) present. Some zoropsids have a flat, sclerotised base plate, to which the scape is attached, in the central depression of the epigyne (Fig. 3c). *Takeoa nishimurai* has the base plate, but lacks a scape (Fig. 3d).

50. *Shape of epigynal scape*: (0) obtusely rectangular; (1) bilobed; (2) lozenge-shaped; (3) ribbon-shaped; (4) mushroom-shaped; (5) arrow-shaped.

51. *Length of epigynal scape*: (0) long (reaching posterior margin of the epigyne); (1) short.

52. *Posterior ends of lateral lobes of epigyne*: (0) separated; (1) touching.

53. *Cavity of spermatheca*: (0) simple; (1) complex: chambered or coiled.

54. *Ovoid bodies*: (0) absent; (1) present. The ovoid bodies (Levy, 1990: 141) are small oval or sausage-shaped cavities situated in the vicinity of the spermathecae. They may be secondary spermathecae. In Fig. 3e, the mushroom-shaped bodies situated left and right of the symmetry axis in the upper (anterior) half of the vulva are the spermathecae, the sausage-shaped cavities immediately adjacent to these and running parallel to their stalks are the ovoid bodies.

55. *Ovoid body position*: (0) median; (1) anterior; (2) lateral.

56. *Insemination duct*: (0) short and simple (1) long and contorted. In the lower (posterior) third of Fig. 3e, the long and contorted insemination duct of *Takeoa nishimurai* can be seen.

Analysis and results

The matrix of character states can be found in Table 2. All characters were run unordered in the analyses performed.

An equally weighted analysis of the data matrix was performed in PAUP with **hsearch addseq=random nreps=1000** (heuristic search with tree bisection and reconnection swapping and 1000 random addition sequences). In order to avoid spurious resolution due to unsupported (Coddington & Scharff, 1994; Wilkinson, 1995) or ambiguously supported (Nixon & Carpenter, 1996) branches, those with a minimum length of zero were collapsed with **condense collapse=minbrlen**. Two shortest trees were found in 883 out of 1000 random addition sequences. An exhaustive tree search using the command **alltrees** found the same shortest trees after evaluating 654729075 trees. One of these trees ("tree 1") is illustrated in Fig. 2. In the second tree ("tree 2"), the two *Acanthoctenus* species branch off in sequence below node 3 (Fig. 2), *Acanthoctenus gaujoni* being in the more basal position. Apart from this, tree 2 is identical to tree1. Both trees have the following statistics in PAUP: length 109, ri 0.6822, rc 0.4694, ci 0.6881, ci excluding phylogenetically uninformative characters 0.6600 and goloboff fit -40.250 (tree 1) or -40.150 (tree 2). NONA 2.0 (commands **amb-** to avoid spurious resolution and **mult*500**, using **max*** was not necessary) found the same shortest trees.

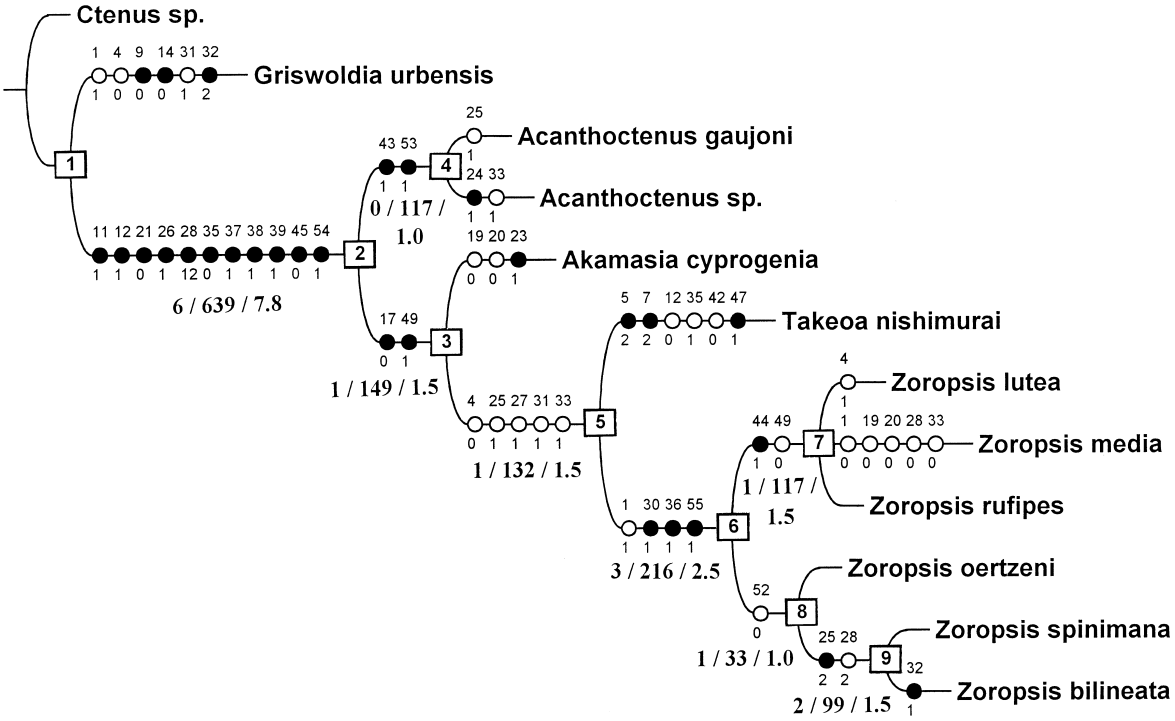

Fig. 2. – Preferred cladogram, with node numbers and unambiguous character state changes indicated. White dots are homoplasious character state changes, black dots non-homoplasious character state changes. Figures below branches are equal weighting, successive weighting, and implied weighting decay values, respectively.

TABLE 2

Character-taxon matrix

Characters	Ctenus sp.	Griswoldia urbense	Acanthoctenus gaujoni	Acanthoctenus sp.	Takeoa nishimurai	Zoropsis spinimana	Zoropsis bilineata	Zoropsis oertzeni	Akamasia cyprogenia	Zoropsis lutea	Zoropsis media	Zoropsis rufipes	Steps	ci	ri	Goloboff fit
1	0	1	0	0	0	1	1	1	0	1	0	1	3	.33	.60	.60
2	1	0	1	1	0	0	0	0	0	0	0	0	2	.50	.50	.75
3	1	0	1	1	0	0	0	0	0	0	0	0	2	.50	.50	.75
4	1	0	1	1	0	[01]	0	01	1	1	0	[01]	3	.33	.50	.60
5	0	0	3	3	2	1	1	1	1	1	1	1	3	1.0	1.0	1.0
6	0	0	3	1	2	2	2	2	2	2	2	2	3	1.0	1.0	1.0
7	0	0	3	?	2	1	1	1	1	1	1	1	3	1.0	1.0	1.0
8	0	0	2	?	1	1	1	1	1	1	1	1	2	1.0	1.0	1.0
9	1	0	[01]	?	1	1	1	1	1	1	1	1	1	1.0	0/0	1.0
10	0	0	[01]	?	1	1	1	1	1	1	1	1	1	1.0	1.0	1.0
11	0	0	1	?	1	1	1	1	1	1	1	1	1	1.0	1.0	1.0
12	0	0	1	?	0	1	1	1	1	1	1	1	2	.50	.50	.75
13	0	0	1	?	0	0	0	0	0	0	0	0	1	1.0	0/0	1.0
14	1	0	1	1	1	1	1	1	1	1	1	1	1	1.0	0/0	1.0
15	1	0	0	0	1	1	1	1	1	1	1	1	2	.50	.50	.75
16	1	0	1	1	0	0	0	0	0	0	0	0	2	.50	.50	.75
17	1	1	1	1	0	0	0	0	0	0	0	0	1	1.0	1.0	1.0
18	1	0	1	1	0	0	0	0	0	0	0	0	2	.50	.50	.75
19	2	1	1	1	1	1	1	1	0	1	0	1	3	.67	0.0	.75
20	2	1	1	1	1	1	1	1	0	1	0	1	3	.67	0.0	.75
21	1	1	0	0	0	0	0	0	0	0	0	0	1	1.0	1.0	1.0
22	-	-	1	1	0	0	0	0	0	0	0	0	1	1.0	1.0	1.0
23	0	0	0	0	0	0	0	0	1	0	0	0	1	1.0	0/0	1.0
24	0	0	0	1	0	0	0	0	-	0	0	0	1	1.0	0/0	1.0
25	0	0	1	0	1	2	2	1	0	1	1	1	3	.67	.75	.75
26	0	0	1	1	1	0	0	0	1	2	2	2	3	.67	.80	.75
27	1	0	0	0	1	1	1	1	0	1	1	1	2	.50	.67	.75
28	0	0	1	2	1	2	2	1	2	1	0	1	5	.40	.40	.50
29	0	0	0	1	1	1	1	1	1	1	1	1	2	.50	.50	.75
30	-	-	-	2	0	1	1	1	0	1	1	1	2	1.0	1.0	1.0
31	0	1	0	0	1	1	1	1	0	1	1	1	2	.50	.67	.75
32	0	2	0	0	0	0	1	0	0	0	0	0	2	1.0	0/0	1.0
33	0	0	0	1	1	1	1	1	0	1	0	1	3	.33	.50	.60
34	0	0	0	0	1	0	0	0	1	0	0	0	2	.50	0.0	.75
35	1	1	0	0	1	0	0	0	0	0	0	0	2	.50	.50	.75
36	0	0	0	0	0	1	1	1	0	1	1	1	1	1.0	1.0	1.0
37	0	0	1	1	1	1	1	1	1	1	1	1	1	1.0	1.0	1.0
38	0	0	1	1	1	1	1	1	1	1	1	1	1	1.0	1.0	1.0
39	0	0	1	1	1	1	1	1	1	1	1	1	1	1.0	1.0	1.0
40	1	0	0	0	0	0	0	0	0	0	0	0	1	1.0	0/0	1.0
41	1	0	1	1	0	0	0	0	0	0	0	0	2	.50	.50	.75
42	0	1	1	1	0	1	1	1	1	1	1	1	2	.50	0.0	.75
43	0	0	1	1	0	0	0	0	0	0	0	0	1	1.0	1.0	1.0
44	0	0	0	0	0	0	0	0	0	1	1	1	1	1.0	1.0	1.0
45	1	1	0	0	0	0	0	0	0	0	0	0	1	1.0	1.0	1.0
46	0	0	0	0	0	1	1	1	1	1	1	1	2	.50	.75	.75
47	0	0	0	0	1	0	0	0	0	0	0	0	1	1.0	0/0	1.0
48	1	0	0	0	-	0	0	0	1	1	1	1	3	.33	.50	.60
49	0	0	0	0	1	1	1	1	1	0	0	0	2	.50	.75	.75
50	0	1	2	2	-	3	3	3	4	5	5	5	5	1.0	1.0	1.0
51	0	1	1	1	-	0	0	0	1	0	0	0	2	.50	.67	.75
52	0	0	0	1	1	0	0	0	1	1	1	1	3	.33	.60	.60
53	0	0	1	1	0	0	0	0	0	0	0	0	1	1.0	1.0	1.0
54	0	0	?	1	1	1	1	1	1	1	1	1	1	1.0	1.0	1.0
55	-	-	?	0	2	1	1	1	2	1	1	1	2	1.0	1.0	1.0
56	0	0	0	0	1	0	0	0	1	0	0	0	2	.50	0.0	.75

Polymorphisms between square brackets.

Missing entries entered as "?".

Unapplicable character states entered as "-".

A few a posteriori character weighting approaches were also applied. Successive approximations character weighting based on ci (CARPENTER, 1988; FARRIS, 1969, 1989) was implemented in NONA 2.0, invoking the command **run[swt amb- mult*500**. The programme stabilised on iteration 2, finding a single most parsimonious tree (tree 1), with weighted length 7487.

Implied weighting (GOLOBOFF, 1993) was performed in PeeWee 2.6 (**amb-; mult*500**, using **max*** was not necessary). With the default value for the concavity constant (**conc 3**) in effect, tree 1 was found as fittest tree in all 500 random addition sequences executed, with fit 402.5. Applying implied weighting under default concavity in PAUP, using **condense collapse=minbrlen** and **pset gpeewee=yes** (emulating peewee) or **pset goloboff=yes** (more accurate) and **hsearch addseq=random nreps=1000** produced the same tree in all 1000 random addition sequences executed, the only difference being that PAUP attributes a negative sign to the fit value calculated. The same fittest tree (tree 1) is found when the concavity constant in PeeWee is increased to 4, 5 or 6. When the concavity constant is lowered to 1, four other fittest trees are found, which differ from tree 1 by details in the relations between the *Zoropsis* species and by the fact that *Akamasia* and *Takeoa* are grouped in one clade, which also includes the *Acanthoctenus* species in two of the trees. With the concavity constant set to 2, a set of five fittest trees is found, encompassing the same four trees found with concavity constant 1, in addition to tree 1. However, under these low concavity values, implied weighting weighs so strongly against homoplastic characters that excessive weight is given to a small set of mutually consistent characters. For that reason, these solutions are not preferred.

Another weighting technique, "Strongest Evidence" (SALISBURY, 1999), was also applied. Strongest Evidence weights characters based on their APS. The APS of a character on a particular tree is the inverse $\log_{10}$ of its probability of being at least as parsimonious on that same tree when its states would be randomly shuffled across the taxa (SALISBURY, 1999: 139). Although this null model-based method has been debated (FARRIS, 2000; SALISBURY, 2000b), it seemed worthwhile to compare its results with the trees obtained by more conventional approaches, especially because the relatively small number of taxa in the matrix under study allowed this computationally-demanding method to be executed within a reasonable period of time. SEPAL 1.1 was run in "Strongest Evidence" mode, with five random addition orders and TBR in effect. SEPAL always returns a strict consensus "Strongest Evidence tree", which had APS 53.930 in this case. This tree is similar to tree 2, having *Griswoldia urbensis, Acanthoctenus gaujoni, Acanthoctenus sp., Akamasia cyprogenia* and *Takeoa nishimurai* branch off in sequence below the clade grouping all *Zoropsis* species. The Strongest Evidence tree differs from tree 2 in details in the arrangement of the individual *Zoropsis* species.

Tree 1, which is found under equal weighting, ci-based successive weighting, and implied weighting with a PeeWee concavity constant of 3-6, is our preferred solution. The node numbers given in Fig. 2, which illustrates the preferred tree, are also used to designate the clades that originate from those nodes. In Table 2, the last four columns give the number of steps and, rounded to two significant digits, ci, ri and Goloboff fit (GOLOBOFF, 1993) for each character on the preferred tree. Cladogram robustness for this tree was examined by calculating decay values ("Bremer support", BREMER, 1988, 1994) in NONA and PeeWee (with concavity 3) using the **bsupport** command. The equal weighting, successive weighting and implied weighting decay values, respectively, are shown in sequence below branches in Fig. 2.

Clade 2 in the preferred tree is supported by a high decay value under all weighting schemes. The most important of the 11 non-homoplasious character state changes that support clade 2 are: presence of a ti I median and terminal pl and rl spine in females (11:1 and 12:1), presence of a cribellum (21:0), absence of a protruding basal half of the tegulum (35:0, reversed in *Takeoa nishimurai*), absence of an isolated sclerite next to the embolus base (37:1), presence of a bristle mat and a rl marginal bump on the cymbium (38:1, 39:1), absence of a lateral tooth on the epigyne (45:0), and presence of ovoid bodies in the vulva (54:1).

The genus *Acanthoctenus* (clade 4) branches off at the base of clade 2 and is supported by the presence of a swollen palpal patella (43:1) and a complex cavity of the spermatheca (53:1). Sister group to clade 4 is clade 3, supported by a shallow trochanter notch (17:0) and the presence of a sclerotised base plate in the central depression of the epigyne (49:1, reversed in clade 7). Clade 3 groups all the taxa in the present study that are considered to belong to the family Zoropsidae. Branching off at the base of clade 3 is the monospecific genus *Akamasia*, which will be described below and which accomodates the species described previously as *Zoropsis cyprogenia*. The most striking feature setting *Akamasia* apart from the rest of Zoropsidae is the absence of a conductor in the male palp (23:1).

Sister group to *Akamasia* is clade 5, supported by five homoplasious changes: absence of a terminal do spine on the male ti I (4:0, parallelled in *Griswoldia urbensis* and reversed in *Zoropsis lutea*), median insertion of the embolus (25:1, parallelled in *Acanthoctenus gaujoni* and transformed to 25:2, apical, in clade 9), presence of an embolar lamella (27:1, also present in *Ctenus sp.*), presence of a MTP (31:1, parallelled in *Griswoldia urbensis*), and a protruding proximal pl part of the tegulum (33:1, parallelled in *Acanthoctenus sp.* and reversed in *Zoropsis media*). *Takeoa nishimurai* branches off at the base of clade 5. This enigmatic species differs from the other

Zoropsidae by the presence of eight vsp on ti I of males and females (5:2, 7:2), the absence of a ti I terminal pl and rl spine in females (12:0), the presence of a protruding basal part of the tegulum (35:1), a mesal RTA (42:0), and an epigyne that lacks a median scape (47:1).

Sister group to *Takeoa nishimurai* is clade 6 (the genus *Zoropsis*), supported by the presence of a male tibial crack (1:1, parallelled in *Griswoldia urbensis* and lacking in *Zoropsis media*), a MA with a bifid tip and an unsclerotised base (30:1, 36:1), and anterior ovoid bodies (55:1).

Discussion

From the results of the cladistic analysis, a taxonomic conclusion is drawn: *Zoropsis cyprogenia* does not fit in *Zoropsis* and is transferred to a new genus, *Akamasia*. *Akamasia cyprogenia* differs from *Zoropsis* by the absence of a conductor, an embolar lamella and a MTP (23:1, 27:0, 31:0); by the presence of a MA with a simple tip and a sclerotised base (30:0, 36:0); a protruding proximal rl part of the tegulum (34:1); a short, mushroom-shaped epigynal scape (51:1, 50:4); laterally positioned ovoid bodies (55:2); and a long, contorted insemination duct (56:1).

Another striking feature of the preferred cladogram obtained is the sister group relationship of the genus *Acanthoctenus* (clade 4) and the family Zoropsidae (clade 3), an arrangement also encountered in cladograms presented by CODDINGTON & LEVI (1991), GRISWOLD (1993), and GRISWOLD et al. (1999). However, no taxonomic conclusions should be drawn from this topology, since many of the close relatives of the genus *Acanthoctenus*, for example the ctenid genera *Nothroctenus* and *Viracucha*, have not been included in the analysis presented here. Only a far more elaborate cladistic analysis including a large number of ctenid genera can give sufficient insight into the relationships and the proper placing of the enigmatic genus *Acanthoctenus*.

DESCRIPTIONS

Akamasia n. gen.
(Figs 3c, 4a-b)

Type species. *Zoropsis cyprogenia* Bosselaers, 1997.

Etymology: the genus name refers to the uninhabited Akamas peninsula on Cyprus, where the holotype of the type species was found.

Diagnosis: differs from all other Zoropsidae by the absence of a conductor and the possession of a short, mushroom-shaped epigynal scape. Differs from *Takeoa* and *Acanthoctenus* by the presence of six instead of eight or nine ti I vsp and from *Takeoa* and *Zoropsis* by the absence of an embolar lamella or a MTP. Can be distinguished from *Zoropsis* by the presence of a fully sclerotised MA with a simple tip and from *Takeoa* by a distally inserted RTA, a flat basal half of the tegulum and the presence of an epigynal scape.

Description

Medium sized spiders (6-8) with linear, bipartite cribellum and oval calamistrum. Carapace yellow-brown, somewhat darker in cephalic part between eyes. Covered with short, dark, forward pointing hairs, except on radial striae. Thoracic groove short but pronounced. Clypeus narrow. Eyes ringed with black, in two rows. In do view anterior row slightly recurved, posterior row strongly recurved. In frontal view, both eye rows slightly recurved. MOQ widest posteriorly. Chelicerae dark greyish brown, with yellow border at fang groove. Three promarginal teeth, small one near fang base, large one in middle, another small one furthest from fang base. Two retromarginal teeth, medium-sized one closest to fang base, large one further from fang base. Sternum shield-shaped, pale yellow with some dark spots, sparsely covered with dark, pointed setae. Labium wider than long, obtuse, rounded anteriorly, with thickened, white margin. Maxillae yellow, parallel-sided. Abdomen dorsally with dark, arrow-shaped median mark on anterior half, dark chevrons on posterior half. Legs stout, heavily spined, yellow with darker markings. Ti I and II with five or six vsp, mt I and II with four vsp. All tarsi bearing scopulae and thick terminal claw tufts. Leg formula 4 1 2 3. Male palp (Figs 4a-b) with simple, distal RTA, simple, slender embolus without lamella and fully sclerotised MA with simple, pointed tip. No conductor, no MTP. Epigyne (Fig. 3c) with central depression containing a flat, sclerotised base plate to which a stalked, mushroom-shaped scape is attached. Lateral sclerotised lobes of epigyne with small pocket, meeting posteriorly, behind stalked scape. Internal genitalia (BOSSELAERS, 1997: Figs 9-10): spermathecae elongated, robust, of uniform texture throughout, laterally flattened. Lateral ovoid body connected to posterior end of each spermatheca. Anterior end of spermatheca connected to long tortuous insemination duct with thick, sclerotised wall and posterior entrance.

Discussion

Akamasia is monospecific and only contains the type species *Akamasia cyprogenia*. The male of this species has recently been discovered and is described here for the first time. The female is redescribed; a few errors in the original description (number of retromarginal cheliceral teeth and an occasional misinterpretation of a spine position) are corrected.

Akamasia cyprogenia (Bosselaers, 1997), n. comb.
(Figs 3c, 4a-b)

Zoropsis cyprogenia BOSSELAERS, 1997: 164, Figs 2-10 (female holotype from Avakas Gorge, Akamas peninsula, Cyprus, examined).

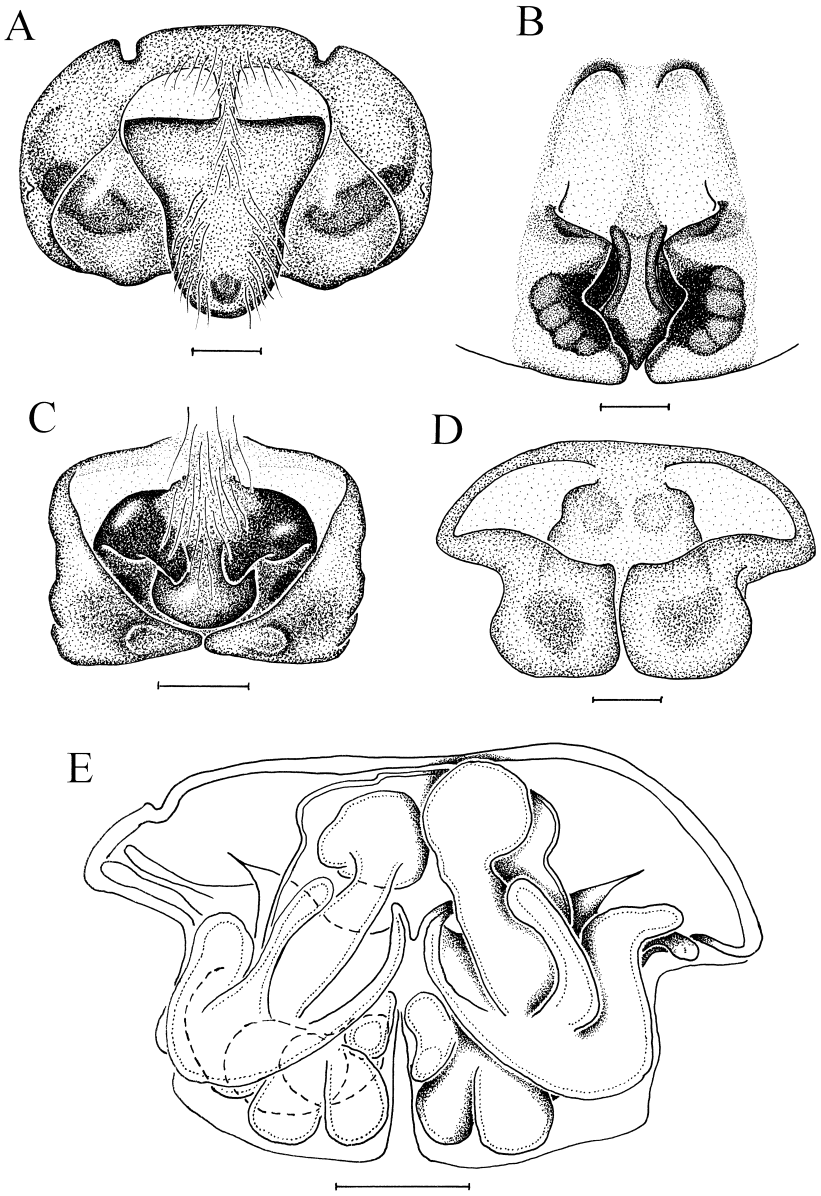

Fig. 3. – A. *Zoropsis media*, epigyne. – B. *Acanthoctenus gaujoni*, epigyne. – C. *Akamasia cyprogenia*, epigyne. – D. *Takeoa nishimurai*, epigyne. – E. *Takeoa nishimurai*, vulva, dorsal view. Scale bars = 0.25 mm.

Material: Holotype female: Cyprus, Akamas peninsula, Avakas Gorge, in leaf litter on the bank of Avgas river, elev. 20 m, 5 April 1997, J. Bosselaers leg. (RBINS 28515). Male: Cyprus, Akamas peninsula, Neo Chorion, March 1998, Martin Askins leg. (CMA).

Description

Male. Total length: 6.15. Carapace length: 3.5; width: 2.65. Colour as in generic description. Width of clypeus: 0.1. Width of anterior eye row: 0.87; width of posterior row: 0.97. Depth of entire ocular field in frontal view: 0.66. MOQ, anterior width: 0.37; posterior width: 0.53; depth: 0.53. Diameter of individual eyes: anterior row, medians: 0.16; laterals: 0.18; posterior row, medians: 0.19; laterals: 0.15. Chelicerae as in generic description. Sternum length: 1.55; width: 1.4. Pale yellow with seven marginal black spots between leg bases and group of seven central black spots forming a circle. Labium length: 0.5; width: 0.6. Maxillae length: 0.95; width: 0.53. Abdomen: do side reddish brown, peppered with black dots, bordered with black, sparsely covered with dark, pointed setae, anterior half with dark brown, more or less arrow-shaped median mark pointing backwards, posterior half almost completely darkened by several wide, black transversal chevrons, ve side black, bordered by pale yellowish brown. Legs yellow, covered with thin grey setae. Femora and patellae with some dark markings. All tibiae and metatarsi with one sub-basal and one terminal, wide, dark ring.

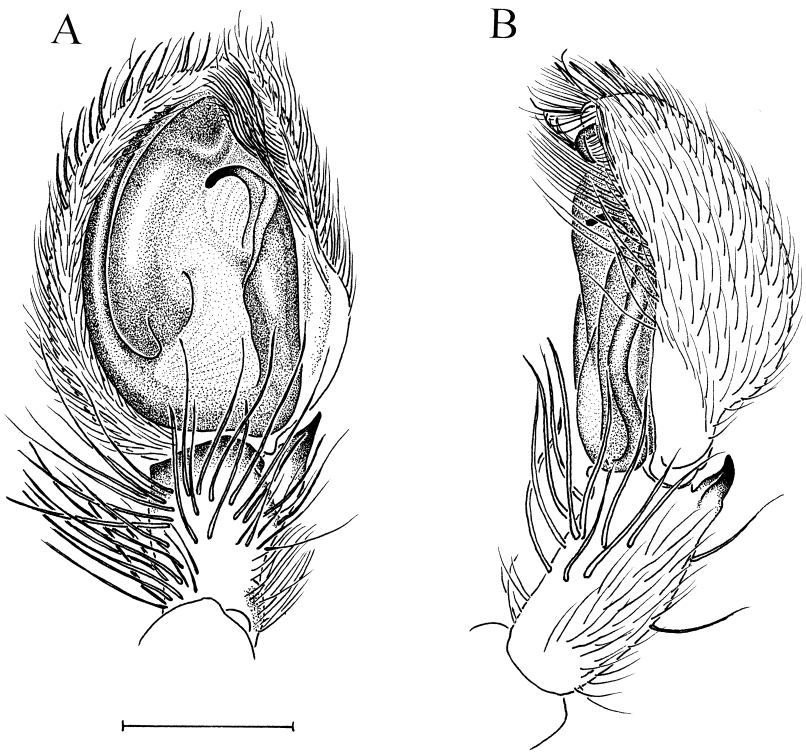

Fig. 4. – *Akamasia cyprogenia*, left male palp. – A. Ventral view. – B. Retrolateral view. Scale bar = 0.5 mm.

Leg measurements:

	fe	pa	ti	mt	ta	total
I	3.40	1.30	3.80	3.45	1.15	13.10
II	3.00	1.30	3.15	2.95	1.05	11.45
III	2.60	1.25	2.30	2.40	0.95	9.50
IV	3.40	1.30	3.35	3.95	1.20	13.20

Leg spination. Leg I: fe with one pl spine and nine do spines in three rows. Pa spineless. Ti with six vsp, two pl and two or three rl spines. Mt with four vsp, one pl and one rl spine. Leg II: fe with ten do spines in three rows. Pa spineless. Ti with six vsp, three pl and three rl spines. Mt with four vsp, one pl and one rl spine. Leg III: fe with ten do spines in three rows. Pa with one rl spine. Ti with three vsp, one do spine, two pl and two rl spines. Mt with two rows of three ve spines and one terminal median ve spine, one do spine, three pl and three rl spines. Leg IV: fe with ten do spines in three rows. Patella with one rl spine. Ti with three vsp, one do spine, two pl and two rl spines. Mt with two rows of three ve spines and one terminal median ve spine, one do spine, three pl and three rl spines. Male palp (Figs 4a-b) as in genus description.

Female. Total length: 8.0. Carapace length: 4.0; width: 3.15. Colour as in generic description. Some small, light grey spots on margin and along radial striae. Width of clypeus: 0.1. Width of anterior eye row: 1.11; width of posterior row: 1.28. Depth of the entire ocular field in frontal view: 0.72. MOQ, anterior width: 0.48; posterior width: 0.59; depth: 0.57. Diameter of individual eyes: anterior row, medians: 0.18; laterals: 0.22; posterior row, medians: 0.21; laterals: 0.19. Chelicerae as in generic description. Sternum length: 1.75; width: 1.65. Pale yellow, with some small grey spots in centre and along margin. Labium length: 0.65; width: 0.75. Maxillae length: 1.03; width: 0.65. Abdomen: do side reddish brown, peppered with black dots and sparsely covered with dark, pointed setae, anterior half with red-brown, more or less arrow-shaped median mark with dark border, pointing backwards, on posterior half several transversal dark chevrons and three pairs of white dots, ve side yellow-brown, sparsely dotted with black spots. Cribellum bipartite. Legs yellow, covered with thin grey setae, femora with some grey spots.

Leg measurements:

	fe	pa	ti	mt	ta	total
I	3.00	1.40	3.15	2.65	0.90	11.10
II	2.75	1.35	2.65	2.35	0.85	9.95
III	2.25	1.25	1.85	1.95	0.85	8.15
IV	3.15	1.30	2.60	3.35	1.00	11.40

Leg spination. Leg I: fe with one pl spine and five do spines in three rows. Pa spineless. Ti with six vsp, two pl and two or three rl spines. Mt with four vsp, one pl and one rl spine. Leg II: fe with eight or nine do spines in three rows. Pa spineless. Ti with six vsp, two pl and three rl spines. Mt with four vsp, one pl and one rl spine. Leg III: fe with nine do spines in three rows. Pa with one rl spine. Ti with three vsp, one do spine, two pl and two rl spines. Mt with two rows of three ve spines and one terminal median ve spine, one do spine, three pl and three rl

spines. Leg IV: fe with six or seven do spines in three rows. Patella with one rl spine. Ti with three vsp, one do spine, two pl and two rl spines. Mt with two rows of three ve spines and one terminal median ve spine, one do spine and oval calamistrum, three pl and three rl spines. Female genitalia: epigyne (Fig. 3c) and internal genitalia as in genus description.

Distribution: Only known from Akamas peninsula, Cyprus.

In the course of this study, the types of *Acanthoctenus gaujoni* Simon, 1906 were seen. Because this species has been described only summarily (SIMON, 1906: 290) and no illustrations have ever been published of it, it is redescribed here.

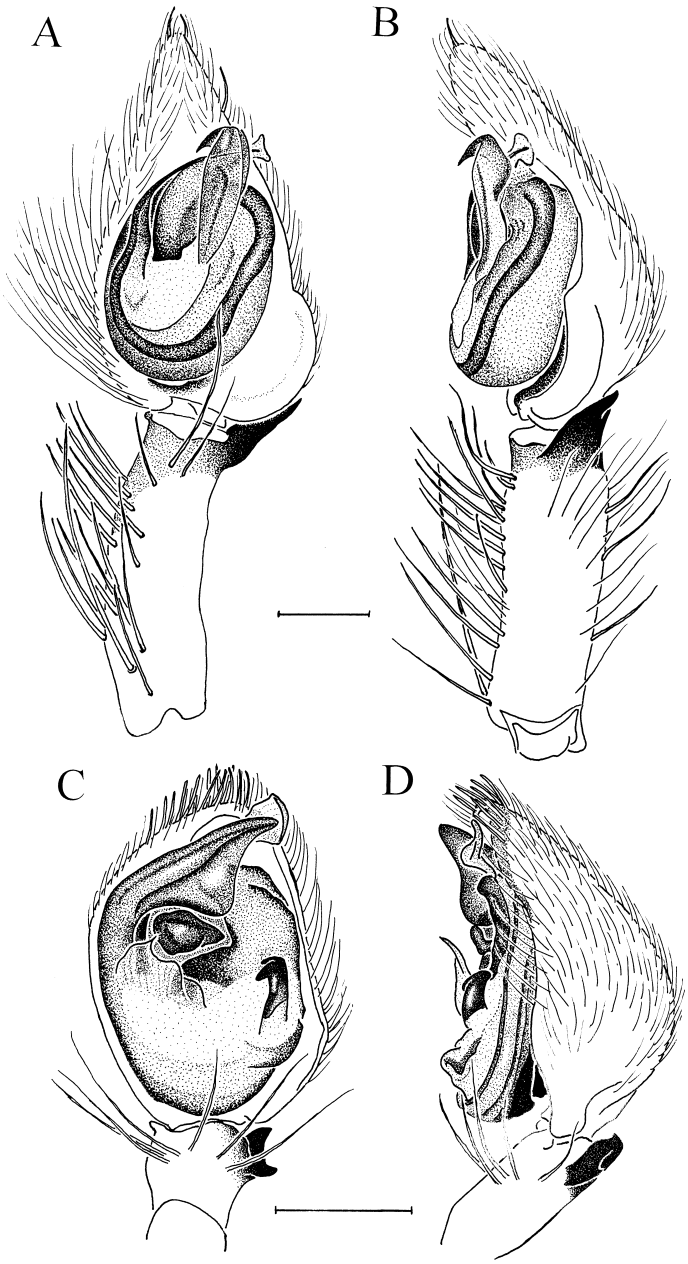

Fig. 5. — *Acanthoctenus gaujoni*, left male palp. — A. Ventral view. — B. Retrolateral view. — *Zoropsis media*, left male palp. — C. Ventral view. — D. Retrolateral view. Scale bars = 0.5 mm.

Acanthoctenus gaujoni Simon, 1906
(Figs 3b, 5a-b)

Acanthoctenus gaujoni SIMON, 1906: 290 (male holotype and female paratype from Zamora, Loja, Ecuador, examined).

Acanthoctenus gaujoni MELLO-LEITÃO, 1936: 194.

Material: Holotype male: Ecuador, Loja, Zamora, Gaujon leg. (MNHN AR5168). Paratype female: Ecuador, Loja, Zamora, Gaujon leg. (MNHN AR5168).

Description

Male. Total length: 12.15. Carapace length: 5.6; width: 4.5. Carapace yellow-brown with two darker longitudinal bands and short but pronounced fovea. Width of clypeus: 0.45. Eyes all ringed with black, in three rows, in typical ctenid pattern: anterior laterals adjacent to posterior medians. Row of long white hairs between median eyes. Width of anterior eye row: 1.65; width of posterior row: 1.75. Depth of entire ocular field in frontal view: 1.5. MOQ, anterior width: 0.9; posterior width: 1.0; depth: 0.95. Diameter of individual eyes: anterior row, medians: 0.37; laterals: 0.24; posterior row, medians: 0.39; laterals: 0.60. Chelicerae greyish brown, with three promarginal teeth, small one near fang base, large one in middle, another small one furthest from fang base, and three subequal retromarginal teeth, largest one closest to fang base, smallest one furthest from fang base. Sternum yellow, shield-shaped, length: 2.8; width: 2.5. Labium mushroom-shaped, with thickened white rim, length: 0.95; width: 0.85. Maxillae widening towards front, length: 1.7; width: 0.9. Abdomen slender, do side yellow-brown with darker, narrow, median triangular mark pointing backward on anterior half and four rows of two circular brown spots on posterior half. Covered with silky white hairs interspersed with shorter, stiff brown hairs. Ve side yellow, bordered by dark brown. Legs yellow-brown, tarsi with dense claw tufts. Leg formula 1 4 2 3.

Leg measurements:

	fe	**pa**	**ti**	**mt**	**ta**	**total**
I	8.50	2.50	10.30	9.90	2.75	33.95
II	7.35	2.50	7.75	7.40	2.30	27.30
III	5.75	2.00	5.10	6.00	2.00	20.85
IV	7.75	2.00	7.25	9.75	3.05	29.80

Leg spination. Leg I: fe with two pl spines and 11 do spines in three rows. Pa with one rl spine. Ti with nine vsp, three pl, three do and two rl spines. Mt with five vsp, two pl and one rl spine. Leg II: fe with one pl spine and ten do spines in three rows. Pa with one rl spine. Ti with nine vsp, three pl, two do and four rl spines. Mt with five vsp, two pl and one rl spine. Leg III: fe with 11 do spines in three rows. Pa with one pl and one rl spine. Ti with

three vsp, three do spines, one pl and three rl spines. Mt with three vsp, four pl and four rl spines. Leg IV: fe with nine do spines in three rows. Patella with one pl and one rl spine. Ti with three vsp, three do spines, three pl and four rl spines. Mt with three vsp, three pl and three rl spines. Male palp (Figs 5a-b) as illustrated, with pronounced rl cymbial bulge, slender, pointed embolus and cup shaped MA.

Female. Total length: 13.15. Carapace length: 5.75; width: 4.4. Carapace coloured as in male. Width of clypeus: 0.38. Eyes all ringed with black, disposed as in male, but no long white hairs between median eyes. Width of anterior eye row: 1.85; width of posterior row: 2.15. Depth of the entire ocular field in frontal view: 1.45. MOQ, anterior width: 0.88; posterior width: 1.05; depth: 0.95. Diameter of individual eyes: anterior row, medians: 0.29; laterals: 0.24; posterior row, medians: 0.37; laterals: 0.45. Chelicerae chestnut brown, somewhat inflated, cheliceral teeth as in male. Sternum yellow, shield-shaped, length: 2.55; width: 2.65. Labium mushroom-shaped, with a thickened white rim, length: 1.0; width: 0.95. Maxillae widening towards front, length: 1.85; width: 1.05. Abdomen oval, coloured as in male. Legs yellow-brown, femora with faint darker rings, tarsi with dense claw tufts.

Leg measurements:

	fe	**pa**	**ti**	**mt**	**ta**	**total**
I	6.20	2.50	6.50	5.00	1.65	21.85
II	5.15	2.15	5.10	4.40	1.50	18.30
III	4.60	1.85	3.85	4.25	1.55	16.10
IV	6.25	2.10	5.00	-	-	-

Leg spination. Leg I: fe with two pl spines and ten do spines in three rows. Pa with one rl spine. Ti with nine vsp, four pl and three rl spines. Mt with five vsp, one pl and one rl spine. Leg II: fe with one pl spine and 11 do spines in three rows. Pa with one rl spine. Ti with nine vsp, four pl and four rl spines. Mt with five vsp, two pl and one rl spine. Leg III: fe with 11 do spines in three rows. Pa with one pl and one rl spine. Ti with three vsp, two do spines, two pl and four rl spines. Mt with three vsp, five pl and four rl spines. Leg IV: fe with nine do spines in three rows. Patella with one pl and one rl spine. Ti with three vsp, three do spines, two pl and two rl spines. Rest of fourth legs missing. Epigyne (Fig. 3b) as illustrated, with lozenge-shaped scape. In order not to damage the unique paratype specimen, the vulva was not studied. But the complex, chambered spermathecae can be observed through the semi-transparent epigyne.

Acanthoctenus gaujoni is obviously closely related to *Acanthoctenus spinipes* Keyserling, 1877, as can be judged by comparing the illustrations presented here (Figs 3a, 5a-b) with those of *Paracantheis virginea* Kraus, 1955 (= *Acanthoctenus spinipes*) in Kraus (1955: Figs 134-137).

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Laboulbeniales (Ascomycota) on Carabidae (Insecta: Coleoptera) from the Galápagos Archipelago

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ABSTRACT. Five species of *Laboulbenia* are recorded from 15 species of Carabidae (Coleoptera) of the Galápagos Archipelago. Laboulbeniales were found on seven of the 15 larger Galápagos Islands.

L. sanjoaquina sp. nov. is described from *Platynus* (subgenus *Dyscolus*) species, endemic to San Cristobal, *L. galapagoensis* sp. nov. from *Tachys* species, occurring on several Galápagos Islands and *L. appendiculata* sp. nov. from a *Bradycellus* species, occurring on Pinzon. The genera *Selenophorus* and *Calosoma* are new host records of *L. flagellata*, this being the first definite record of a *Laboulbenia*-infested Carabini under natural conditions. In general, most of the Laboulbeniales have been observed in more humid conditions, mainly from coastal littoral habitats and the fern-sedge vegetation zone at higher elevation on Galápagos islands and volcanoes. *L. galapagoensis* and *L. appendiculata* are assumed to be dioecious. Together with *L. inflata*, three presumably dioecious species thus occur on the Galápagos Islands. Characters, a distribution map and an identification key are given for the *Laboulbenia inflata* species-group.

KEY WORDS: Carabidae, Laboulbeniales, Galápagos Islands.

INTRODUCTION

The Galápagos Archipelago is situated in the Pacific Ocean, 1000 km west of the Ecuadorian coast. Composed of many small and 15 larger islands, the archipelago is of volcanic origin. The age of the islands has been estimated between 0.7 (westernmost islands, e.g. Isla Isabela) and 3 million years (eastern islands such as San Cristobal) (SIMKIN 1984).

Despite the recent age and the strong isolation of Galápagos, some 40 species of Carabidae (Coleoptera), belonging to 15 genera, are at present known from the islands (DESENDER et al. 1992a; DESENDER, unpubl.). Most of these carabid species are endemic to the islands, while their biogeographical origin is assumed to be in western South America (DESENDER et al. 1992b).

During several recent expeditions, the junior author and co-workers sampled all main islands and volcanoes of the archipelago in order to study carabid beetles, herbivorous beetles and spiders (BAERT et al. 1994). As a consequence, systematic, ecological and molecular studies are in progress for several carabid genera (e.g. DESENDER et al. 1990, 1991, 1992a,b, 1999; DESENDER & VERDYCK 2000).

One aim of the recent study is the examination of the Laboulbeniales (Ascomycota) infesting Carabidae from the Galápagos Archipelago in order to obtain information about species numbers and the degree of endemism as well as the rate of parasitism of the ground beetle communities and populations. Such ecological data are very scarce from tropical regions but are needed to answer open questions of host specificity and transfer mechanisms of the parasites.

We will (1) summarise the results of our studies of Laboulbeniales on Galápagos carabids, with a description of three new species (in annex), (2) discuss the distribution and host-parasite relationships and (3) present an identification key to the *Laboulbenia inflata* species group.

HOST-PARASITE RELATIONSHIPS BETWEEN GROUND BEETLES AND LABOULBENIALES

Laboulbeniales are parasitic fungi infesting insects, diplopods and mites. Ground beetles represent one of the

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most frequently infested host groups. Sixteen genera of Laboulbeniales are known from Carabidae including *Laboulbenia* Montagne & Robin with several hundred ground beetle-infesting species.

Host specificity and mechanisms of parasite transfer of many taxa, especially those from tropical regions, are still unclear. There are few specific studies of these problems of population ecology. DE KESEL (1997) concluded from transmission experiments with a *Pogonus*-infesting *Laboulbenia* species that direct infections are much more frequent than soil-borne infections. This is due to the extremely low pick-up probability of spores left on the substrate. SCHELOSKE (1976a,b) demonstrated in hydrophilid beetles the transfer of Laboulbeniales during the copulation of the hosts. The parasitic fungi cover specific regions of the body (posterior margin of elytra in females, ventral side of mesothorax in males) in these hosts. Several carabid hosts bear Laboulbeniales on the same body areas, which suggests the transfer of parasites during copulation. In other cases, the fungi infest the mouth parts of their hosts (see below, e.g. *Calosoma linelli* Mutchler) and were obviously transferred during feeding on infested prey. Often co-occurring carabids have the same parasite species. ARNDT (unpubl.) recognized the same *Laboulbenia* species in three co-occurring ground beetles (two *Notiobia* species, one *Selenophorus* species) on fruit fall areas in a Venezuelan rain forest site. The parasite infested these genera, overlapping all members of a spermatophagous carabid community. This phenomenon leads to the problem of host specificity. While most known Laboulbeniales infest only one species, a species group or one genus, there are some carabid-infesting fungi that are extremely polyphagous. Whereas e.g. *Laboulbenia palmella* Thaxter is restricted to *Mormolyce phyllodes* Hagenbach in South East Asia, and *L. pheropsophi* Thaxter to the genus *Pheropsophus* Solier worldwide, *L. flagellata* Peyritsch, *L. polyphaga* Thaxter and *L. vulgaris* Peyritsch are world-wide occurring carabid parasites, each infesting more than 25 host genera. The basis for this range in host specificity is not clear. DE KESEL (1996, 1997) showed that *L. slackensis* Cépède & Picard, under natural conditions monophagous on *Pogonus* species, can infest several carabids under laboratory conditions, even members of the genus *Carabus*, which are not known as a regular hosts of any Laboulbeniales.

A close host specificity can give information on the history of infection as well as on biogeographical aspects. TAVARES (1985) showed a co-evolution between the parasitic genus *Rhachomyces* Thaxter and their hosts in the carabid tribe Trechini. Trechini species migrated into Europe several times pre- and postglacial. Every migration was combined with its specific *Laboulbenia*- or *Rhachomyces* infection. Later, cross infections occurred between species of Trechini of different migrations and *Bembidion* species. TAVARES (1985) also summarized the knowledge on Laboulbeniales of Carabidae from the Hawaii archipelago. On these islands, a considerable evolution and radiation of colonizing ground beetles took place, but the number of Laboulbeniales species remained low. With the exception of *L. vulgaris*, all recent species appear to be descendants from a single ancestor that invaded the Hawaii archipelago with its original host species.

MATERIAL AND METHODS

During several recent expeditions to the Galápagos Islands, more than 25,000 specimens of Carabidae have been collected from all of the larger Galápagos Islands and examined for the presence of Laboulbeniales (Dept. Entomology, Royal Belgian Institute of Natural Sciences, Brussels: BAERT, DESENDER, MAELFAIT & VERDYCK, 1986, 1988, 1991, 1996, 1998, 2000; complemented with Galápagos carabid material sampled by I. and H. Schatz, University of Innsbruck, Austria and by S. B. Peck and coworkers, Carleton University, Ottawa, Canada). On the whole, 480 carabid beetles, belonging to 15 species appeared to be infected. From these specimens, subsamples from all different localities were investigated in more detail.

Most beetles have been preserved in ethanol. Parasitic thalli were carefully removed from the host surface with the help of an insect pin (size 3). The thalli were mounted on a microscope slide in a mixture of glycerol+lactid acid+acid fuchsine and ringed using finger nail polish. The fungi were studied using a phase contrast microscope (Carl Zeiss Jena) at magnifications up to 600x.

Morphological terms are used according to SANTAMARIA (1998).

RESULTS AND DISCUSSION

Laboulbeniales on Carabidae from Galápagos

Laboulbeniales were recorded on 15 carabid species belonging to seven genera (Tab. 1). Host specimens occurred on seven of the 15 larger islands (Fig. 1).

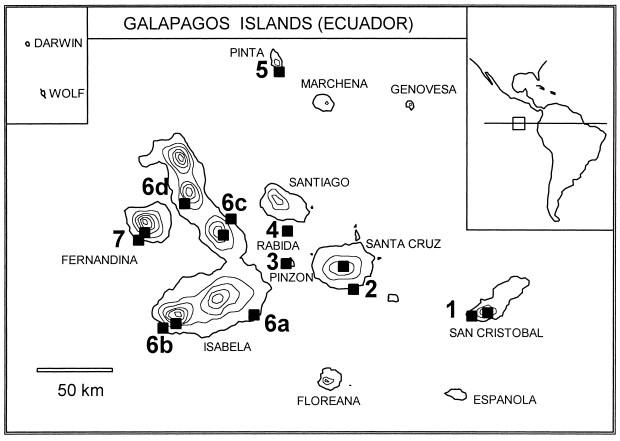


Fig. 1. – Sampling sites of Carabidae infested with Laboulbeniales in the Galápagos Archipelago: 1= San Cristobal, 2= Santa Cruz, 3= Pinzon, 4= Rabida, 5= Pinta, 6= Isabela: 6a= Volcan Sierra Negra, 6b= Volcan Cerro Azul, 6c= Volcan Alcedo, 6d= Volcan Darwin, 7= Fernandina.

TABLE 1

List of recorded *Laboulbenia* species, their host insects and infection rate. T – total number of collected host specimens in the sample. I – Number of infected specimens in sample.

Laboulbenia species	Host name	Island and volcano	exposition	m. a. sl.	vegetation zone	T	I
appendiculata	*Bradycellus insularis*	Pinzon	SE	380	dry arid zone	24	2
?flagellata	*Bembidion galapagoensis*	Isabela Volcan Alcedo	SE	1060	fern sedge zone	28	1
flagellata	*Calosoma linelli*	San Cristobal	W	675	fern sedge zone	7	2
flagellata	*Platynus albemarli*	Isabela Volcan Cerro Azul	WSW	680	fern sedge zone	1	1
flagellata	*Platynus darwini*	San Cristobal	W	700	fern sedge zone	5	1
flagellata	*Pterostichus calathoides*	San Cristobal	W	675	fern sedge zone	37	37
flagellata	*Pterostichus leleuporum*	Santa Cruz	S	875	fern sedge zone	18	10
flagellata	*Pterostichus leleuporum*	Santa Cruz	E	570	fern sedge zone	6	6
flagellata	*Selenophorus galapagoensis*	Pinta	SSE	2	litoral zone	2	1
flagellata	*Selenophorus galapagoensis*	Pinta	SSE	2	litoral zone	18	3
flagellata	*Selenophorus obscuricornis*	Fernandina	SW	400	dry arid zone	46	3
flagellata	*Selenophorus obscuricornis*	Isabela Volcan Alcedo	SE	600	dry arid zone	1	1
flagellata	*Selenophorus obscuricornis*	Isabela Volcan Darwin	SW	600	dry arid zone	24	3
galapagoensis	*Tachys erwini*	Fernandina	SW	5	litoral zone	24	5
galapagoensis	*Tachys erwini*	Pinta	SSE	2	litoral zone	22	5
galapagoensis	*Tachys nov. spec. 1*	San Cristobal	W	2	litoral zone	2	2
galapagoensis	*Tachys nov. spec. 2*	Isabela Volcan Alcedo	NE	2	litoral zone	3	1
galapagoensis	*Tachys vittiger*	Isabela Volcan Sierra Negra	SE	2	litoral zone	17	1
galapagoensis	*Tachys vittiger*	Rabida	NW	2	litoral zone	35	12
galapagoensis	*Tachys vittiger*	Santa Cruz	S	0	litoral zone	403	40
inflata	*Bradycellus insularis*	Isabela Volcan Alcedo	SE	1060	fern sedge zone	8	3
inflata	*Bradycellus insularis*	Pinzon	SE	380	dry arid zone	24	6
inflata	*Bradycellus spec.*	Santa Cruz	S	5	dry arid zone	2	2
inflata	*Bradycellus spec.*	Santa Cruz	S	20	dry arid zone	5	1
?inflata	*Bradycellus spec.*	Santa Cruz	S	5	dry arid zone	1	1
sanjoaquina	*Platynus chathami*	San Cristobal	W	530	fern sedge zone	3	2
sanjoaquina	*Platynus chathami*	San Cristobal	W	700	fern sedge zone	12	12
sanjoaquina	*Platynus darwini*	San Cristobal	W	700	fern sedge zone	5	5

One juvenile *Laboulbenia* specimen infested *Bembidion* (*Notaphus*) *galapagoensis* (Waterhouse) from Isla Isabela, Volcan Alcedo. This *Laboulbenia* species cannot be identified beyond doubt.

Laboulbenia flagellata **Peyritsch**
(Fig. 2)

Studied hosts and localities (+ sample codes, elevation and sampling date or period):

Calosoma linelli Mutchler, 1925, San Joaquin, Isla San Cristobal (A92/35, 675m, 21.02.92) (two specimens).

Pterostichus (*Blennidius*) *calathoides* (Waterhouse, 1845), San Joaquin, Isla San Cristobal (A92/33, 675m, 21.02.-01.03.92, A92/32, 530m, same period).

Pterostichus (*Blennidius*) *leleuporum* Reichardt, 1976, top of Isla Santa Cruz (A91/A20, 875m, 16.10.-15.11.92); Isla Santa Cruz, Los Gemelos (R00/01, 650m, 17.12.97).

Platynus (*Dyscolus*) *albemarli* (Van Dyke, 1953), Isla Isabela, Volcan Cerro Azul (P91/163, 680m, 21.-25.05.91) (only one female).

Platynus (*Dyscolus*) *darwini* (Van Dyke, 1953), San Joaquin, Isla San Cristobal (A92/38, 700m, 20.02.-01.03.92). (Only one male, *L. flagellata* on the same host as *L. sanjoaquina* sp. nov.).

Selenophorus obscuricornis (Waterhouse, 1845), Isla Fernandina (B91/765, 400m, 04.05.91); Isla Isabela, Volcan Darwin (P92/189, 600m, 16.05.92); Isla Isabela, Volcan Alcedo (R00/06, 600m, 07.-10.04.99).

Selenophorus galapagoensis (Waterhouse, 1845), Isla Pinta (P92/43, 2m, 13.-23.03.92); Isla Pinta (B00/108A, 2m, 31.03.00).

L. flagellata occurs on the whole body surface of male and female beetles, often in groups of large numbers on *P. calathoides*, *P. leleuporum* and an infested male of *C. linelli*. The fungi grow very often pair-wise. The *Selenophorus* species are infested less numerously (scattered individuals, only one host specimen with 50 individuals of *Laboulbenia*; fungi mostly on elytra, very few individuals on ventral side and legs), whereas the *Platynus* species bear only scattered individuals on the elytra.

L. flagellata is on average longer and more slender on *Pterostichus* (full-grown: 220-280µm) than on *Selenophorus* and *Calosoma* species (140-200µm), but no other morphological differences were found.

L. flagellata is regarded as one of the most polyphagous and widespread species of the Laboulbeniales. MAJEWSKI (1994) mentioned that at least 80 gen-

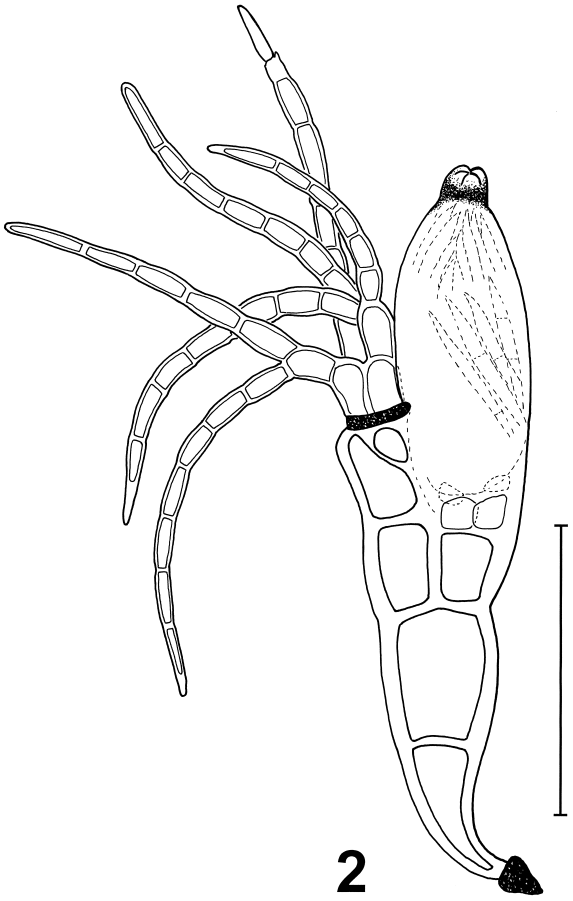


Fig. 2. – *Laboulbenia flagellata* Peyritsch from *Selenophorus galapagoensis* (Waterhouse). Bar 100µm.

era of Carabidae are hosts of this species. However, this number can vary because of different opinions on the generic state of several subgenera belonging to the large groups *Pterostichus* Bonelli (in widest sense) and *Platynus* Bonelli (in widest sense).

Selenophorus and *Calosoma* species are new host genera of this fungus. This is the first definite record of an infested Carabini under natural conditions; infection of *Carabus* spec. in laboratory experiments have already been described by De Kesel (1997). However, *Calosoma linelli* is probably a secondary host in the sense of Scheloske (1969). *C. linelli* co-occurs in the fern-sedge zone at higher elevations of Isla San Cristobal with the much more frequently-parasitised species *Platynus calathoides* as well as with *P. darwini* (cf. Tab. 2). Head and mouthparts of one of the *Calosoma* specimens were heavily infested.

Pterostichus leleuporum is the most frequent host species in Santa Cruz. It is endemic to the relatively more humid highlands of Isla Santa Cruz (fern-sedge zone). We could not find fungi on *Pterostichus williamsi,* the other endemic species of the same genus, occurring in the dry arid zone at low elevation on the same island.

All infested specimens of *Selenophorus galapagoensis* are from the littoral zone of Isla Pinta, one of the northernmost islands. Although this carabid species occurs on several other islands in the archipelago, parasites could not be found elsewhere. *Selenophorus obscuricornis*, the second infested species of this genus, lives in the dry arid zone of the central and western islands, although it is also found at higher elevation, especially on the younger volcanoes of Isabela and Fernandina. However, this species

TABLE 2

Carabid communities from three different localities (two habitat types) with recorded parasitic *Laboulbenia* species and infection rate. T – total number of collected carabid specimens in the sample. I – Number of infested specimens in the sample.

Carabid community	**T**	**Infesting *Laboulbenia* species**	**I**
Santa Cruz, South exposition, 5-20m a.s.l., dry arid zone, 21.3.98			
Bradycellus spec.	8	*L. inflata*	4
Selenophorus obscuricornis	330	-	0
Calosoma granatense	34	-	0
Pentagonica flavipes	2	-	0
Isabela Volcan Cerro Azul, WSW exposition, 680m a.s.l., fern sedge zone, 21.-25.05.91			
Platynus albemarli	1	*L. flagellata*	1
Scarites williamsi	7	-	0
Bembidion galapagoensis	9	-	0
Bradycellus insularis	1	-	0
Pterostichus insularis	3	-	0
San Cristobal, West exposition, 530-700m a.s.l., fern sedge zone, 20.02.-01.03.92			
Pterostichus calathoides	96	*L. flagellata*	95
Calosoma linelli	7	*L. flagellata*	2
Platynus darwini	6	*L. flagellata* + *L. sanjoaquina*	6
Platynus chathami	18	*L. sanjoaquina*	17
Scarites galapagoensis	5	-	0

is only abundant in the 'dry arid zone' during periods with unusual rainfall (e.g. during El Niño events).

In conclusion, *L. flagellata* occurs in the Galápagos Archipelago from the littoral over the dry arid zone to the fern-sedge zone in the highlands, on eight different host ground beetle species (four genera). A large majority of infected beetles were found in humid habitats. All of the infected species are endemic to the archipelago. Five of these carabid species are limited to the higher and more humid parts of a single island (three species from San Cristobal, one from Santa Cruz and one from Isabela). Interestingly, we did not find any Laboulbeniales on carabids of the southernmost islands Floreana and Española. These islands are indeed known to be strongly isolated from the other Galápagos Islands by sea-currents, which mostly go westwards from San Cristobal towards Santa Cruz and Isabela, and then bend northwards. The same currents explain how *L. flagellata* could have reached the latter islands (see above).

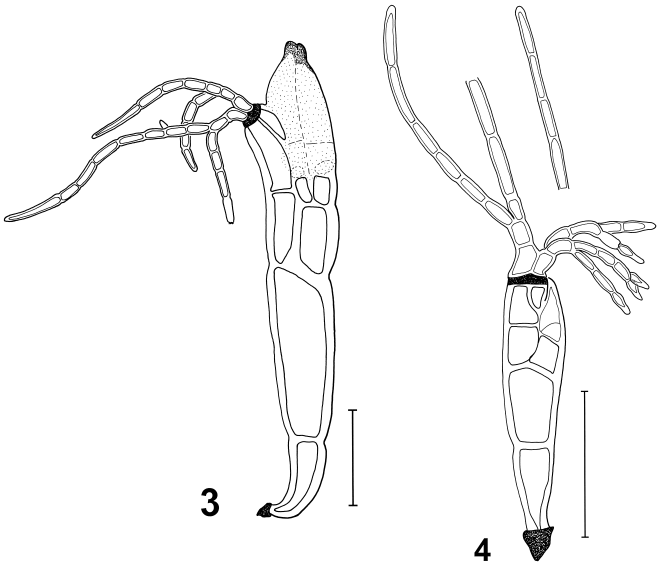
***Laboulbenia sanjoaquina* sp. nov.**
(Figs 3, 4)

Studied hosts and localities:

Platynus (*Dyscolus*) *chathami* (Van Dyke, 1953), several infested specimens from San Joaquin near the top of Isla San Cristobal (A92/38, 700m, 20.02.-01.03.92; A92/30, 530m).

Platynus (*Dyscolus*) *darwini* (Van Dyke, 1953), several specimens, Isla San Cristobal, San Joaquin, same date (A92/38, 700m). One female also infected with *L. flagellata*.

Hosts often infested with more than 100 *L. sanjoaquina* on dorsal and ventral sides of body. Usually females are infested on dorsal side (thorax, elytra), and males on ventral side (sternites, trochanter), suggesting regular infestation during copulation. Antennae and legs are mostly free from parasites. Several *L. sanjoaquina* of *P. darwini* are secondarily infested with nematode cysts. The hosts co-occur in the fern-sedge zone of Isla San Cristobal.



Figs 3, 4. – *Laboulbenia sanjoaquina* sp. nov. from *Platynus* (*Dyscolus*) *chathami* (Van Dyke). Fig. 3. Fully-grown specimen. Fig. 4. Juvenile specimen. Bars 100µm.

***Laboulbenia galapagoensis* sp. nov.**
(Figs 5, 6)

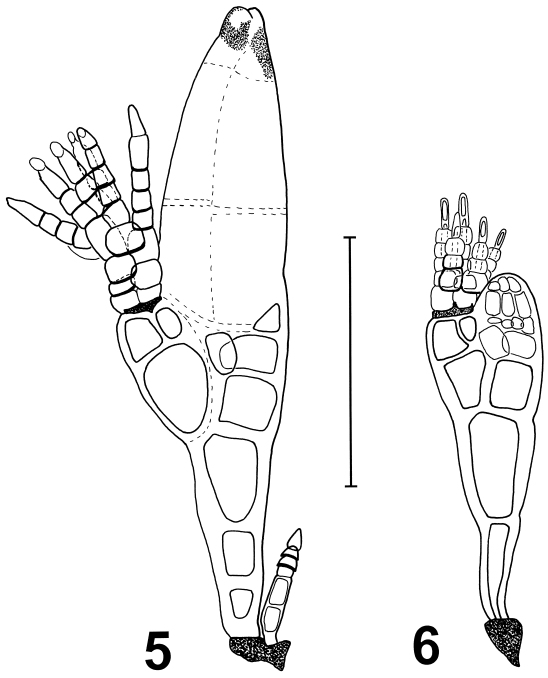
Tachys vittiger LeConte, 1851, female from Isla Santa Cruz (B91/890, 0m, 28.-29.05.91); Isla Rabida (P92/90, 2m, 03.03.92); Isla Isabela, Volcan Sierra Negra (B91/884, 2m, 26.05.91).

Tachys erwini Reichardt, 1976, Isla Fernandina (P91/115, 5m, 03.05.91); Isla Pinta (P92/55, 2m, 19.03.92).

Tachys nov. sp. 1, Isla San Cristobal (P96/27, 2m, 16.03.96).

Tachys nov. sp. 2, Isla Isabela, Volcan Alcedo (P96/77, 2m, 01.-03.04.96).

About 30 host specimens were examined. Hosts bear usually 1-2, very rarely up to five *L. galapagoensis*. The parasites occur most frequently on elytra or margin of thorax, rarely on sternites and legs of males and females.



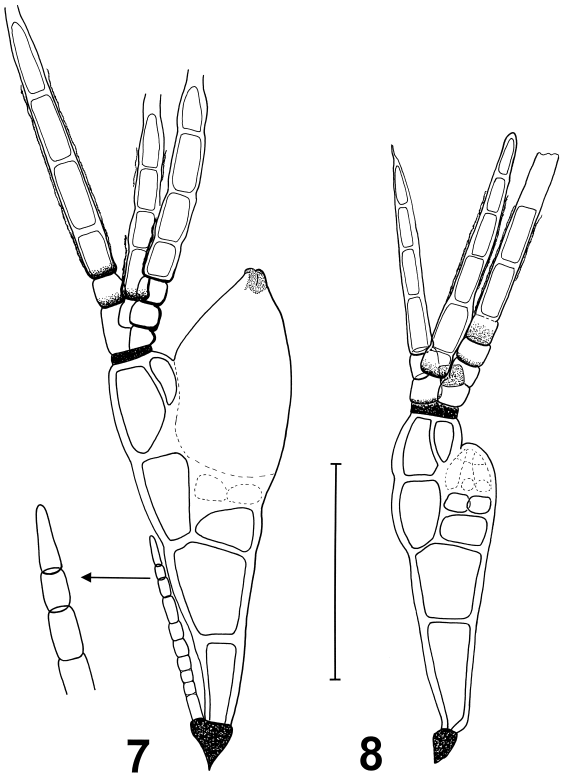
Figs 5, 6. – *Laboulbenia galapagoensis* sp. nov. from *Tachys vittiger* LeConte. Fig. 5. Fully-grown presumed-female thallus and small male. Fig. 6. Juvenile presumed-female thallus. Bar 100µm.

***Laboulbenia appendiculata* sp. nov.**
(Figs 7, 8)

Studied hosts and distribution:

Bradycellus insularis Reichardt, 1976, four specimens from Isla Pinzon (P91/255, 380m, 27.06.91).

From a group of seven co-occurring host specimens, six were infested by *L. inflata* (see below) and two by this new species on elytra and sternites. Two specimens of *L. appendiculata* occurred together with one specimen of *L. inflata* on one of the hosts.



Figs 7, 8. – *Laboulbenia appendiculata* sp. nov. from *Bradycellus* sp. Fig. 7. Fully-grown presumed female thallus and small male. Fig. 8. Juvenile presumed-female thallus. Bar 100µm.

Laboulbenia inflata Thaxter

Studied hosts and distribution:

Bradycellus insularis Reichardt, 1976, 3 specimens, Isla Isabela, Volcan Alcedo (P91/250, 1060m, 22.06.91); 6 specimens from Isla Pinzon, P91/255, 27.06.91. *L. inflata* occurred together with *L. appendiculata* sp. nov. on one male host specimen of *Bradycellus insularis*.

Bradycellus sp., 4 specimens from Santa Cruz (B98/049A, 21.3.98, B98/065A, 22.3.98, 2 specimens, B98/096A, 30.3.98, 5-20 m).

13 infested hosts were examined, each with one, rarely three, parasites. *L. inflata* occurred on elytra, sternites, thorax and tarsi.

Distribution and host-parasite relationships of the *Laboulbenia inflata* species group

Three of the *Laboulbenia* species occurring on the Galápagos archipelago are presumably dioecious, which is an extreme exception in this genus. Moreover, we suggest that these species (*L. inflata*, *L. appendiculata*, and *L. galapagoensis*) together with four other taxa form a monophyletic species group of *Laboulbenia* (see Annex for morphological details and arguments of the monophyly of this group).

L. inflata is regarded as the most primitive representative of the group; it also has the widest distribution. The known records of the *L. inflata*-group show a wide distribution in the New World and in Europe (Fig. 9). However, they also show a very scattered distribution due to inadequate collecting.

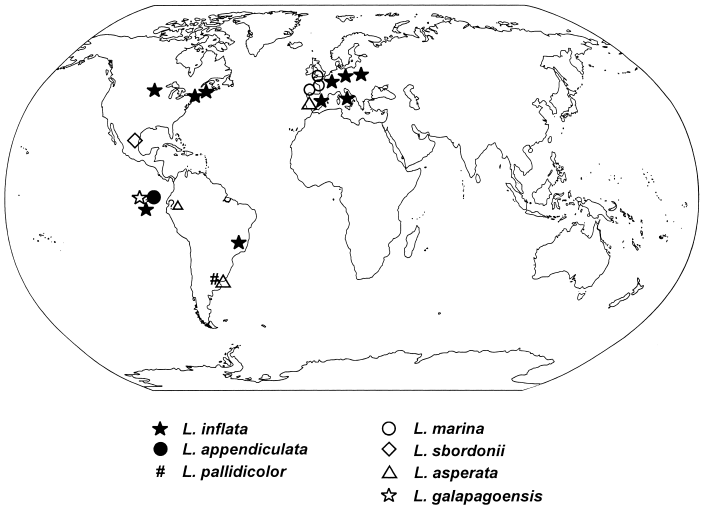


Fig. 9. – Distribution map of the species of the *Laboulbenia inflata*-group.

The *L. inflata*-group infests two groups of hosts (see key for details of host genera): Stenolophini, a small group of the subfamily Harpalinae with world-wide distribution, and the tachyine-trechine-complex of the subfamily Trechinae. The host species of the latter group seem to prefer littoral habitats, whereas species of the former also occur in riparian, but not necessarily saline, habitats. Host specificity of the *L. inflata*-group is high, most species infecting only one ecologically-restricted host species or a group of related hosts in a geographically-restricted area. This may indicate host-parasite co-evolution. In the case of the *Tachys* species from Galápagos Islands, the species group-specificity apparently arose on the continent. A comparable situation was described for *Laboulbenia* species infesting Carabidae on Hawaii (Tavares 1985, Thaxter 1908).

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ANNEX

Description of new *Laboulbenia* species and taxonomic remarks

Laboulbenia sanjoaquina sp. nov.

(Figs 3, 4)

Thallus gracilis, 500-650µm longus. Cellulae receptaculi elongatae, flavo-fusca; cellula V parva. Cellula insertionis atra. Appendix externa elata semel ramosa, appendix interna brevis in plurima antheridia desinens. Perithecium ovatum semiliberum, fuliginosum. Parasitus Platynus (Dyscolus) *(Coleoptera, Carabidae, Agonini). Typus EA02/33 (coll. Arndt, Anhalt University).*

Description: Mature thallus slender, from foot to perithecial tip 500-650µm. Thallus pale olive-brown, perithecium dark brown, preostiolar spots black, the posterior spot occupying most of the respective lips. Insertion cell black, opaque; both appendages of pale colour.

Cells I-IV very slender, each 2.5-3.8 times longer than wide; cell V narrow oval, about half as long as cell IV. Septum IV-V straight to slightly curved. Insertion cell slightly constricting the posterior margin of thallus, situated towards the middle of posterior margin of perithecial wall, but separated from it.

Basal cells of appendages of similar size. Outer appendage consisting of two branches arising from the suprabasal cell, exceeding the perithecial apex, up to 320µm long. Inner appendage divided twice, rarely more above the basal cell, with antheridia at the tip of branches (Fig. 4), forming sterile branches exceeding the perithecial apex in mature thalli (Fig. 3). Cell VI more than twice as long as wide, trapezoid in section; cell VII longer than wide, square to trapezoid.

Perithecium relatively narrow, at the level of insertion cell 64-80µm wide, adnate to cells IV and V for 2/3 of its length. Perithecium is about 1/3 of length of the thallus. Apex asymmetrical, with prominent rounded posterior lips. Ascospores 81-90µm.

Holotype: on *Platynus* (*Dyscolus*) *chathami* (Van Dyke), female from San Joaquin near the top of Isla San Cristobal (A92/38, 700m, 20.02.-01.03.92, collection number EA02/33).

Paratypes: same host species, several infested specimens from same date and locality; same host species, several infested specimens from Isla San Cristobal, same date (A92/30, 530m, EA02/31-32, EA02/72-74). *Platynus* (*Dyscolus*) *darwini* (Van Dyke), several specimens, Isla San Cristobal, San Joaquin, same date (A92/38, 700m, EA02/19-25).

Remarks. *L. sanjoaquina* is similar to *L. collae* Majewski from a platynine host in Europe and the group of *L. pseudomasei* Thaxter and related species from pterostichine hosts. *L. collae* has a smaller inner appendage basal cell and an insertion cell adnate to the side of the perithecium in contrast to *L. sanjoaquina* with basal appendage cells of similar size and an insertion cell which is separated from the perithecium. *L. pseudomasei* has a smaller inner appendage basal cell and phialides occurring laterally along the appendage branches, rather than forming terminal clusters as in *L. sanjoaquina*.

Laboulbenia galapagoensis sp. nov.

(Figs 5, 6)

Dioecious. Mas. Hyalinus, elongatus, gracilis, e 6 cellulis confectus, 32-40µm longus. Receptaculum cellularum superpositarum constans. Antheridium unum simplex apicale.

Femina. Thallus 176-250 µm longus, melleo pro parte maxima. Cellula I divisa aut duplo-triplo longior quam latior; II sesqui longior quam latior; III obovata; cellula IV cellulam V amplitudine parum superat. Perithecium circiter duplo-triplo longius quam latius. Cellula insertionis atra. Appendices perithecio breviores, fuscis septis separatae. Parasitus Tachys *(Coleoptera, Carabidae, Tachyini). Holotypus EA02/26 (coll. Arndt, Anhalt University).*

Description: Presumably dioecious. Male and female thalli often occurring in pair, attached at their respective feet (Fig. 5). Both thalli including appendages pale yellowish except the black, opaque insertion cell and black preostiolar spots, the latter more or less merging with the paler colour of the perithecal walls by preapical shading.

Male thallus consisting of one row of apparently six cells, 32-40µm or slightly longer than cell I of female thallus. Basal cell extends into the opaque region of foot, cells 2 and 3 longer than wide and longer than following cells; cell 4 wider than long, cells 5, 6 cap-shaped, septa between cells 4-6 black, ultimate cell (presumed antheridium) triangular to flask-shaped.

Mature presumed female thallus comparably short and stout, from foot to perithecial tip 176-250µm long, only in rare cases more slender with a length up to 296µm.

Cell I often (=60% of examined specimens) divided in two small cells; cells I (if undivided) and II of similar length, cell II more or less wedge-shaped; cell III large, rounded, less than 1.5 times longer than wide, much larger than cell IV; cell V comparably large, not much smaller than cell IV; septum IV-V more or less straight, not turned inward. Insertion cell thin, at posterior margin of thallus strongly constricted; insertion cell attached to the base of perithecium.

Basal cells of appendages of similar size, outer basal cell only slightly larger. Both appendages of similar structure, dividing into several branchlets from suprabasal and following cells above; branches robust, short, with a maximum length of 90µm, not exceeding the middle third of the perithecium. Cells of basal and mesal parts of the branches with black septa (Figs 5, 6).

Perithecium relatively large, about half of thallus length. Perithecium with maximum width of 50-65µm. Apex nearly symmetrical with prominent rounded posterior lips. Cell VI about as long as wide, cell VII wider than long.

Holotype: on *Tachys vittiger* LeConte, female from Isla Santa Cruz (B91/890, 0m, 28.-29.05.91, collection number EA02/26).

Paratypes: same host species, date and locality (EA02/28-29, 39-40); Isla Rabida (P92/90, 2m, 03.03.92, EA02/36-38); Isla Isabela, Volcan Sierra Negra (B91/884, 2m, 26.05.91, EA02/63).*Tachys erwini* Reichardt, Isla Fernandina (P91/115, 5m, 03.05.91, EA02/17-18); Isla Pinta (P92/55, 2m, 19.03.92,

EA02/39). *Tachys* nov. sp. 1, Isla San Cristobal (P96/27, 2m, 16.03.96, EA 02/62). *Tachys* nov. sp. 2, Isla Isabela, Volcan Alcedo (P96/77, 2m, 01.-03.04.96, EA02/27).

Remarks. *L. galapagoensis* belongs to the *Laboulbenia inflata*-group (see below). The shape of the appendages and the character combination [small cell IV+ pale colour +often divided cell I] distinguish it from all other species known to us.

All known *Tachys* species from Galápagos are infected by this *Laboulbenia* species, suggesting coevolution at the level of host genus. All recorded hosts are littoral species from mangrove and salt marshes. *Tachys* nov. sp. 2 has a more or less marine way of life. The biology of this species (as far as it is known) resembles that of *Aepopsis robini* (Laboulbène), which is infested by *Laboulbenia marina* Picard, a representative of the same species group.

One of the host species of *L. galapagoensis*, *Tachys vittiger,* is also distributed on the mainland. It is known from the coastal region of Ecuador, and from Guatemala northwards up to British Columbia (Canada). We found one infested *T. vittiger* with one specimen of *Laboulbenia* from mainland Ecuador (Prov. Guayas, Bahia, Agangue, 18-30.VIII.1964, N. & J. Leleup leg.). However, the fungus is in poor condition and cannot be determined beyond doubt. It is most similar to the widespread *Laboulbenia asperata* Thaxter.

Laboulbenia appendiculata **sp. nov.**
(Figs 7, 8)

Dioecious. Mas. Hyalinus, elongatus, gracilis, e 10 cellulis confectus, 70-85 µm longus. Receptaculum cellularum superpositarum constans. Antheridium unum simplex apicale.

Femina. Thallus 178-220 µm longus, melleo colore pro parte maxima. Cellula I duplo-triplo longior quam latior; II paulo longior quam latior; cellula III cellulam IV amplitudine adaequat, cellulam V superat. Perithecium ovatum semiliberum, circiter sesqui-duplo longius quam latius. Cellula insertionis atra. Appendices longior quam perithecii, cum septa e basi nigrum. Parasitus Bradycellus insularis *(Coleoptera, Carabidae, Harpalini). Holotypus EA02/65 (coll. Arndt, Anhalt University).*

Description: Presumably dioecious. Male and presumed female thalli often occurring in pairs, attached at their respective feet (Fig. 7). Both thalli pale yellowish; only preostiolar spots, insertion cell and basal part of first cells of appendages dark brown to black.

Male thallus slender, consisting of one row of 10 cells of different length, from foot to apex 70-85µm long. The basal cell longer than following cells; cells of middle and apical part with dark septa constricting the male thallus; apical cell (presumed antheridium) slender and flask-shaped.

Mature presumed female thallus relatively short and stout, from foot to perithecial tip 178-220µm. Cell I two to three times longer than wide; cell II slightly longer than wide, widened upward; cells III and IV of similar size, about two times longer than wide; cell V more than half as long as cell IV, septum IV-V more or less straight, slightly oblique. Insertion cell thick, opaque, the posterior margin of thallus strongly constricting; insertion cell separated from the perithecial wall, situated above the middle of perithecium.

Inner and outer appendages of similar structure, quite robust. Basal cell of outer appendage two times longer and slightly wider than basal cell of inner appendage. All examined specimens with three branchlets at least in the juvenile specimens; outer appendage consisting of two branches arising from the basal cell; inner appendage mostly simple. First three or four cells of appendages with blackish septa and basal region partly darkened. Often new branches arising within deteriorating walls of old branches (Figs 7, 8). All branchlets exceeding the tip of the perithecium, branchlets of inner appendage with maximum length of 140µm, branchlets of outer appendage with maximum length of 180µm.

Cell VI wider than long, triangular to rhomboidal in optical section. Perithecium large, about half of thallus length. Perithecium with maximum width of 48-56µm. Apex nearly symmetrical with prominent rounded lips.

Holotype: on *Bradycellus insularis* Reichardt, male from Isla Pinzon (P91/255, 380m, 27.06.91, collection number EA02/65).

One juvenile (same host, same collection number) and three further adult specimens from another *Bradycellus insularis*, female, same date and locality (EA02/68).

Remarks. *L. appendiculata* belongs to the *Laboulbenia inflata*-group. Besides *L. inflata,* this is the only known species of the group infesting Stenolophini. It is, however, clearly different from *L. inflata* because of its pale colour, large cell V, broadly rounded perithecium with narrow apex, and cell VI that is wider than tall. Also it is paired with a long, thin supposed male thallus (Fig. 7). Appendages may arise in older branches and resemble those of *L. marina* Picard. However, they are much longer in *L. appendiculata*. *L. appendiculata* could be more closely related to *L. asperata*, a species of Tachyini/Trechini, than to *L. inflata* (see SANTAMARIA 1999).

Laboulbenia inflata **Thaxter**

Remarks. The specimens of *L. inflata* from the Galápagos Archipelago are very similar to specimens from North America and Western Europe described e.g. by SANTAMARIA (1998).

Taxonomic characters, phylogenetic relationships and identification key of the presumable dioecious *Laboulbenia inflata* species group

Dioecism is an extreme exception in the genus *Laboulbenia* Montagne & Robin. It was first described for *L. formicarum* Thaxter, an ant-infesting species from North America (BENJAMIN & SHANOR 1950a, b). This is one of the smallest and most peculiar species of *Laboulbenia*. With a total length of only 70-80µm, it has a comparably large perithecium, which is longer than the remaining part of the female thallus. The appendages are about as long as the rest of the female thallus; the outer appendage is simple, the inner one consists of two branches. The first three cells of both appendages show constricted, dark septa. The male thallus is similar in structure to the female thallus in *L. formicarum*.

More recently, *L. asperata* Thaxter, *L. inflata* Thaxter, and *L. marina* Picard have been shown to be dioecious (SANTAMARIA 1996, 1998, 1999). Dioecism was not described explicitly for *L. sbordonii* W. Rossi & Cesari. However, its characters and the

illustrated thalli (Rossi & Cesari Rossi 1977) leave little doubt that *L. sbordonii* belongs to this group. The new species *L. galapagoensis* and *L. appendiculata* from Galápagos are probably dioecious species because of the characters shared with *L. asperata* and *L. inflata*. All these *Laboulbenia* species are parasites of Carabidae.

A perithecium-bearing thallus paired with a small filiform thallus was also described for *L. lecoareri* (Balazuc) Huldén. However, the structure of these thalli (Balazuc 1974: 305) differs from those of the above-mentioned species group by the extreme reduction in size of the basal cell of the inner appendage, the branches of which are extremely small or even absent. Most probably the other seven species form a monophyletic group (*L. inflata*-group) within the large genus *Laboulbenia*. We regard dioecism as a synapomorphic character of this group. A second autapomorphic character of the group is the organisation of the male thallus, which differs strictly from that of *L. formicarum*. The male thallus consists of a simple series of superposed cells, similar to the male thalli of some other dioecious genera of Laboulbeniales (Benjamin 1995, Santamaria 1996). The preapical cells have black basal septa. Male and female thalli occur together with their feet attached to the host side-by-side. A further apomorphic character of the *L. inflata*-group is the black basal septa of the lower cells of their appendages. Simple long appendages or two simple branches respectively, as in *L. inflata,* are the supposed plesiomorphic state of appendages. The two new species from Galápagos as well as *L. marina* from a marine carabid species have derived appendages. The host of *L. marina* and the hosts of the new species from Galápagos live in coastal habitats.

The *L. inflata*-group may have been derived from a monoecious ancestor with similar appendages and upper receptacle. *Laboulbenia tachyis* Thaxter might appear to be such a species; however, no evidence has been published that antheridia are borne on the perithecial thallus in *L. tachyis*.

Identification key to the species of the *Laboulbenia inflata-group*

1 Appendages of mature female thalli much shorter than perithecium. Hosts in littoral habitats 2

- Appendages exceeding the perithecium distinctly 3

2 Outer appendage simple, very wide. Cell V equal to or larger than cell IV, cells III and VI subequal in size. Male thallus consisting of 6 cells. Host: *Aepopsis robini* (Laboulbène) (subfamily Trechinae). .....*L. marina* Picard

- Outer appendage consisting of more than one branch arising from the second or third cell (Figs 5, 6). Cell V not larger than cell IV, cell III much larger than cells IV-VI. Male thallus consists of 6 cells. Host: *Tachys* Stephens (subfamily Trechinae) .....*L. galapagoensis* sp. nov.

3 Insertion cell transparent, reddish and outer basal wall cells of perithecium roughened. Male thallus consists of 5 cells. Host: *Tachys* Stephens (subfamily Trechinae).....*L. asperata* Thaxter

- Insertion cell dark, opaque and/or outer basal wall cells smooth.....4

4 Appendages long and filiform, as long as female thallus or longer. Cells of appendages pale, not darkened in basal part. Male thallus not longer than cell I of female, with less than 10 cells 5

- Appendages robust, shorter than female thallus with basally darkened cells in basal part of appendages (Figs 7, 8). Male thallus longer than cell I+II of female thallus, consisting of 10 cells. Host: *Bradycellus* Erichson.....*L. appendiculata* sp. nov.

5 Female thalli unicoloured pale, slender, total length 280-463µm. Host: *Mexaphaenops intermedius* Barr, restricted to Central America (subfamily Trechinae).....*L. sbordonii* W. Rossi & Cesari

- Female thalli bicoloured, perithecium brown, basal part pale, less slender, maximum length 266µm, average length 220µm. Male thallus consists of 6-7 cells. Hosts: *Bradycellus* Erichson, *Acupalpus* Latreille, *Stenolophus* Latreille (Stenolophini, subfamily Harpalinae) .....*L. inflata* Thaxter

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Effects of forest fragmentation and local habitat structure on densities of winter moth (*Operophtera brumata* L.)

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ABSTRACT. We study the effects of habitat fragmentation (habitat area, isolation, distance from forest edge) and local habitat structure (size and phenology of host tree and density of herb cover) on winter moth densities in oak forests near Antwerp, N. Belgium, focussing on both effects simultaneously and comparing males and females. In this way, we can study how adult dispersal strategies – active male flight vs. flightless females – affect the distribution of individuals. The analyses show that both the degree of fragmentation and local habitat structure affect moth densities and that the effects differ between males and females. Failing to correct for sex-differences and for various aspects of local habitat structure would have lead to the conclusion that there were no effects of fragmentation on winter moth densities. Thus, structured populations in which dispersal rates vary between individuals need careful evaluation of the effects of fragmentation, separately for the different groups of individuals.

KEY WORDS: Habitat Fragmentation, Density, winter moth, dispersal, local adaptation.

INTRODUCTION

Habitat fragmentation – the process that reduces the area of natural habitats and thereby increases distances between suitable patches – is increasingly endangering many organisms and is one of the major threats to global biodiversity (e.g. SAUNDERS et al., 1991; STEFFAN-DEWENTER & TSCHARNTKE, 2000). Not surprisingly, habitat fragmentation has been a central theme in conservation biology since the field began (HARRISON & BRUNA, 1999). In spite of a very large body of literature studying the various effects of fragmentation, there are many obstacles relating to the design and replication of experiments that hamper the development of a solid general framework (HARRISON & BRUNA, 1999). Nevertheless, in their recent review HARRISON & BRUNA (1999) identified a number of general patterns. First, fragmented habitats often appear to be of impoverished quality, and second, physical and biological edge effects appear to be predominant in forest habitats as opposed to non-forest areas. Edge effects may render large natural habitats of hundreds of hectares equivalent to virtually all edge. These conclusions stand in sharp contrast to many theoretical models, which put a lot of emphasis on the importance of dispersal among fragments (HARRISON & BRUNA, 1999).

In addition to these large scale fragmentation effects, subtle features of local habitat structure, such as habitat suitability or nutritional quality, can also influence the distribution of organisms in the landscape. Furthermore, behavioural traits, such as dispersal and habitat selection, need to be taken into account to understand variation in densities at any geographical scale. Finally, the scale of sampling and inference is of crucial importance (DIDHAM, 1997). Fragmentation takes place at the level of habitat patches, while many processes affecting densities and species distributions occur at a much smaller scale (e.g. host individuals). Therefore, local processes should be taken into account when studying regional patterns.

In this paper we investigate the distribution of winter moths (*Operophtera brumata* L.) in a highly fragmented landscape. We determine densities on individual host trees (pedunculate oak, *Quercus robur* L.) and study the effects of individual tree characteristics and their surrounding habitat, as well as the effects of habitat area and degree of isolation of the different woodlots. The winter moth is an interesting model system to investigate the effects of frag-

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mentation and host characteristics for several reasons. First, winter moths show phenological adaptation to individual hosts within oak stands (VAN DONGEN et al., 1997). Therefore, local tree and habitat characteristics are likely to affect the distribution of this species. This is likely to occur at the level of the caterpillars, which feed upon the host and are affected directly by the degree of synchrony between egg hatching and leaf flush. Second, dispersal abilities differ markedly between adults and larvae, as well as between adult males and females. Adult females show almost no dispersal, which could predispose the species to be vulnerable to the effects of fragmentation. Nevertheless, larval wind dispersal can occur over several kilometres and could dilute effects. In addition effects on males and females could differ since males show active flight up to several hundreds of meters. These variations in dispersal abilities render the winter moth an interesting model species to study the effects of fragmentation at different geographic scales. We show that, unless the associations between densities on the one hand and both the effects of forest fragmentation and local habitat structure on the other hand are modelled carefully for males and females separately, patterns may be overlooked.

MATERIAL AND METHODS

Study species and density estimation

The winter moth is a univoltine moth species with adults active mainly in November in Northern Belgium. Adults emerge after pupation in the soil with the winged males flying towards trees while the brachypterous non-flying females crawl towards the nearest vertical object. Shortly after dusk, females emit pheromones to attract males and copulation takes place on the tree trunk. Adults crawl into the canopy where they lay eggs in bark crevices and on lichens. Males return to the ground and hide until the next evening. Eggs overwinter and hatch in early spring, in synchrony with host budburst. This synchrony has important fitness consequences, as early hatching results in starvation or wind dispersal of first instar caterpillars, while late hatching presents developing larvae with leaves of lower quality. We studied winter moths in oak forests, the primary host of this species. Within each forest patch, individual oaks show high variation in budburst dates, a pattern that is consistent across years (CRAWLEY & AKTERUZAMANN, 1988; VAN DONGEN, 1997; VAN DONGEN et al., 1997; and unpublished results). Consequently, optimal hatching dates for caterpillars vary on a very small geographical scale, resulting in some degree of local adaptation to the phenology of individual oak trees (VAN DONGEN et al., 1997).

Winter moth densities were determined in November 2000. We chose 12 oak forest patches that differed in size and degree of isolation. Degree of isolation was defined as the distance to a forest larger than 10 ha (VAN DONGEN et al., 1994, 1998). Forests larger than 10 ha were automatically assigned a degree of isolation of zero. In each area, five to15 trees were randomly selected four weeks prior to density determination. Afterwards, for each tree its size (trunk diameter at breast height and canopy radius, i.e. average distance from the trunk to the edge of the canopy), distance from the forest edge (in meters), budburst date (date when 50% of the buds had burst open with small leaves occasionally becoming visible; VAN DONGEN et al., 1997) and degree of herb-cover (proportion of the soil covered by small herbaceous plants; VAN DONGEN et al., 1994) were determined. Because trunk diameter and canopy radius were highly correlated, both were combined in a single measure of tree size. This measure was calculated as the average of trunk diameter and canopy radius after standardisation (i.e. subtraction of the mean, and division by the standard deviation). Thus, this tree size measure will have mean zero.

Each forest fragment was visited seven times between the 8th of November and the 12th of December, from 18.00-20.00 hours (the peak of adult activity). The numbers of male and female winter moths were counted by inspecting each tree trunk up to a height of 2.5 meters for about 1 minute. Details of the 12 forest fragments and the number of trees and visits are given in Table 1. The sequence of visits to the different forest fragments was randomised to avoid confounding the data with temporal variation in densities.

Statistical analysis

Because forest fragments larger than 10 ha were assigned a degree of isolation of zero, isolation and surface area were correlated (r_s=-0.72, p=0.008). Effects of area and isolation are therefore difficult to disentangle statistically. To avoid multicolinearity problems in our analyses, we grouped the areas into three categories (further called isol-area): LARGE fragments greater than 10 ha, SMALL-CLOSE fragments smaller than 10 ha with a degree of isolation less than 300 meters, and SMALL-DISTANT fragments of which the degree of isolation was higher (Table 1, Fig. 1). This choice was arbitrarily made ensuring a reasonably balanced dataset – three, four and five forest fragments in each category respectively (Table 1) – and the criteria were not changed during the analyses.

Prior to the analysis of winter moth densities, we compared the degree of herb cover, the budburst date and tree size between the three area-classes (isol-area) and among the different fragments using a two-way mixed model nested ANOVA (fragment nested within isol-area). In addition, correlations between these three characteristics were investigated (after correction for plot effects) to avoid multicolinearity problems in the linear model described below. Next, male and female densities were analysed in relation to fragment-specific and tree-specific explanatory variables in a mixed-effects linear model. Male and female densities were calculated for each tree by averaging the counts over the seven visits, and were

TABLE 1

Summary of forest fragment characteristics and the number of trees that were sampled. Fragments were grouped into three isol-area classes reflecting a combined effect of surface area and isolation (see text for details).

Area code	area (ha)	isolation (m)	# trees	Isol-area
KB	12	0	15	large
LO	17.6	0	15	large
ZZ	16.7	0	15	large
KL	1.8	90	8	small-close
LW	1.0	200	8	small-close
ZW	0.4	200	7	small-close
ZN	1.7	100	8	small-close
LS	0.5	900	8	small-distant
VS	1.7	350	8	small-distant
HM	0.8	400	5	small-distant
HN	3.2	450	9	small-distant
LI	0.7	1150	7	small-distant

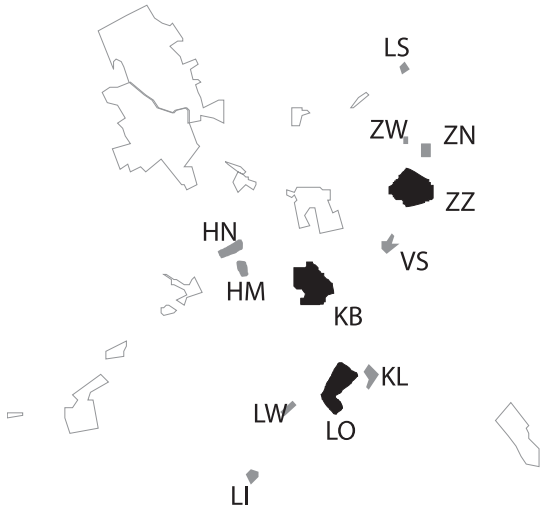

Fig. 1. – Map of forest patches in the study area. Fragments larger than 10 ha are indicated in black (i.e. Isol-area=LARGE), smaller areas located nearby a larger plot are indicated in dark grey (i.e. Isol-area=SMALL-CLOSE), and small and more isolated plots are indicated in light grey (i.e. Isol-area=SMALL-DISTANT). White forest fragments were not monitored.

log-transformed (log(density+0.1)) to achieve approximate normality (as tested by the Shapiro-Wilks test, see results section). As we aimed to compare the effects of the explanatory variables between both sexes – i.e. to measure the impact of the different dispersal behaviour of males and females on their distribution – male and female density was analysed in a single model and interactions with sex were tested. A significant interaction of a factor with sex indicates that the effect of this explanatory variable is different for males and females. However, considering male and female densities simultaneously complicates the method since repeated measures on individual trees are analysed. We corrected for this statistical dependency by adding tree as a random effect to our model. Furthermore, fragment is also added as a random effect to account for the fact that data from individual trees within a forest fragment do not represent independent data for tests of fragment-specific effects (i.e. the Isol-area effect). Degrees of freedom of the fixed effect were approximated using Satterthwaite's procedure (VERBEKE & MOLENBERGHS, 2000). Effects of individual explanatory variables are visualised using residual plots. Additionally, for comparison, the effect isol-area was tested ignoring all tree-specific variables using a one-way ANOVA model.

RESULTS

The one-way ANOVA model did not show a significant isol-area effect ($F_{2,112}$=1.81, p=0.17). Before starting with the more elaborate mixed model analysis of winter moth densities, we first compared variation in herb cover, tree size and tree budburst among forest fragments. Neither herb cover, nor tree size nor budburst date differed between the three area-classes (Table 2). Only herb cover showed significant variation among fragments within area-classes (Table 2). In addition, a plot of the mean values and standard deviations (Fig. 2) suggests that most variability can be found within the individual forest fragments. Herb cover, tree size and budburst date were not significantly correlated (all p>0.05). These three explana-

TABLE 2

Two-way nested ANOVA's models analysing variation in herb cover, tree size and tree budburst between the three groups of fragments (for details see Table 1) and among fragments nested within these three groups. The fixed isol-area effect was tested using a traditional F-test, while the random nested fragment effect was tested using a likelihood ratio test. Among fragment variation is given as a variance component with the residual variance between brackets.

Variable	isol-area	fragment (nested within isol-area)
Herb cover	$F_{2,9}$=0.34, p=0.72	σ^2=0.036 ($\sigma^2_{residual}$=0.07) (χ^2_1=19.3, p<0.0001)
Tree size	$F_{2,9}$=1.61, p=0.25	σ^2=0.10 ($\sigma^2_{residual}$=0.79) (χ^2_1=2.5, p=0.11)
Tree budburst	$F_{2,9}$=2.61, p=0.13	σ^2=0.78 ($\sigma^2_{residual}$=58) (χ^2_1=0.07, p=0.80)

tory variables can therefore be added simultaneously in a statistical model without causing multicolinearity problems.

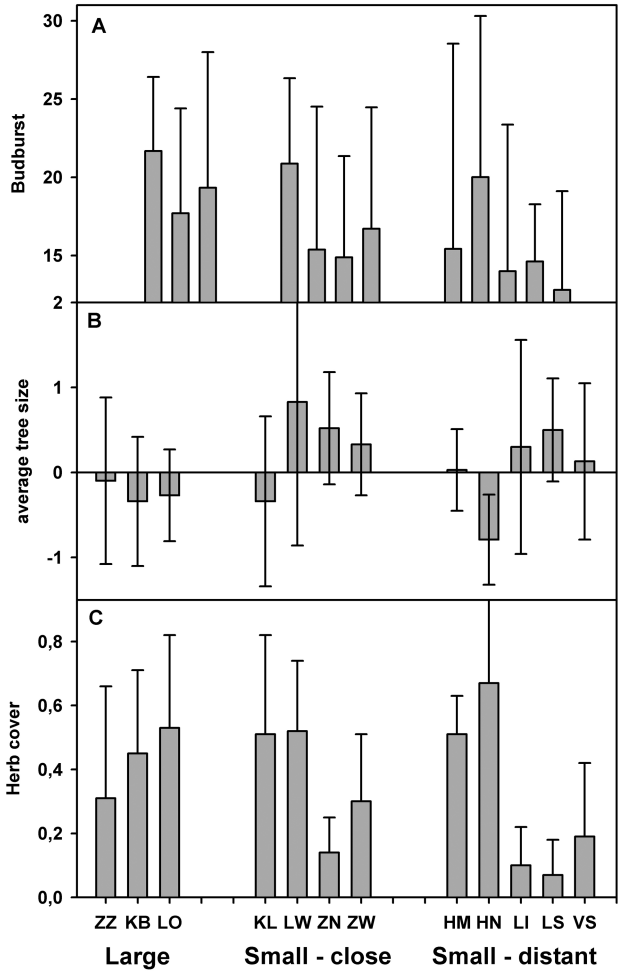

Fig. 2. – Between fragment variation in average budburst (A), tree size (B) and degree of herb cover (C). Error bars indicate standard deviations of the fragment-specific distributions.

Table 3 summarises the significance tests of the linear model relating winter moth densities to the different explanatory variables of interest. Graphical representations of the different associations are given in Figs 3 and 4. With the exception of tree size, which showed a positive association with density (Fig. 3), the effects of all other explanatory variables differed between the two sexes (Table 3). Densities decreased with date of budburst for females (t_{107}=-2.76, p=0.007) but not for males (t_{107}=-0.70, p=0.49) (Fig. 3). Winter moth densities decreased with distance from the forest edge for both sexes, but the effect was significantly stronger for females as shown by the significant sex×edge interaction (Table 3) (females: t_{107}=-4.74, p<0.0001; males t_{107}=-2.69, p=0.008; Fig. 3). It is also worth noting that graphical inspection of this relationship revealed that the edge effect was only present in large areas because variation in distances to the edge were much smaller in the small fragments (Fig. 3). Densities decreased with herb cover, but only significantly so in males (females: t_{107}=-0.94, p=0.34; males: t_{107}=-3.18, p=0.002). Finally, the effect of isol-area differed between males and females as well (Table 3, Fig. 4). For males, there was only a significant difference between large and small-distant fragments, while for females all small fragments showed a lower density relative to the larger ones (Fig. 4).

TABLE 3

Summary of tests of the fixed effects relating winter moth density to a suite of explanatory variables (significant results in bold).

Source	F-value	num. d.f.	den. d.f.	p-value
Sex	**63.0**	**1**	**107**	**<0.0001**
Herb-cover	**5.27**	**1**	**102**	**0.02**
Tree size	**17.4**	**1**	**98**	**<0.0001**
Budburst	**3.95**	**1**	**98**	**0.05**
Edge	**17.6**	**1**	**99**	**<0.0001**
Isol-area	3.92	2	9	0.06
Herb-cover×sex	**6.43**	**1**	**107**	**0.01**
Budburst×sex	**4.38**	**1**	**107**	**0.04**
Edge×sex	**4.90**	**1**	**107**	**0.03**
Isol-area×sex	**6.30**	**2**	**107**	**0.003**
Tree size×sex	2.43	1	106	0.12
Edge×isol-area	0.90	2	104	0.41
Edge×isol-area×sex	1.05	2	102	0.35

Besides the variability in winter moth densities among trees and forest fragments, there was still a significant amount of unexplained variation left. The random fragment effect explained 57% of the total variation (σ^2=0.62, Z=1.97, p=0.02) while the random tree effect explained 20% (σ^2=0.22, Z=4.27, p<0.0001). The amount of unexplained residual variance after correction for these fragment and tree effects was 0.25. The residual values of this full model were approximately normally distributed (Shapiro Wilks' W=0.97).

DISCUSSION

Our study shows that both habitat fragmentation and local habitat structure affect winter moth densities. In several cases, these effects appear to differ between males and females. The differences in dispersal abilities through the habitat are likely to explain the observed differences in associations. In contrast to the flightless females, males show active flight. Effects of local habitat structure that affect larval distribution could therefore persist in females but disappear in males. For example, the fact that the edge-effects as well as the effect of tree budburst were stronger for females could be due to the fact that the factors determining densities operate at the larval stage, which is still reflected in the distribution of females, but only to a lesser extent in the flying males. The fact that the association between herb cover and density is only significant for males could indicate that this factor did not affect larval densities and therefore did

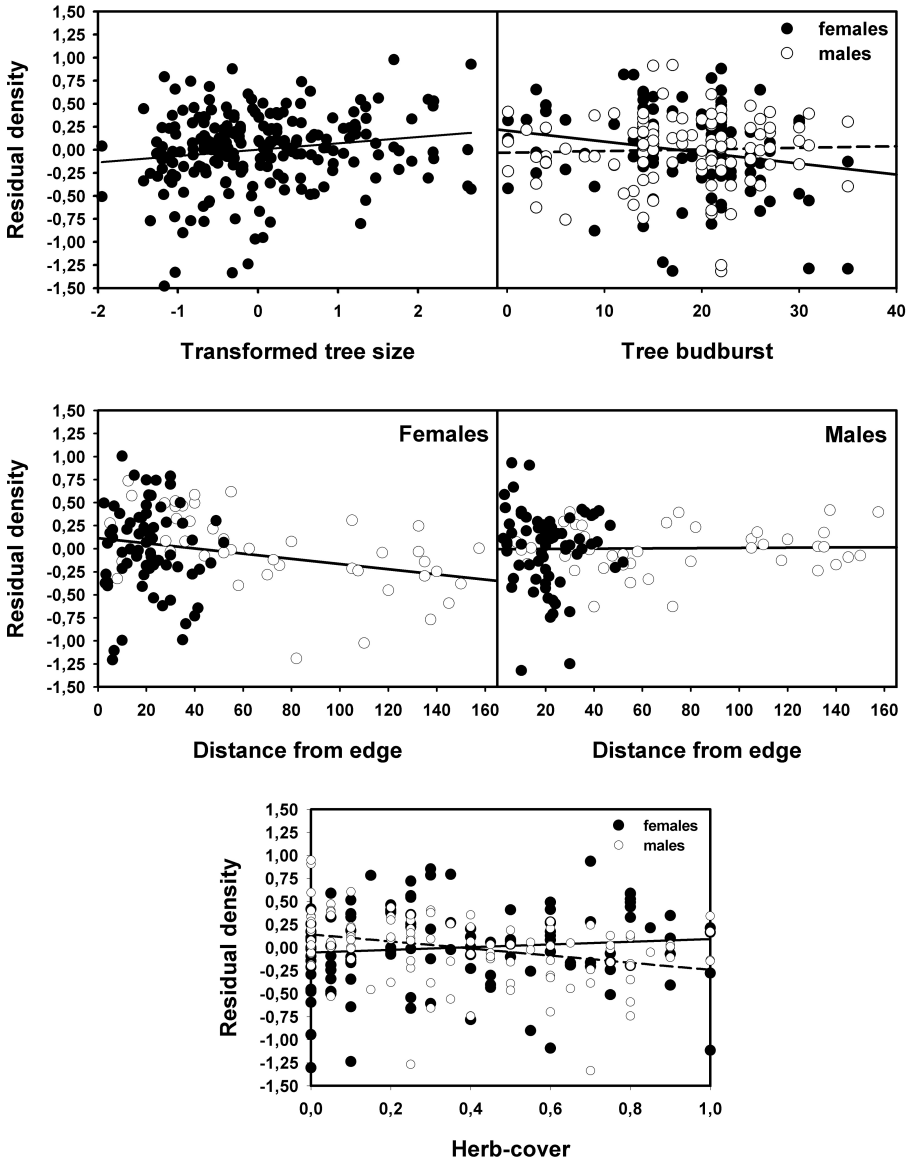

Fig. 3. – Relationship between winter moth density and tree size (A), tree budburst (B), distance from the edge (C1 & C2) and herb-cover (D). For explanatory variables that showed a significant interaction with sex, plots were constructed to highlight the importance of the interaction effect by splitting data by sex (females: solid line; males dashed line). For the association between density and distance from the edge, solid symbols represent data from small fragments, and open symbols observations from large fragments.

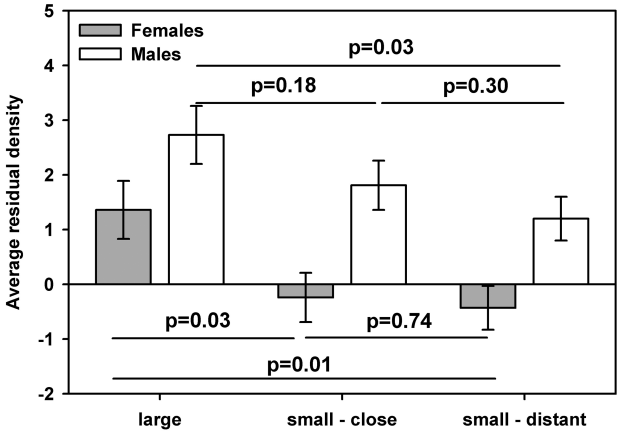

Fig. 4. – Differences in male and female winter moth densities between area-classes. P-values are corrected for multiple comparisons using Tukey's method.

not affect the distribution of females. We can only speculate about why only male densities are lower with denser herb cover. Possibly, the lower male densities on those trees are the result of the fact that males also use the herb plants as substrate to wait for females. This behaviour has been observed (data not shown), but was not quantified because of practical difficulties in assigning particular herb plants to the area around a tree. Finally, the effects of fragmentation also appeared to differ between males and females. For females, densities were significantly lower in smaller forest fragments, irrespective of their degree of isolation. In males, densities were only lower in the more remote fragments. Thus, we provide evidence that both surface area and isolation affect densities, but that male dispersal could help to prevent or lower these effects in fragments located close to larger forests. Winter moth males do not appear to fly over

distances large enough to reach other areas when measured within a forest (VAN DONGEN et al., 1996). However, dispersal behaviour in open areas has never been studied in detail. In addition, hedgerows and linear tree-rows could act as corridors increasing connectivity, but their importance has never been investigated.

What mechanistic processes lie beneath the effects of surface area, isolation and distance from the edge are difficult to determine and require an experimental approach. With regard to the positive edge effect, it has been argued that this could be due to the larger sizes of host trees at the edge of forests (MURCIA, 1995). However, we still find this edge effect after controlling for the effect of tree size. In more isolated fragments, winter moths have been shown to exhibit lower degrees of genetic variability and lower fecundity (VAN DONGEN et al., 1994, 1998) which could have led to lower densities. In addition, it has been hypothesised that in more isolated forest fragments, winter moths would have problems synchronising their egg hatching with oak budburst (VAN DONGEN et al., 1997), which could further reduce fitness and densities. Since synchrony appears to be a major factor determining mortality of caterpillars and fecundity, we suggest that minute investigation of synchrony and of its spatial and temporal variation could lead to better insights into how fragmentation affects the population structure of the winter moth (VAN DONGEN, 1997).

Winter moth densities are affected by both regional fragmentation as well as local processes (habitat structure). The scale of sampling and analysis is very important in such situations. When all tree-specific variables were ignored, the effect of fragmentation was insignificant. Thus, controlling for various other effects at a local scale increases the statistical power and accuracy of pattern detection at a regional scale. In spite of the fact that several covariates were included in the model, there was still a large amount of unexplained variation in densities at both the level of the tree and of the habitat patches.

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SHORT NOTES

The invasive occurrence of the Mediterranean dwarfspider *Diplocephalus graecus* (O.-P. Cambridge, 1872) in Belgium (Araneae: Linyphiidae)

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KEY WORDS: *Diplocephalus graecus*, Araneae, first occurrence, Mediterranean species, coastal dunes, climate change, invasion, expansion

Diplocephalus graecus (O.-P. Cambridge, 1872) is known as one of the most common erigonid spiders of the European Mediterranean region (9). Until now the species has been recorded from Northern Africa (Algeria, Morocco, Tunisia; 9, 10, 16, 17, 29, 21), Southern (France, Italy, Spain, Greece; 11, 12, 13, 14, 15, 16, 18, 19, 20, 22) and Eastern Europe (Romania; 18), reaching its northern limit near Paris (17, 20). The recent captures in Belgium indicate that its range is expanding further to the north.

Males measure 1.5 to 1.9 mm, females are a little larger: 1.8 to 2.2 mm. *D. graecus* is a brownish species with a dark grey abdomen; chelicerae and legs are yellowish to orange-brown and the sternum is of a darker brown. The placements of the trichobotrium and spines on the legs are characteristic for the genus *Diplocephalus*. As in most members of the Erigoninae subfamily, males of this species are easily distinguished by the shape of the male cephalic tubercle (Fig. 1A). Certainty about identification can be gained by checking the shape of the male palpal tibia, which lacks typical apophyses, in contrast to other European members of the genus (Fig.1B). The epigyne of the females has a typical wide median fissure with median constriction (Fig.1C).

In the Mediterranean region, *D. graecus* occurs in a wide variety of man-made and man-influenced habitats such as gardens, pastures, arable fields and short mountain grasslands. The species is also found in more natural habitats such as maquis, rough grassland, lake-borders and saltmarshes (9, 16, 17).

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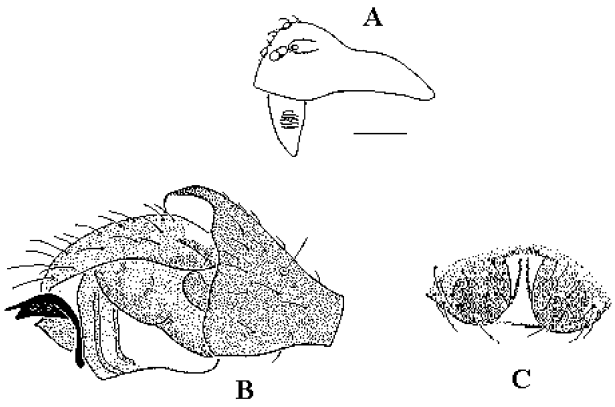

Fig. 1. – Male cephalothorax (A), palp structure (B) and epigyne (C) of *Diplocephalus graecus* (O.-P.-Cambridge) – scale line=0.2 mm (9).

Most individuals in Belgium were found in pitfall traps used for monitoring studies in the Flemish coastal dunes. Another specimen was captured with an arboreal eclector trap (3) in a recently installed forest reserve in the Province of East-Flanders. The first individuals were collected in the winter of 1999 in the Westhoek dune reserve at De Panne (18 males and two females in the period between September 10th, 1999 and February 6th, 2000) and in the fossil dunes of Ghyvelde-Adinkerke, on the French-Belgian Border (20 males in the period between October 12th, 1999 and February 6th, 2000). During this period, no individuals were collected in other sampled dune areas in Nieuwpoort and Oostduinkerke. Surprisingly, the following summer one male was found on a dead tree-trunk in an open Beech-forest (*Fagus sylvatica*) without substantial undergrowth (Kluisbergen). In October and November of 2000, *D. graecus* (three males) was for the first time found in the grey dune-area of the IJzer-estuary in Nieuwpoort, although this site has been monitored for terrestrial invertebrates since 1990 (2). During the same period, 15 males were captured in a dune slack of the Westhoek Nature Reserve. All individuals,

except for one, were captured in thermophilous, sandy habitats (dune slacks, grey dunes, short dune grasslands and recently cut scrubland with a large amount of bare sand). However, no other specimens were collected in other suitable habitats (heathland, inland dunes) sampled elsewhere in Belgium from early 1999 until October 2000 (8,12).

In Fig. 2 we visualise the captures of the species in Belgium. If we pool our data from the two years, it becomes clear that the species reaches its highest activity in the winter-period, although it can be present during summer and autumn (Fig. 3). For the Mediterranean region the same trend can be recognized: most individuals of *D. graecus* were collected during the period October-April (9). In general, summer records are very rare in the south (9,20), which makes our phenology data highly concordant with those from southern countries.

Our data show that the species expands its range northward along the coastline in an invasive way, a phenomenon also observed in other southern insect and arachnid species (4,5,6,7,21). Our observations and the fact that in southern regions *D. graecus* occurs in disturbed habitats, indicate that the species has a high dispersal power by way of ballooning. The milder winter-temperatures along the coast in comparison with those of inland habitats (1), probably enables the species to survive and spread in our region. Because of its assumed high dispersal capacities, the follow-up of its expansion should reveal interesting data on climate change and its effect on the spread of southern species.

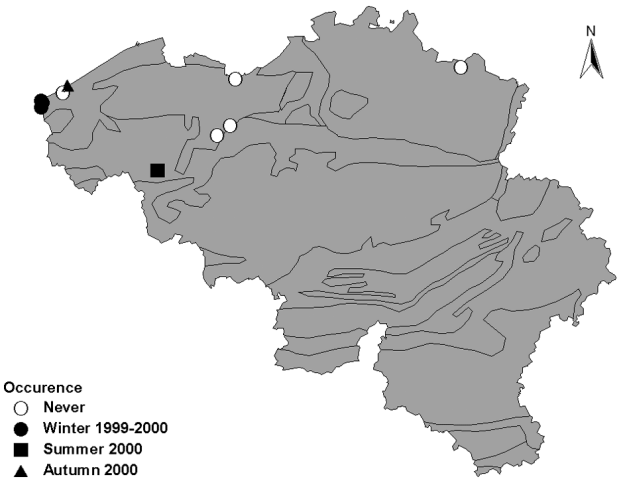

Fig. 2. – Captures of *Diplocephalus graecus* in Belgium in the sampled suitable habitats in the period 1999-2000.

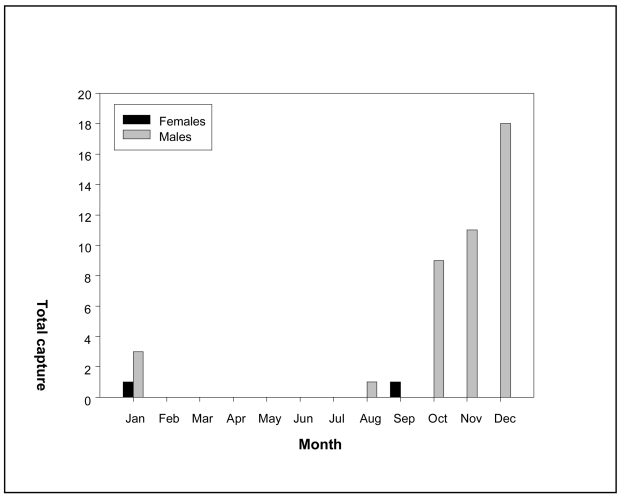

Fig. 3. – Phenology of *Diplocephalus graecus* in Belgium in the period 1999-2000.

We would like to thank Dr. R. Bosmans for checking the identification.

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A novel epithelial intramandibular gland in the ant *Pyramica membranifera* (Hymenoptera, Formicidae)

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Social insects in general, and ants in particular, are known for the overwhelming development of their exocrine system (3, 9). As all exocrine glands are ectodermal in origin, they are always in one way or another associated with cuticle, which has long made it difficult to study their occurrence and structural organisation. Glands found in close association with the rigid exoskeleton, and especially with its hardest parts such as the mandibles or antennae, sting or legs, have therefore often long remained unknown. The use of plastic embedding, however, has made it possible to section through the hardest cuticle, which has resulted in the discovery of several hitherto unknown glands (e.g. 2, 3, 4, 10, 11). Among these discoveries is also the intramandibular gland, which occurs as a common exocrine structure within the mandibles of all major ant subfamilies (14). It corresponds to the gland type with bicellular units (1), each unit consisting of a duct cell and a secretory cell (type 3 glandular cells according to the classification by NOIROT & QUENNEDEY: 12). During a histological investigation of the mandibles of the tiny dacetonine ant *Pyramica* (formerly *Trichoscapa*) *membranifera*, our attention was drawn to the occurrence of an intramandibular glandular epithelium, which we here describe, and which description represents the first report on the existence of an epithelial gland inside the mandibles in ants.

Pyramica membranifera (Emery, 1869) workers were collected in Sant Cugat (Barcelona), Spain. The anterior parts of their heads were fixed in 2% glutaraldehyde and postfixed in 2% osmium tetroxide (buffered at pH 7.3 with Na-cacodylate). After dehydration in a graded acetone series, they were embedded in araldite and sections made with a Reichert Ultracut E microtome. Semithin sections for light microscopy were stained with methylene blue and thionin; double-stained thin sections were viewed in a Zeiss EM900 electron microscope.

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The mandibles of workers of the small dacetonine ant *Pyramica membranifera* show at their inner distal margin a conspicuous glandular epithelium with a thickness of 10 µm (Fig. 1). The columnar epithelial cells display an obvious microvillar differentiation of the apical cell membrane and a basal position of the rounded nuclei (Fig. 2). The cytoplasm is characterized by the presence of numerous mitochondria, and an abundance of rounded electron-lucid vesicles with a diameter between 0.5 and 2 µm (Fig. 2). Endoplasmic reticulum could not be discerned, neither of the smooth nor of the granular type. The cuticle overlaying the epithelium is perforated by numerous vertical pore canals with a diameter of approx. 0.2 µm, that open at the external surface (Fig. 3).

Exocrine structures inside the mandibles of ants have been reported for many species (14), but these intramandibular glands invariably were formed by bicellular units (type 3 cells according to NOIROT & QUENNEDEY: 12) with duct cells opening at the upper mandibular surface. Epithelial glands (type 1 cells according to NOIROT & QUENNEDEY: 12) so far have never been found inside ant mandibles, and among social insects have only been described for some stingless bee species (5). The function of this novel gland in ants so far remains unknown. The numerous mitochondria and secretory vesicles are indicative of an active metabolism, while the apical microvilli and cuticular pores (13) would facilitate the discharge of the secretory products to the outside. One possible function for the secretion could be related to the predatory habits of Dacetini. This group of ants is highly specialised in capturing mainly Collembola and other soil microfauna (7, 15). Freshly-captured entomobryomorph Collembola were present in the nests of *P. membranifera* at the collection site in Sant Cugat, in a small cell close to the colony. During the capture process, dacetine ants approach prey very slowly and the hypothesis has been proposed that foraging workers attract or appease Collembola via an allomone (6). This author proposed two possible origins for that allomone, the petiolar spongiform appendages or the labrum. The intramandibular gland here described could be the source of this substance.

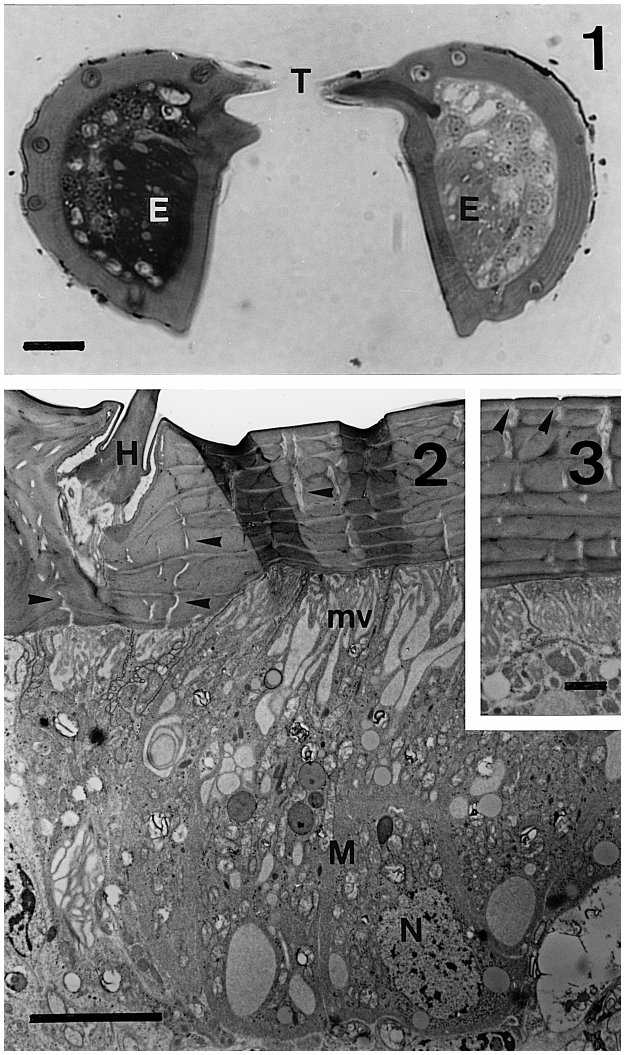

Fig. 1. – Semithin section through distal tip of mandibles of *P. membranifera* worker. E = glandular epithelium, T = mandibular tooth, scale bar 10 μm.

Fig. 2. – Electron micrograph showing epithelial gland and overlaying cuticle. Note narrow pores crossing cuticle (arrowheads), apical microvilli (mv), mitochondria (M), electron-clear vesicles and basally-located nuclei (N); H = hair. Dark zones in cuticle represent artefact folds due to sectioning. Scale bar 5 μm.

Fig. 3. – Detail of cuticular pores opening at external surface (arrowheads), scale bar 1 μm.

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