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Osteology and myology of the cephalic region and pectoral girdle of *Cetopsis coecutiens* Spix & Agassiz, 1829, comparison with other cetopsids, and comments on the synapomorphies and phylogenetic position of the Cetopsidae (Teleostei : Siluriformes)

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ABSTRACT. The cephalic and pectoral girdle structures of the cetopsid *Cetopsis coecutiens* (Cetopsinae) are described and compared with those of another species of the subfamily Cetopsinae, *Hemicetopsis candiru*, and of one species of the single genus of the subfamily Helogeninae, *Helogenes marmoratus*, as well as of several other catfishes. Our observations and comparisons support Mo's 1991 and de Pinna's 1998 phylogenetic hypothesis, according to which the cetopsids occupy a rather basal position within the Siluriformes. In addition, our observations and comparisons pointed out three new, additional characters to diagnose the family Cetopsidae, namely : 1) presence of a muscle 6 of the mandibular barbels; 2) medial branchiostegal rays long and stout; 3) mandibular barbels originate on the posteroventral surface of their irregularly shaped basal cartilages.

KEY WORDS : catfish, cephalic region, Cetopsidae, *Cetopsis*, comparative morphology, pectoral girdle, phylogeny, Siluriformes.

INTRODUCTION

The Siluriformes, or catfishes, with about 437 genera and more than 2700 species, represent about 32% of all freshwater fishes (Teugels, 2003). Although some controversy and several uncertainties remain concerning the higher-level phylogeny of the Siluriformes (de Pinna, 1998; Diogo, 2003), some studies (Mo, 1991; de Pinna, 1998) suggest that the cetopsids occupy a markedly basal position within the non-diplomystid siluriforms (the Diplomystidae are considered as the most plesiomorphic catfishes : Regan, 1911; Alexander, 1965; Arratia, 1987, 1992; Mo, 1991; de Pinna, 1998; Diogo & Chardon, 2000bc; Diogo et al., 2000b, 2001ab; etc.). Therefore, information on the cetopsid catfishes could probably be very useful for understanding the evolution and phylogeny of the Siluriformes. However, the anatomy of the family Cetopsidae, which includes the subfamilies Cetopsinae and Helogeninae (de Pinna & Vari, 1995), is relatively poorly known. In fact, despite the large number of works concerning catfish anatomy (Regan, 1911; Alexander, 1965; Chardon, 1968; Gosline, 1975; Lundberg 1975, 1982; Howes, 1983ab, 1985; Arratia, 1987, 1990, 1992; Mo, 1991; Bornbusch, 1995; Diogo et al., 1999, 2000ab, 2002; Diogo & Chardon, 2000abc; etc.), the only published papers dealing with the morphology of cetopsids are those of Chardon (1968), Lundberg (1975), de Pinna & Vari (1995), Lundberg & Py-Daniel (1994) and Ferraris (1996). Moreover, the descriptions given in these papers are usually brief and incomplete. In fact, the configuration of some osteological structures of the cetopsids (as, e.g., those of the pectoral girdle) are poorly known, and the myology of these fishes is practically unknown.

The aim of this work is to describe the bones, muscles and ligaments of the cephalic region (branchial apparatus excluded) and pectoral girdle of a species belonging to the type genus of the Cetopsidae, *Cetopsis coecutiens* Spix & Agassiz, 1829 (Cetopsinae). We will compare these structures with those of another species of this subfamily, namely *Hemicetopsis candiru* (Spix & Agassiz, 1829), and of one species of the single genus of the subfamily Helogeninae, *Helogenes marmoratus* Günther, 1863, as well as of members from the other siluriform families. This will thus serve as a foundation for a discussion on the synapomorphies and phylogenetic position of the Cetopsidae.

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 National Museum of Natural History of Washington (USNM), and from the South African Institute for Aquatic Biodiversity (SAIAB) and the Albany Museum of Grahamstown (AMG). Ana-

tomical 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 alcohol fixed (alc), trypsin-cleared and alizarine-stained (c&s), or simply alizarine-stained (s) condition of the studied fishes is given in parentheses following the number of specimens dissected. A list of the specimens dissected is given below.

Akysidae : *Akysis baramensis* LFEM, 2 (alc). *Akysis leucorhynchus* USNM 109636, 2 (alc). *Parakysis anomalopteryx* USNM 230307, 2 (alc); LFEM, 1 (alc).

Amblycipitidae : *Amblyceps caecutiens* LFEM, 2 (alc). *Amblyceps mangois* USNM 109634, 2 (alc). *Liobagrus reini* USNM 089370, 2 (alc).

Amphiliidae : *Amphilius brevis* MRAC 89-043-P-403, 3 (alc); MRAC 89-043-P-2333, 1 (c&s). *Andersonia leptura* MNHN 1961-0600, 2 (alc). *Belonoglanis tenuis* MRAC P.60494, 2 (alc). *Doumea typica* MRAC 93-041-P-1335, 1 (alc). *Leptoglanis rotundiceps* MRAC P.186591-93, 3 (alc). *Paramphilius trichomycteroides* LFEM, 2 (alc). *Phractura brevicauda* MRAC 90-057-P-5145, 2 (alc); MRAC 92-125-P-386, 1 (c&s). *Phractura intermedia* MRAC 73-016-P-5888, 1 (alc). *Trachyglanis ineac* MRAC P.125552-125553, 2 (alc). *Zaireichthys zonatus* MRAC 89-043-P-2243-2245, 3 (alc).

Ariidae : *Arius hertzbergii* LFEM, 1 (alc). *Arius heudelotii* LFEM, 4 (alc). *Bagre marinus* LFEM, 1 (alc); LFEM, 1 (c&s). *Genidens genidens* LFEM, 2 (alc).

Aspredinidae : *Aspredo aspredo* USNM 226072, 1 (alc). *Aspredo sicuephorus* LFEM, 1 (alc). *Bunocephalus knerii* USNM 177206, 2 (alc). *Xyliphius magdalenae* USNM 120224, 1 (alc).

Astroblepidae : *Astroblepus phelpis* LFEM, 1 (alc); USNM 121127, 2 (alc).

Auchenipteridae : *Ageneiosus vittatus* USNM 257562, 1 (alc). *Auchenipterus dentatus* USNM 339222, 1 (alc). *Centromochlus hechelii* USNM 261397, 1 (alc).

Austroglanididae : *Austroglanis gilli* LFEM, 3 (alc); SAIAB 58416 (c&s). *Austroglanis sclateri* AMG, 1 (c&s); SAIAB 68917 (s).

Bagridae : *Bagrichthys macropterus* USNM 230275, 1 (alc). *Bagrus bayad* LFEM, 1 (alc); LFEM, 1 (c&s). *Bagrus docmak* MRAC 86-07-P-512, 1 (alc); MRAC 86-07-P-516, 1 (c&s). *Hemibagrus nemurus* USNM 317590, 1 (alc). *Rita chrysea* USNM 114948, 1 (alc).

Callichthyidae : *Callichthys callichthys* USNM 226210, 2 (alc). *Corydoras guianensis* LFEM, 2 (alc).

Cetopsidae : *Cetopsis coecutiens* USNM 265628, 2 (alc). *Helogenes marmuratus* USNM 264030, 2 (alc). *Hemicetopsis candiru* USNM 167854, 2 (alc).

Chacidae : *Chaca bankanensis* LFEM, 3 (alc). *Chaca burmensis* LFEM, 2 (alc). *Chaca chaca* LFEM, 2 (alc).

Clariidae : *Clarias anguillaris* LFEM, 2 (alc). *Clarias batrachus* LFEM, 2 (alc). *Clarias ebriensis* LFEM, 2 (alc). *Clarias gariepinus* MRAC 93-152-P-1356, 1 (alc), LFEM, 2 (alc). *Heterobranchus bidorsalis* LFEM, 2 (alc). *Heterobranchus longifilis* LFEM, 2 (alc). *Uegitglanis zammaronoi* MRAC P-15361, 1 (alc).

Claroteidae : *Auchenoglanis biscutatus* MRAC 73-015-P-999, 2 (alc). *Auchenoglanis occidentalis* LFEM, 2 (alc). *Chrysichthys auratus* UNB, 2 (alc); UNB, 2 (c&s). *Chrysichthys nigrodigitatus* UNB, 2 (alc); UNB, 2 (c&s). *Clarotes laticeps* MRAC 73-13-P-980, 2 (alc).

Cranoglanididae : *Cranoglanis bouderius* LFEM, 2 (alc).

Diplomystidae : *Diplomystes chilensis* LFEM, 3 (alc).

Doradidae : *Acanthodoras cataphractus* USNM 034433, 2 (alc). *Anadoras weddellii* USNM 317965, 2 (alc). *Doras brevis* LFEM, 2 (alc). *Doras punctatus* USNM 284575, 2 (alc). *Franciscodoras marmoratus* USNM 196712, 2 (alc).

Erethistidae : *Erethistes pusillus* USNM 044759, 2 (alc). *Hara filamentosa* USNM 288437, 1 (alc).

Heteropneustidae : *Heteropneustes fossilis* USNM 343564, 2 (alc); USNM 274063, 1 (alc); LFEM, 2 (alc).

Ictaluridae : *Amiurus nebolosus* USNM 246143, 1 (alc); USNM 73712, 1 (alc). *Ictalurus furcatus* LFEM, 2 (alc). *Ictalurus punctatus* USNM 244950, 2 (alc).

Loricariidae : *Hypoptopoma bilobatum* LFEM, 2 (alc). *Hypoptopoma inexspectata* LFEM, 2 (alc). *Lithoxus lithoides* LFEM, 2 (alc). *Loricaria cataphracta* LFEM, 1 (alc). *Loricaria loricaria* USNM 305366, 2 (alc); USNM 314311, 1 (alc).

Malapteruridae : *Malapterurus electricus* LFEM, 5 (alc).

Mochokidae : *Mochokus niloticus* MRAC P.119413, 1 (alc); MRAC P.119415, 1 (alc). *Synodontis clarias* USNM 229790, 1 (alc). *Synodontis schall* LFEM, 2 (alc). *Synodontis sorex* LFEM, 2 (alc).

Nematogenyidae : *Nematogenys inermis* USNM 084346, 2 (alc); LFEM, 2 (alc).

Pangasiidae : *Helicophagus leptorhynchus* USNM 355238, 1 (alc). *Pangasius larnaudii* USNM 288673, 1 (alc). *Pangasius sianensis* USNM 316837, 2 (alc).

Pimelodidae : *Calophysus macropterus* USNM 306962, 1 (alc). *Goeldiella eques* USNM 066180, 1 (alc). *Hepapterus mustelinus* USNM 287058, 2 (alc). *Hypophthalmus edentatus* USNM 226140, 1 (alc). *Microglanis cottoides* USNM 285838, 1 (alc). *Pimelodus blochii* LFEM, 2 (alc). *Pimelodus clarias* LFEM, 2 (alc); USNM 076925, 1 (alc). *Pseudopimelodus raninus* USNM 226136, 2 (alc). *Pseudoplatystoma fasciatum* USNM 284814, 1 (alc). *Rhamdia guatemalensis* USNM 114494, 1 (alc).

Plotosidae : *Cnidoglanis macrocephalus* USNM 219580, 2 (alc). *Neosilurus rendahli* USNM 173554, 2 (alc). *Paraplotosus albilabris* USNM 173554, 2 (alc). *Plotosus anguillaris* LFEM, 2(alc). *Plotosus lineatus* USNM 200226), 2 (alc).

Schilbidae : *Ailia colia* USNM 165080, 1 (alc). *Laides hexanema* USNM 316734, 1 (alc). *Pseudeutropius brachypopterus* USNM 230301, 1 (alc). *Schilbe intermedius* MRAC P.58661, 1 (alc). *Schilbe mystus* LFEM, 3 (alc). *Siluranodon auritus* USNM 061302, 2 (alc).

Scoloplacidae : *Scoloplax distolothrix* LFEM, 1 (alc); USNM 232408, 1 (alc).

Siluridae : *Silurus aristotelis* LFEM, 2(alc). *Silurus glanis* LFEM, 2 (alc). *Silurus asotus* USNM 130504, 2 (alc). *Wallago attu* USNM 304884, 1 (alc).

Sisoridae : *Bagarius yarreli* USNM 348830, 2 (alc); LFEM, 1 (c&s). *Gagata cenia* USNM 109610, 2 (alc). *Glyptosternon reticulatum* USNM 165114, 1 (alc). *Glyptothorax fukiensis* USNM 087613, 2 (alc).

Trichomycteridae : *Hatcheria macraei* LFEM, 2 (alc). *Trichomycterus areolatus* LFEM, 2 (alc). *Trichomycterus banneaui* LFEM, 2 (alc). *Trichomycterus immaculatus* USNM 301015, 2 (alc).

RESULTS

In this section we will describe the cephalic and pectoral girdle structures of *Cetopsis coecutiens* (Cetopsinae) and compare these structures with those of another cetopsin species, *Hemicetopsis candiru*, as well as of one representative of the single genus of the subfamily Helogeninae, *Helogenes marmoratus*. In the anatomical descriptions, the nomenclature for the osteological structures of the cephalic region follows basically that of ARRATIA (1997). However, for the several reasons explained in detail in our recent papers (DIOGO et al., 2001a; DIOGO & CHARDON, 2003), with respect to the skeletal components of the suspensorium we follow DIOGO et al. (2001a). The nomenclature of the cephalic muscles is mainly based on WINTERBOTTOM (1974). However, for the different adductor mandibulae sections, we follow DIOGO & CHARDON (2000b). In relation to the muscles associated with the mandibular barbels, which were not studied by WINTERBOTTOM (1974), we follow DIOGO & CHARDON (2000c). With respect to the nomenclature of the pectoral girdle muscles, we follow DIOGO et al. (2001b).

Cetopsis coecutiens (adult specimens)

Osteology

Mesethmoid. It is situated on the anterodorsal surface of the neurocranium (Figs 1, 2). Each of its prominent anterolateral arms is ligamentously connected to the premaxilla.

Lateral ethmoid. Large bone (Fig. 1), which exhibits a laterally directed articulatory facet for the palatine. Its posterodorsolateral surface presents a prominent lateral projection that sutures with a somewhat similar lateral projection of the anterodorsolateral surface of the pterosphenoid (Fig. 1).

Prevomer. T-shaped bony plate lying underneath the ethmoideal region and presenting two prominent, posterolaterally directed anterolateral arms (Fig. 2). Anteroventrally, the prevomer bears numerous prominent teeth (Fig. 1).

Orbitosphenoid. Large bone lying posterior to the lateral ethmoid (Figs 1, 2). It does not contact the frontal (Fig. 1).

Pterosphenoid. It is posterior to the orbitosphenoid (Figs 1, 2), covering, together with this bone, the gap between the frontals and the parasphenoid.

Parasphenoid. This is the longest bone of the cranium (Fig. 2). It bears a pair of prominent ascending flanges, which suture with the pterosphenoids and prootics.

Frontal. The paired frontals are very long bones (Fig. 1). A great part of their main body forms part of the prominent dorsomedial crest of the cranial roof, on which originates part of the muscle adductor mandibulae (see below). There is no fontanel between the dorsomedian margins of the paired frontals.

Sphenotic. It bears, together with the pterotic, an articulatory facet for the hyomandibulo-metapterygoid (Fig. 2) and presents a prominent, anteriorly oriented anterodorsal process (Figs 1, 2 : sph-adp).

Pterotic. Its dorsal surface is somewhat rectangular and has about the same size as the dorsal surface of the sphenotic (Fig. 1). The pterotic has short, lateral triangular process posteriorly to its articulatory surface for the hyomandibulo-metapterygoid (Fig. 2).

Prootic. Large bone (Fig. 2). Together with the pterosphenoid, it borders the foramen of the trigemino-facial nerve complex (Fig. 2).

Epioccipital. Small bone situated on the posteroventral surface of the neurocranium, lateral to the posterior surface of the exoccipital and medial to the posterior surface of the pterotic (Fig. 2).

Exoccipital. The paired exoccipitals are small bones situated laterally to the basioccipital (Fig. 2). The posteroventromedial surfaces of the exoccipitals are firmly connected, by means of connective tissue, to the ventromedial limbs of the posttemporo-supracleithra (Fig. 2).

Basioccipital. Unpaired bone, which forms the posteriormost part of the floor of the neurocranium and presents two prominent posteroventrolateral processes (Fig. 2).

Parieto-supraoccipital. Large bone (Fig. 1) with two prominent, thin, posterolaterally directed, posterodorsolateral arms and a large, posteriorly directed posterodorsomedian process. The anteromedian surfaces of the parieto-supraoccipital are largely separated by a somewhat rectangular dorsal fontanel.

Extrascapular. Small bone situated between the dorsomedian surface of the posttemporo-supracleithrum, the posterodorsal surface of the pterotic and posterodorsolateral surface of the parieto-supraoccipital (Fig. 1).

Angulo-articular. This bone (Figs 1, 3), together with the dentary bone, coronomeckelian bone and Meckel's cartilage, constitute the mandible (Fig. 3). The anterodorsal surface of the angulo-articular, together with the posterodorsal surface of the dentary bone, form a prominent dorsal process (processus coronoideus) (Figs 1, 3), which is linked to the maxilla by means of a massive, long ligament. Posterodorsally, the angulo-articular has an articulatory surface for the quadrato-symplectic. The angulo-articular presents a prominent, circular, medially directed posteromedial process (Fig. 3 : ang-art-pmp).

Dentary bone. Anterodorsally, it presents a broad dorsolateral lamina (Fig. 3 : den-dl), which covers a considerable portion of the lateral surface of the mandibular teeth in lateral view (see Fig. 1).

Coronomeckelian bone. Small bone lodged in the medial surface of the mandible (Fig. 3). Posterodorsally it bears a crest for attachment of the adductor mandibulae A3'-d (see below).

Premaxilla. The premaxillae (Figs 1, 2) are a pair of large triangular plates lying underneath and attaching to

the mesethmoidal cornua via ligamentous tissue. Ventrally, each premaxilla bears numerous small teeth (Fig. 1) having their tips slightly turned backward.

Maxilla. It is markedly compressed proximodistally and articulates with the anterior cartilage of the autopalatine by means of a single proximal articulatory head (Fig. 4). As in most catfishes, the maxilla barbels are supported by the maxillae.

Autopalatine. The autopalatine (Fig. 4) is markedly compressed dorsoventrally. Its anterior end is tipped by a large cartilage (Fig. 4), which is markedly extended mesially, bordering inclusively part of the anteromedial margin of the bony portion of the autopalatine. This cartilage presents an anterolateral concavity to receive the proximal articulatory head of the maxilla. The posterior end of the autopalatine, which is expanded transversely, is capped by a small cartilage. Medially, the autopalatine articulates with the lateral ethmoid (Fig. 4). The autopalatine articulates mesially with the lateral ethmoid (Fig. 4).

Hyomandibulo-metapterygoid. Dorsally, this bone articulates synchondrally with both the pterotic and the sphenotic (Figs 1, 2). Anterior to this articulation, it presents a prominent, anteriorly pointed anterodorsal extension, which, however, does not articulate synchondrally with the neurocranium (Fig. 1). Laterodorsally, it presents a prominent, broad, somewhat quadrangular lateral crest for the attachment of the posterior section of the muscle levator arcus palatini (see below). Posteriorly, the hyomandibulo-metapterygoid presents a large articulatory facet for the opercle, which is significantly elongated dorsoventrally (Fig. 4).

Sesamoid bone 1 of suspensorium. Roughly triangular in shape (Figs 1, 2). Its dentate posterior margin is firmly attached, by means of a very short, strong ligament, to the also dentate anterolateral margin of the entopterygoideo-ectopterygoid, thus giving the impression that these two bones are partially sutured (see Fig. 2). Its anterior margin is ligamentously connected to the prevomer (Fig. 2). The sesamoid bones 2 and 3 of the suspensorium are absent.

Entopterygoideo-ectopterygoid. Posteriorly the broad, somewhat rectangular entopterygoideo-ectopterygoid (see DIOGO et al., 2001a) is connected, by a large cartilaginous band and by bony sutures, to both the quadrato-symplectic and the hyomandibulo-metapterygoid (Fig. 1).

Quadrato-symplectic. Triangular bone that articulates anteroventrally with the mandible (Fig. 1).

Preopercle. Long and large bone firmly sutured to the hyomandibulo-metapterygoid and to the quadrato-symplectic (Fig. 1).

Opercle. Very large, irregular bone, with its ventral margin being significantly broader than its dorsal margin (Fig. 1).

Interopercle. The interopercle is a broad, dorsoventrally elongated bone roughly triangular in shape (Fig. 1). Its posterior margin, which is connected to the opercle by means of connective tissue, is situated medial to this latter bone (Fig. 1). Its anterodorsal margin is linked, by means of thick ligament (Fig. 1 : l-ang-iop) to the angulo-articular. Medially, the interopercle is firmly connected, via connective tissue, to the posterolateral surface of the ceratohyal (Figs 1, 4, 5).

Interhyal. The interhyal (Fig. 2) is a small bone attached, by means of ligaments, to both the posterior ceratohyal and the medial surface of the suspensorium (hyomandibulo-metapterygoid and quadrato-symplectic).

Posterior ceratohyal. This triangular bone (Figs 1, 5) is linked by ligaments to the angulo-articular, interhyal and interopercle.

Anterior ceratohyal. Stout bone (Fig. 1) that, together with the posterior ceratohyal, supports the large branchiostegal rays, which are, including the inner ones, remarkably elongated anteroposteriorly (see Fig. 5).

Ventral hypohyal. Small bone (Fig. 1). Each ventral hypohyal contains a ventral concavity to receive one of the anterolateral edges of the parurohyal.

Dorsal hypohyal. It is a small bone. It lies dorsally to the ventral hypohyal, to which it is connected by a thin cartilage.

Parurohyal. The parurohyal (see ARRATIA & SCHULTZE, 1990) is a somewhat triangular bone with two prominent posterolateral processes and a small posteromedial process. It lies medially behind the ventromedial surfaces of the ventral hypohyals and is connected to these bones by means of two strong, thick ligaments.

Posttemporo-supracleithrum. The medial and posterior margins of the thin ventromedial limb of this large bone are firmly attached, by means of connective tissue, to the posteroventromedial surface of the exoccipital and to the anterior surface of the fourth parapophysis of the complex vertebra (Fig. 2). Its posteroventrolateral limb is forked, forming, together with the anterolateral surface of this parapophysis, an articulating groove (Fig. 2) for the upper edge of the cleithrum (see Fig. 1).

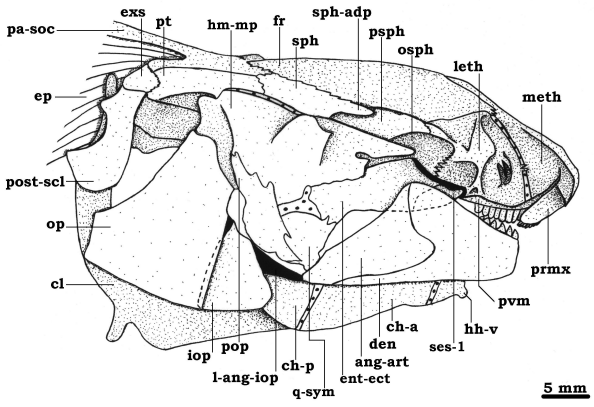

Fig. 1. – Lateral view of the cranium and pectoral girdle of *Cetopsis coecutiens*. The muscle epaxialis is also illustrated. *ang-art,* angulo-articular; *ch-a,* anterior ceratohyal; *ch-p,* posterior ceratohyal; *cl,* cleithrum; *den,* dentary bone; *ent-ect,* entopterygoideo-ectopterygoid; *ep,* muscle epaxialis; *exs,* extrascapular; *fr,* frontal; *hh-v,* ventral hypohyal; *hm-mp,* hyomandibulo-metapterygoid; *iop,* interopercle; *l-ang-iop,* angulo-interopercular ligament; *leth,* lateral-ethmoid; *meth,* mesethmoid; *op,* opercle; *osph,* orbitosphenoid; *pa-soc,* parieto-supraoccipital; *pop,* preopercle; *post-scl,* posttemporo-supracleithrum; *prmx,* premaxilla; *psph,* pterosphenoid; *pt,* pterotic; *pvm,* prevomer; *q-sym,* quadrato-symplectic; *ses 1,* sesamoid bone 1 of suspensorium; *sph,* sphenotic; *sph-adp,* anterodorsal process of sphenotic.

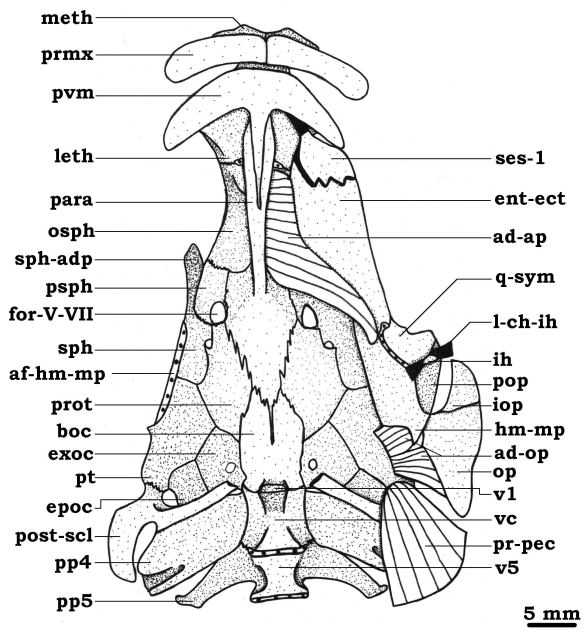

Fig. 2. – Ventral view of the neurocranium of *Cetopsis coecutiens*. On the right side the suspensorium, as well as the adductor arcus palatini, adductor operculi and protractor pectoralis, are also illustrated. Both the premaxillary and the prevomerine teeth were removed. *ad-ap,* muscle adductor arcus palatini; *ad-op,* muscle adductor operculi; *af-hm-mp,* articulatory facet for hyomandibulo-metapterygoid; *boc,* basioccipital; *ent-ect,* entopterygoideo-ectopterygoid; *epoc,* epioccipital; *exoc,* exoccipital; *for-V-VII,* trigemino-facialis foramen; *hm-mp,* hyomandibulo-metapterygoid; *ih,* interhyal; *iop,* interopercle; *l-ch-ih,* ceratohyalo-interhyale ligament; *leth,* lateral ethmoid; *meth,* mesethmoid; *op,* opercle; *osph,* orbitosphenoid; *para,* parasphenoid; *pop,* preopercle; *post-scl,* posttemporo-supracleithrum; *pp4*, *pp5,* parapophysises 4 and 5; *pr-pec,* muscle protractor pectoralis; *prmx,* premaxilla; *prot,* prootic; *psph,* pterosphenoid; *pt,* pterotic; *pvm,* prevomer; *q-sym,* quadrato-symplectic; *ses-1,* sesamoid bone 1 of suspensorium; *sph,* sphenotic; *sph-adp,* anterodorsal process of sphenotic; *v1, v5,* vertebrae 1 and 5; *vc,* complex vertebrae.

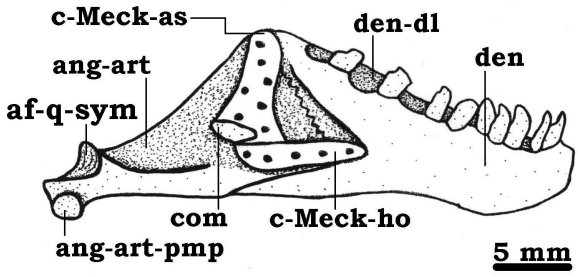

Fig. 3. – Medial view of the left lower jaw of *Cetopsis coecutiens*. *af-q-sym* articulatory facet for quadrato-symplectic, *ang-art* angulo-articular, *ang-art-pmp* posteromedial process of angulo-articular, *c-Meck-as, c-Meck-ho* ascending and horizontal portions of Meckel's cartilage, *com* coronomeckelian, *den* dentary bone, *den-dl* dorsal lamina of dentary bone.

Cleithrum. The cleithrum (Figs 1, 6) is a large, well-ossified stout structure forming the major part of the pectoral girdle and the posterior boundary of the branchial chamber. It has a single dorsal process (Fig. 6 : cl-dp), which articulates (Fig. 1) with both the posttemporo-supracleithrum and the fourth parapophysis of the complex vertebra. The humeral process is absent (Figs 1, 6). The two cleithra are attached in the anteromedial line via massive connective tissue.

Scapulo-coracoid. This is an elongated bony plate, of which the anteromedial and the posterolateral margins are firmly attached with the cleithrum (Fig. 6). It presents a short, thin median arm (see Diogo et al., 2001b), which does not reach the median line (Fig. 6), and, thus, does not suture with its counterpart medially. Anterolaterally, the scapulo-coracoid presents a prominent, anteroventrolaterally directed process, usually called the coracoid bridge (see Diogo et al., 2001b), which extends anteroventrally to the ventral surface of the cleithrum, fusing with a ventral ridge of this bone (Fig. 6 : cor-bri). This coracoid bridge is prolonged posteromesially by a prominent posteroventral laminar process (Fig. 6 : cor-bri-pvp). Posterolaterally, the scapulo-coracoid bears two condyles, which articulate, respectively, with the pectoral spine and the complex radial (see Mo, 1991). The mesocoracoid arch (see Diogo et al., 2001b) is present and markedly expanded transversally.

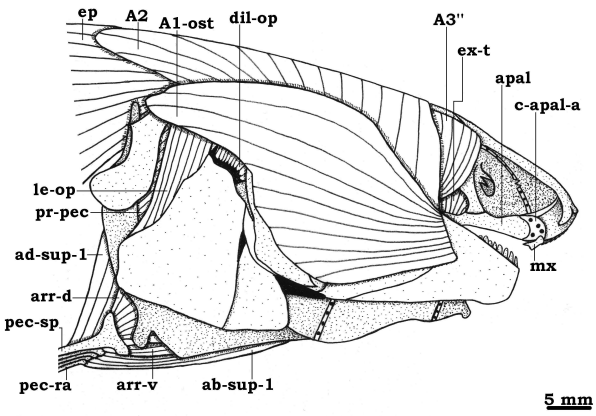

Fig. 4. – Lateral view of the cephalic and pectoral girdle musculature of *Cetopsis coecutiens*. *A1-ost, A2, A3'',* sections of muscle adductor mandibulae; *ab-sup-1,* section of muscle abductor superficialis; a*d-sup-1,* section of muscle adductor superficialis; *apal,* autopalatine; *arr-d,* muscle arrector dorsalis; *arr-v,* muscle arrector ventralis; *c-apal-a,* anterior cartilage of autopalatine; *dil-op,* muscle dilatator operculi; *ep,* muscle epaxialis; *ex-t,* muscle extensor tentaculi; *le-op,* muscle levator operculi; *mx,* maxilla; *pec-ra,* pectoral rays; *pec-sp,* pectoral spine; *pr-pec* muscle protractor pectoralis (for the other osteological structures, see abbreviations on Fig. 1).

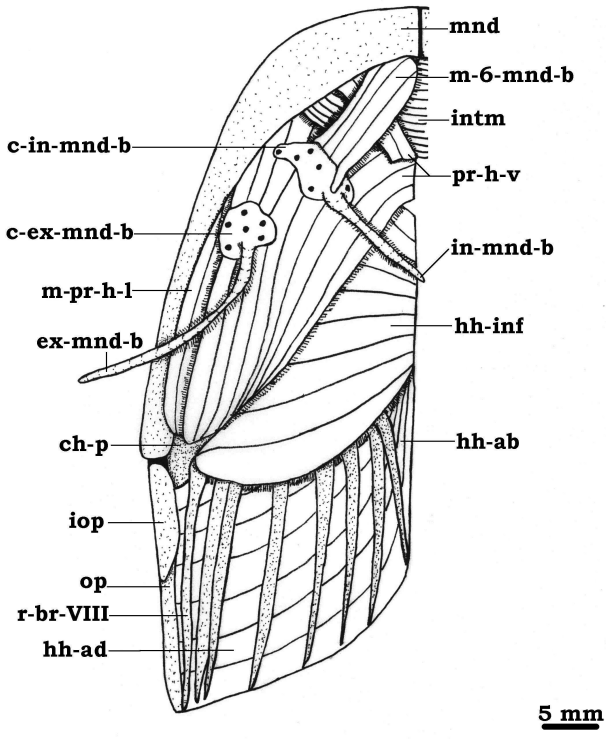

Fig. 5. – Ventral view of the cephalic musculature of *Cetopsis coecutiens*. *c-ex-mnd-b, c-in-mnd-b,* basal cartilages of external and internal mandibular barbels; *ch-p,* posterior ceratohyal; *ex-mnd-b, in-mnd-b,* external and internal mandibular barbels; *hh-ab,* muscle hyohyoideus abductor; *hh-ad*, muscle hyohyoideus adductor; *hh-inf,* muscle hyohyoideus inferior; *intm,* muscle intermandibularis; *iop,* interopercle; *m-6-mnd-b,* muscle 6 of the mandibular barbels; *mnd,* mandible; *op,* opercle; *pr-h-l, pr-h-v,* pars lateralis and ventralis of muscle protactor hyoideus; *r-br-VIII,* branchiostegal ray VIII.

Myology

Adductor mandibulae. The adductor mandibulae A1-ost (DIOGO & CHARDON, 2000b) originates on the parieto-supraoccipital, posttemporo-supracleithrum, extrascapular, pterotic, hyomandibulo-metapterygoid and preopercle and inserts on the lateral surface of both the dentary bone and the angulo-articular (Fig. 4). The A2 (Fig. 4), which lies dorsomesially to the A1-ost, originates on the prominent dorsomedian crest of the cranial roof formed by both the frontal and the parieto-supraoccipital and inserts, by means of a thick tendon, on the posteroventromedial surface of the dentary bone. It covers almost all the posterodorsal surface of the neurocranium (Fig. 4). It covers almost all the posterodorsal surface of the neurocranium (Fig. 4), and also covers the most anterior fibers of the epaxialis. However, it should be noted that there is no aponeurosis between the A2 and the epaxialis. The adductor mandibulae A3' runs from the quadrato-symplectic, hyomandibulo-metapterygoid, entopterygoideo-ectoperygoid and preopercle to both the angulo-articular and the coronomeckelian bone. The deeper bundle of the adductor mandibulae, the A3" (Fig. 4) originates on both the anterodorsal surface of the frontal and the posterodorsal surface of the lateral ethmoid and inserts, by means of a massive tendon, to the medial surface of the angulo-articular. The Aω, which is small and situated on the mesial surface of the mandible, contacts anteriorly the tendon of the A2 and posteriorly the anteriormost fibers of the A3'.

Levator arcus palatini. This hypertrophied muscle is differentiated into posterior and anterior large sections. The posterior section originates on the dorsal surfaces of both the sphenotic, frontal and lateral ethmoid and inserts on a prominent lateral crest of the hyomandibulo-metapterygoid (see above). The anterior section runs from the dorsal surface of the lateral ethmoid to the lateral surfaces of both the hyomandibulo-metapterygoid and the entopterygoideo-ectopterygoid.

Adductor arcus palatini. It extends from the lateral sides of the parasphenoid, pterosphenoid and orbitosphenoid to the medial sides of the hyomandibulo-metapterygoid and entopterygoideo-ectopterygoid (Fig. 2).

Adductor operculi. It runs from the ventral surface of the pterotic to both the dorsomedial surface of the opercle and the posterodorsomedial surface of the hyomandibulo-metapterygoid (Fig. 2).

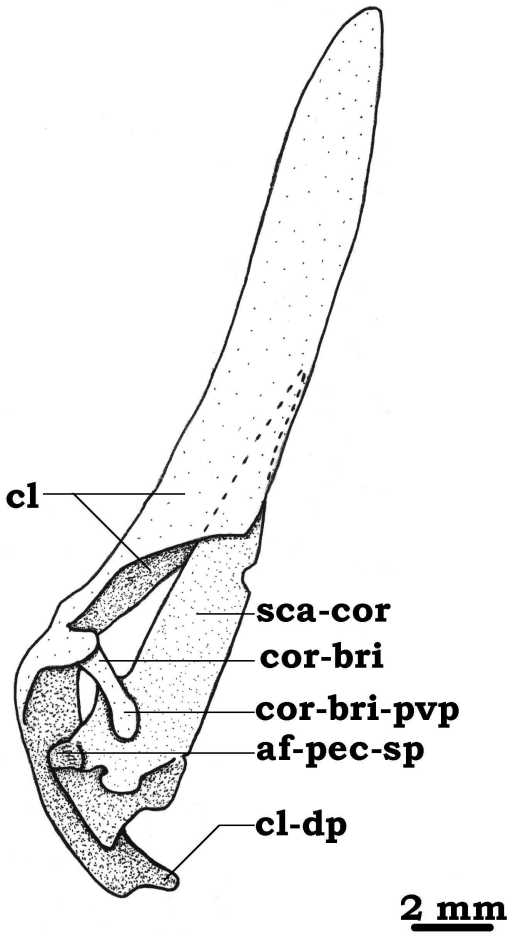

Fig. 6. – Ventral view of the pectoral girdle of *Cetopsis coecutiens*. *af-pec-sp,* articulatory surface for pectoral spine; *cl,* cleithrum; *cl-dp,* dorsal process of cleithrum; *cor-bri,* coracoid bridge; *cor-bri-pvp,* posteroventral process of coracoid bridge; *sca-cor,* scapulo-coracoid.

Dilatator operculi. Thick muscle running from the pterotic, sphenotic, frontal, pterosphenoid, orbitosphenoid and lateral ethmoid, as well as from the anterodorsal surface of the hyomandibulo-metapterygoid, to the anterodorsal edge of the opercle (medial to the preopercle but lateral to the articulatory facet of the opercle for the hyomandibulo-metapterygoid) (Fig. 4).

Levator operculi. It originates on the ventrolateral margin of the pterotic and inserts on the dorsal edge of the opercle (Fig. 4).

Extensor tentaculi. It runs from the ventromedial surface of the lateral ethmoid to the posterior portion of the autopalatine (Fig. 4).

Protractor hyoidei. This muscle has 3 parts. The pars ventralis (Fig. 5 : m-pr-h-v) lodges the large, irregular cartilages associated with the mandibular barbels, which are not differentiated into a moving and a supporting part (see DIOGO & CHARDON, 2000c), with the mandibular barbels originating on their posteroventral surfaces (Fig. 5). It runs from both the anterior and the posterior ceratohyals to the dentary bone, meeting its counterpart in a large median aponeurosis (Fig. 5). The pars lateralis (Fig. 5 : m-pr-h-l) originates on the posterior ceratohyal, inserting, by means of a thick tendon, on the ventromedial face of the dentary bone. The pars dorsalis originates tendinously on the anterior ceratohyal and inserts tendinously on the dentary bone, with some of its anterior fibers being mixed with those of the intermandibularis.

Intermandibularis. This muscle joins the two mandibles (Fig. 5).

Retractor tentaculi mandibularis interni. Small muscle lying dorsal to the muscle 6 of the mandibular barbels (see below) and running from the cartilage associated with the inner mandibular barbel to the dentary bone.

Retractor tentaculi mandibularis externi. Small muscle attaching anteriorly on the dentary bone and posteriorly on the cartilage associated with the outer mandibular barbel.

Protractor tentaculi mandibularis externi. Small muscle lying dorsal to the pars ventralis of the protractor hyoidei and running from the posterior ceratohyal to the cartilage associated with the external mandibular barbel.

Muscle 6 of the mandibular barbels. This muscle (Fig. 5 : m-6-mnd-b), which is not homologous with the muscles 1, 2, 3, 4 and 5 of the mandibular barbels described by DIOGO & CHARDON (2000c) (see below), originates on the dentary bone. It passes ventrally to both the intermandibularis, the retractor tentaculi mandibularis interni and the pars ventralis of the protractor hyoidei and inserts on the ventrolateral surface of the cartilage associated with the internal mandibular barbel.

Hyohyoideus inferior. This thick muscle (Fig. 5) attaches medially on a median aponeurosis and laterally on the ventral surfaces of the ventral hypohyal, anterior ceratohyal and posterior ceratohyal.

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.

Hyohyoideus adductor. Each hyohyoideus adductor (Fig. 5) connects the branchiostegal rays of the respective side, with the most lateral fibers of this muscle also attaching to the mesial surface of the opercular bone.

Sternohyoideus. It originates on the anterior region of the cleithrum and inserts on the posterior region of the parurohyal. Posteriorly, the fibers of the sternohyoideus are deeply mixed with those of the epaxialis.

Arrector ventralis. Thin, anteroposteriorly elongated muscle attaching anteriorly on both the cleithrum and the scapulo-coracoid and posteriorly on the anteroventral surface of the pectoral spine (Fig. 4).

Arrector dorsalis. The arrector dorsalis, although constituted by a single section (see DIOGO et al., 2001b) that inserts posteriorly on the anterodorsal surface of the pectoral spine (Fig. 4), is bifurcated anteriorly, with its anteroventral fibers attaching to the ventral surface of the pectoral girdle and its anterodorsal fibers attaching to the dorsal surface of the pectoral girdle.

Abductor profundus. Small muscle, it originates on the posterolateral surface of the coracoid and inserts on the anterodorsomedial surface of the pectoral spine.

Adductor superficialis. It is differentiated into two sections. The larger section (Fig. 4 : ad-sup-1) originates on the posterior surfaces of both the cleithrum and the scapulo-coracoid, as well as on the posterior margin of the mesocoracoid arch and inserts on the anterodorsal margin of the dorsal part of the pectoral fin rays. The smaller section originates on the posterior surface of the scapulo-coracoid, the posteroventral surface of the mesocoracoid arch and the dorsal surface of the proximal radials and inserts on the anteroventral margin of the dorsal part of the pectoral fin rays.

Abductor superficialis. This muscle is also differentiated into two sections. The larger section (Fig. 4 : ab-sup-1) attaches anteriorly on the ventral face of both the cleithrum and the scapulo-coracoid and posteriorly on the anteroventral margin of the ventral part of the pectoral fin rays. The smaller section runs from both the posterolateroventral edge of the scapulo-coracoid and the ventral surface of the proximal radials to the anterodorsal margin of the ventral part of the pectoral fin rays.

Protractor pectoralis. This thick muscle (Figs 2, 4) runs from the ventral surfaces of both the pterotic, the epioccipital and the posttemporo-supracleithrum to the anterodorsal surfaces of both the cleithrum and the scapulo-coracoid.

Hemicetopsis candiru *(adult specimens)*

Osteology

In a general way, the configuration of the osteological structures of the pectoral girdle and cephalic region of this species resembles that of *Cetopsis coecutiens*, although there are some differences : *1)* the anterolateral arms of the prevomer are considerably more expanded anteroposteriorly in *H. candiru* than in *C. coecutiens*; *2)* there are two, and not only one, large fontanels on the dorsal surface of the cranial roof in *H. candiru*; *3)* in *H. candiru* the dorsomedian crest of the cranial roof is not as large as in *C. coecutiens*; *4)* the interopercle of *H. candiru* is considerably less expanded dorsoventrally than that of

C. coecutiens; *5)* in *H. candiru* the dorsolateral lamina of the dentary bone is significantly broader than that of *C. coecutiens*, with this dorsomedial lamina covering the main part of the lateral surface of the mandibular teeth in lateral view; *6)* contrarily to *C. coecutiens*, there is no posteromedian circular process of the angulo-articular in *H. candiru*.

Myology

The configuration of the cephalic and pectoral girdle muscles of *H. candiru* resembles that of *C. coecutiens*, although there are some differences between these species concerning these muscles : *1)* the adductor mandibulae A2 of *H. candiru* is still more developed than that of *C. coecutiens*, covering inclusively all the lateral surface of the A3" in lateral view; *2)* the levator arcus palatini is not as voluminous in *H. candiru* than in *C. coecutiens*; *3)* contrarily to *C. coecutiens*, in *H. candiru* the dilatator operculi does not contact the anterodorsal surface of the hyomandibulo-metapterygoid.

Helogenes marmoratus
(adult specimens)

Osteology

There are differences between the configuration of the osteological structures of the cephalic region and pectoral girdle of *H. marmoratus* and those of *C. coecutiens* : *1)* the premaxilla of *H. marmoratus* (Fig. 7) is considerably more expanded anteroposteriorly than that of *C. coecutiens*; *2)* anteroventrally the mesethmoid of *H. marmoratus* presents a broad, horizontal lamina (Fig. 7), which borders a significant part of the dorsal surface of the premaxilla; *3)* in the examined specimens of *H. marmoratus* there is a large cartilaginous band between the posterodorsal margin of the lateral ethmoid and the anterodorsal margin of the sphenotic (see Fig. 7), and the mesethmoid is unossified posterodorsomesially; *4)* the posterior arm of the T-shaped prevomer of *H. marmoratus* is significantly shorter than that of *C. coecutiens*; *5)* the sesamoid bone 1 of the suspensorium of *H. marmoratus* (Fig. 7) is smaller than that of *C. coecutiens*; *6)* in *H. marmoratus* there is no prominent anterodorsal expansion of the hyomandibulo-metapterygoid; *7)* the articulatory surface between the hyomandibulo-metapterygoid and the opercle is not as expanded dorsoventrally in *H. marmoratus* as it is in *C. coecutiens*; *8)* the parieto-supraoccipital of *H. marmoratus* (Fig. 7) is markedly compressed anteroposteriorly, and does not present, as in *C. coecutiens* (see above), two prominent, thin posterolateral arms; *9)* in *H. marmoratus* there are two, and not only one, large fontanels on the dorsomedian surface of the cranial roof, and there is no median crest on the dorsomedian surface of the neurocranium; *10)* in *H. marmoratus* the anterolateral teeth of the mandible are significantly larger than the remaining mandibular teeth (Fig. 7); *11)* the mandible of *H. marmoratus* is significantly more compressed dorsoventrally (Fig. 7) than that of *C. coecutiens*; *12)* there is no dorsolateral lamina of the dentary bone in *H. marmoratus* (Fig. 7); *13)* contrary to *C. coecutiens*, in *H. marmoratus* there is no connection (Figs 7, 8) between the posterior ceratohyal and the interopercle; *14)* in *H. marmoratus* the ventromedial limb of the posttemporo-supracleithrum is firmly attached to the posterolateral surface of the basioccipital, and not to the posteroventromedial surface of the exoccipital; *15)* the mesocoracoid arch of *H. marmoratus* is not significantly expanded transversally.

Myology

There are some differences between the configuration of the cephalic and pectoral girdle muscles of *H. marmoratus* and those of *C. coecutiens* : *1)* the adductor mandibulae A1-ost and the adductor mandibulae A2 of *H. marmoratus* (Fig. 7) are significantly less developed than those of *C. coecutiens*; *2)* the adductor mandibulae A3" is absent in *H. marmoratus*; *3)* in *H. marmoratus* the levator arcus palatini does not cover part of the dorsal surface of the cranial roof; *4)* contrary to *C. coecutiens*, in *H. marmoratus* the dilatator operculi does not contact the anterodorsal surface of the hyomandibulo-metapterygoid; *5)* the muscle 6 of the mandibular barbels of *H. marmoratus* (Fig. 8) is significantly more expanded transversally than that of *C. coecutiens*; 6) in *H. marmoratus* the protractor externi mandibularis tentaculi (Fig. 8 : pr-ex-mnd-t) lies ventral to the pars lateralis and the pars ventralis of the protractor hyoidei, thus being visible in ventral view (see Fig. 8).

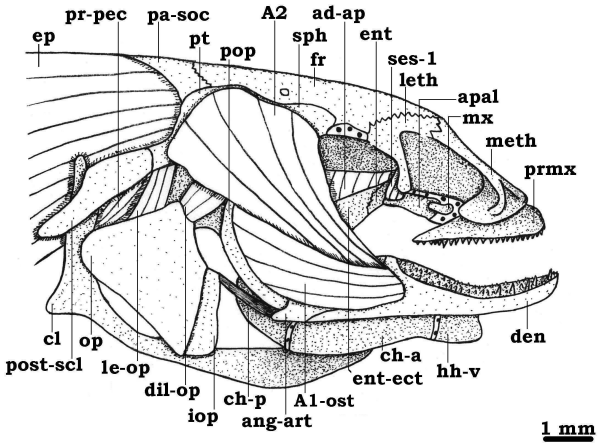

Fig. 7. – Lateral view of the cephalic musculature of *Helogenes marmoratus*. *A1-ost, A2,* sections of muscle adductor mandibulae; *ad-ap,* muscle adductor arcus palatini; *ang-art,* angulo-articular; *apal,* autopalatine; *ch-a, ch-p,* anterior and posterior ceratohyals; *cl,* cleithrum; *den,* dentary bone; *dil-op,* muscle dilatator operculi; *ent-ect,* entopterygoideo-ectopterygoid; *ep,* muscle epaxialis; *ex-t,* muscle extensor tentaculi; *fr,* frontal; *hh-v,* ventral hypohyal; *iop,* interopercle; *le-op,* muscle levator operculi; *leth,* lateral-ethmoid; *meth,* mesethmoid; *mx,* maxilla; *op,* opercle; *pa-soc,* parieto-supraoccipital; *pop,* preopercle; *post-scl,* posttemporo-supracleithrum; *pr-pec,* muscle protractor pectoralis; *prmx,* premaxilla; *pt,* pterotic; *ses 1,* sesamoid bone 1 of suspensorium; *sph,* sphenotic.

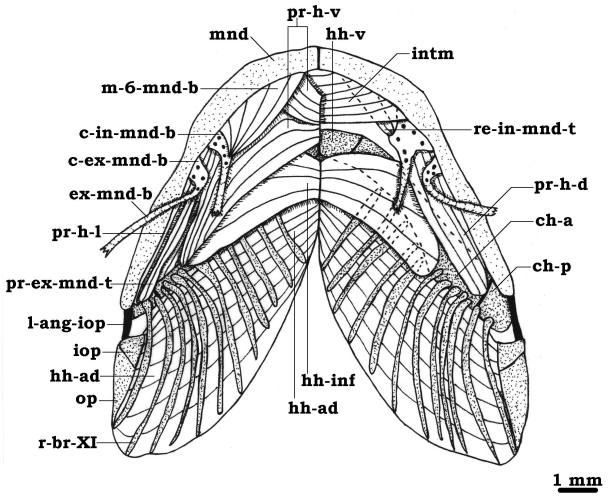

Fig. 8. – Ventral view of the cephalic musculature of *Helogenes marmoratus*. On the right side the protractor externi mandibularis tentaculi, the muscle 6 of the mandibular barbels and the pars ventralis and lateralis of the protractor hyoidei were removed. *c-ex-mnd-b, c-in-mnd-b,* basal cartilages of external and internal mandibular barbels; *ch-a, ch-p,* anterior and posterior ceratohyals; *ex-mnd-b,* external mandibular barbel; *hh-ab,* muscle hyohyoideus abductor; *hh-ad,* muscle hyohyoideus adductor; *hh-inf,* muscle hyohyoideus inferior; *hh-v,* ventral hypohyal; *intm,* muscle intermandibularis; *iop,* interopercle; *l-ang-iop,* angulo-interopercular ligament; *m-6-mnd-b,* muscle 6 of the mandibular barbels; *mnd,* mandible; *op,* opercle; *pr-ex-mnd-t,* muscle protractor externi mandibularis tentaculi; *pr-h-d, pr-h-l, pr-h-v,* pars dorsalis lateralis and ventralis of muscle protactor hyoideus; *r-br-XI,* branchiostegal ray XI; *re-in-mnd-t,* muscle retractor interni mandibularis tentaculi.

DISCUSSION

De Pinna & Vari (1995 : 4-7) listed nine characters to support the monophyly of the Cetopsidae (including the subfamilies Cetopsinae and Helogeninae), of which eight concern the configuration of structures examined in this work, namely : *I)* "maxilla with a single proximal head"; *II)* "posterior portion of palatine depressed, expanded lateromesially"; *III)* "anterior distal cartilage of palatine extending onto mesial surface of bone"; *IV)* "anterior cartilage of palatine expanded anteriorly"; *V)* "lap joint present between opercle and interopercle"; *VI)* "attachment of interoperculo-mandibular ligament on dorsal portion of interopercle"; *VII)* "interopercle expanded along dorsoventral axis, deeper than long"; *VIII)* "metapterygoid elongate, roughly rectangular in shape" [the other character was the "shaft of second basibranchial expanded laterally, with strongly convex lateral margins"]. Our observations and comparisons not only confirmed these eight synapomorphies, but also pointed out three other characters that are found in the three cetopsid genera examined, that is, in members of the two subfamilies of the family Cetopsidae, and in no other catfish examined or described in the literature. These, thus, constitute very likely additional characters to diagnose this family :

1- Presence of a muscle 6 of the mandibular barbels. Although several catfishes have small, specialised muscles directly associated with the movements of the mandibular barbels (see Diogo & Chardon, 2000c : 465-475), a muscle 6 of the mandibular barbels is exclusively present in the cetopsid catfishes examined (see Figs 5, 8).

2- Large, stout medial branchiostegal rays. Characteristically among catfishes the inner branchiostegal rays are relatively thin, being markedly thinner than the more lateral ones (see, e.g., Tilak, 1963 : figs 1, 12, 30; Grande, 1987 : fig. 5B; Arratia & Schultze, 1990 : figs 6B, 8A, 8B, 13D; Mo, 1991 : fig 16D; Diogo et al., 1999 : fig. 5; Diogo & Chardon, 2000a : fig. 4; Oliveira et al., 2001 : fig. 8B; etc.). However, the helogenines (see Fig. 8), and particularly the cetopsines (see Fig. 5) examined present large, stout median branchiostegal rays.

3- Mandibular barbels originate on the posteroventral surface of their irregularly shaped basal cartilages. Characteristically in those catfishes having mandibular barbels the cartilages associated with these barbels are differentiated into an anterior, short "supporting" part and a posterior, long "moving" part (Diogo & Chardon, 2000c), with the mandibular barbels originating on the anteroventral surface of these cartilages (see, e.g., Diogo & Chardon, 2000c : fig. 1). However, in both the cetopsines (see Fig. 5) and the helogenines (see Fig. 8) examined the mandibular barbels originate on the posteroventral, and not on the anteroventral, margin of the basal cartilages, which are irregularly shaped and not differentiated into a "supporting" and a "moving" part.

So far, the only published cladistic papers dealing with the phylogenetic position of the cetopsids within the order Siluriformes were those of Mo (1991) and de Pinna (1998). Both these papers suggest that the cetopsid catfishes occupy a markedly basal position within the siluriforms. In Mo's paper the cetopsids appear as the most basal non-diplomystid catfishes. The cetopsids and diplomystids are separated from the remaining catfishes by a "computer node" in Mo's cladogram 1 (see Mo, 1991 : fig. 4) and by the fact that in these two groups the "ramus mandibularis nerve (does not run) inside hyomandibular for a distance" (although Mo considered the Cetopsidae of the present study a non-monophyletic group, both the genus *Hemicetopsis*, the remaining cetopsines and the helogenines appear in a more basal position than all the other non-diplomystid catfishes, including the fossil hypsidorids, in Mo's 1991 cladograms). With respect to the work of de Pinna (1998 : fig. 1), it suggests that the cetopsids, together with the fossil hypsidorids, are the most basal non-diplomystid catfishes. These because "they lack some synapomorphies of all other catfishes except for diplomystids and in some stances also hypsidorids", without specifying, however, which are these synapomorphies (de Pinna, 1998 : 292).

Our observations and comparisons strongly support the hypotheses of Mo (1991) and de Pinna (1998) concerning the phylogenetic position of the cetopsids within the Siluriformes. In fact, although the cetopsids are characterised by numerous derived, synapomorphic features (see above), they lack some apomorphic features that are present in the vast majority of the non-diplomystid catfishes, which are described below. It is important to notice that these phylogenetic results were corroborated by an explicit phylogenetic comparison of 440 morpho-

logical characters, concerning the bones, muscles, cartilages and ligaments of both the cephalic region and the pectoral girdle, in 87 genera representing all the extend catfish families (DIOGO, 2005).

Pronounced ankylosis between the cleithrum and the scapulo-coracoid. As stated by DIOGO et al. (2001b), the plesiomorphic condition for catfishes is that in which the scapulo-coracoid is just loosely ankylosed with the cleithrum. Such a plesiomorphic condition is found in diplomystids (DIOGO et al., 2001b) and cetopsids (see, e.g., Fig. 6). In the vast majority of the catfishes, including the fossil hypsidorids, there is a pronounced ankylosis between the cleithrum and the scapulo-coracoid (see DIOGO et al., 2001b).

Abductor profundus originated in the medial surface of the pectoral girdle. In catfish closest relatives, as well as in the diplomystids (DIOGO et al., 2001b) and the cetopsids, the abductor profundus, although well-developed, does not reach medially to the median line (it should be noted that, due to the state of conservation of the fossil hypsidorids reported so far, it is not possible to appraise the configuration of the adductor profundus in these catfishes). In the vast majority of the other catfishes, excluding the plotosids (DIOGO et al., 2001b) and the nematogenyids, silurids and heptapterines (pers. obs), the abductor profundus originates on the medial surface of the pectoral girdle, at the level of the interdigitations between the scapulo-coracoids (see DIOGO et al., 2001b).

Arrector dorsalis differentiated into two broad, separated sections. In diplomystid (DIOGO et al., 2001b) and cetopsid (see, e.g., Fig. 4) catfishes the muscle arrector ventralis is made up of a single mass of fibers partially bifurcated medially, a configuration that, according to DIOGO et al. (2001b) represents the plesiomorphic condition for the Siluriformes (it should be noted that, due to the state of conservation of the fossil hypsidorids reported so far, it is not possible to appraise the configuration of the adductor profundus in these catfishes). In the vast majority of the catfishes the arrector dorsalis is divided into two broad, separated sections (DIOGO et al., 2001b).

Broad scapulo-coracoid suturing medially with its counterpart. Plesiomorphically in siluriforms the scapulo-coracoid is a slender structure with a thin median process, which does not suture with its counterpart medially (BORNBUSCH, 1995; GRANDE & DE PINNA, 1998; DIOGO et al., 2001b). Such a configuration of the scapulo-coracoid is only found in the diplomystids, cetopsids (see, e.g., Fig. 6), trichomycterids, nematogenyids, astroblepids and silurids. The other catfishes, including the fossil hypsidorids, present a broad scapulo-coracoid suturing medially with its counterpart (see DIOGO et al., 2001b). According to BORNBUSCH (1995) and GRANDE & DE PINNA (1998) the slender scapulo-coracoid with no mesial suture with its counterpart present in the trichomycterids, nematogenyids, astroblepids and silurids is very likely the result of a secondary homoplastic reversion of the apomorphic situation found in the vast majority of the catfishes.

These characters, together with the papers of MO (1991) and DE PINNA (1998), strongly suggest that the Cetopsidae occupy a rather basal position within the Siluriformes. As noted by DE PINNA (1998 : 292), this "may seem contradictory, since cetopsids are considered as highly specialised catfishes in most of the specialised literature". In fact, the cetopsids present several peculiar apomorphic morphological features, as it is clearly indicated by the numerous cetopsid synapomorphies described in DE PINNA & VARI's 1995 study and in the present paper (see above). However, as argued by DE PINNA (1998 : 292), the presence of numerous apomorphic features in the cetopsids is not contradictory with a rather basal position of these catfishes within the Siluriformes, since it indicates, precisely, that the cetopsids "have a long history independent of that of other catfishes".

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On the osteology and myology of the cephalic region and pectoral girdle of *Nematogenys inermis* (Ghichenot, 1848), with comments on the autapomorphies and phylogenetic relationships of the Nematogenyidae (Teleostei : Siluriformes)

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ABSTRACT. The cephalic and pectoral girdle structures of *Nematogenys inermis* are described and compared to those of other catfishes, as the foundation for a discussion on the autapomorphies and phylogenetic relationships of the Nematogenyidae. Our observations and comparisons indicate that nematogenyids are defined, at least, by two unique, autapomorphic characters, namely : 1) anterior margin of prevomer markedly extended anteriorly, at about the same level of anterior margin of mesethmoid; 2) anterior ceratohyal with prominent, posteriorly pointed, posterodorsal projection bordering a significant part of the dorsal margin of the posterior ceratohyal. With respect to the phylogenetic relationships of the Nematogenyidae, this study supports Mo's 1991 and de Pinna's 1992 phylogenetic hypotheses according to which the nematogenyids and the trichomycterids are sister-groups.

KEY WORDS : catfish, cephalic region, Loricarioidea, morphology, myology, Nematogenyidae, osteology, pectoral girdle, phylogeny, Siluriformes.

INTRODUCTION

The Siluriformes are one of the most economically important groups of fresh and brackish water fishes in the world and, in many countries, form a significant part of inland fisheries (Teugels, 1996). Among the 35 siluriform families (Ferraris & de Pinna, 1999), the Nematogenyidae, a small family of Chilean catfishes including a single species, *Nematogenys inermis* (Ghichenot, 1848), is surely one of the less studied, with "little being known about the anatomy" of these fishes (de Pinna, 1998 : 295). In fact, despite the large number of works concerning catfish anatomy (e.g., McMurrich, 1884; Regan, 1911; de Beer, 1937; Alexander, 1965; Gosline, 1975; Ghiot, 1978; Ghiot et al., 1984; Arratia, 1990; Mo, 1991; Diogo & Chardon, 2000ab; Diogo et al., 2000, 2001ab; etc.), the only papers describing the morphology of nematogenyids with some detail are those of Arratia & Chang (1975), Arratia (1990), Arratia & Huaquin (1995) and Azpelicueta & Rubilar (1998). Moreover, as these descriptions are almost exclusively restricted to the osteology and external anatomy of the nematogenyids, important aspects of the morphology of these fishes are poorly known, such as the configuration of their pectoral girdle, the structures associated with their mandibular barbels, or the muscles and ligaments of their cephalic region and their pectoral girdle. The lack of studies concerning the morphology of the nematogenyids probably explains why, although these fishes are commonly grouped in a separate family, Nematogenyidae, not even one single unique, autapomorphic character has been suggested so far to define this family (see Diogo, 2003).

The aim of this work is, thus, to study the osteological and myological structures of the cephalic region (branchial apparatus excluded) and pectoral girdle of *Nematogenys inermis*, and to compare these structures with those of members of all other siluriform families as the foundation for a discussion on the autapomorphies and phylogenetic relationships of the Nematogenyidae. It is also hoped that this study could increase the knowledge of the anatomy and phylogeny of the catfishes in general, as well as pave the way for future works concerning the comparative anatomy, evolution, functional morphology, palaeontology, eco-morphology and particularly the phylogeny of these fishes.

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 National Museum of Natural History of Washington (USNM), and from the South African Institute for Aquatic Biodiversity (SAIAB) and the Albany Museum of Grahamstown (AMG). 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 alcohol fixed (alc), trypsin-cleared and alizarine-stained (c&s), or simply alizarine-stained (s) condition of the studied fishes is given in parentheses following the number of specimens dissected. A list of the specimens dissected is given below.

Akysidae : *Akysis baramensis* LFEM, 2 (alc). *Akysis leucorhynchus* USNM 109636, 2 (alc). *Parakysis anomalopteryx* USNM 230307, 2 (alc); LFEM, 1 (alc).

Amblycipitidae : *Amblyceps caecutiens* LFEM, 2 (alc). *Amblyceps mangois* USNM 109634, 2 (alc). *Liobagrus reini* USNM 089370, 2 (alc).

Amphiliidae : *Amphilius brevis* MRAC 89-043-P-403, 3 (alc); MRAC 89-043-P-2333, 1 (c&s). *Andersonia leptura* MNHN 1961-0600, 2 (alc). *Belonoglanis tenuis* MRAC P.60494, 2 (alc). *Doumea typica* MRAC 93-041-P-1335, 1 (alc). *Leptoglanis rotundiceps* MRAC P.186591-93, 3 (alc). *Paramphilius trichomycteroides* LFEM, 2 (alc). *Phractura brevicauda* MRAC 90-057-P-5145, 2 (alc); MRAC 92-125-P-386, 1 (c&s). *Phractura intermedia* MRAC 73-016-P-5888, 1 (alc). *Trachyglanis ineac* MRAC P.125552-125553, 2 (alc). *Zaireichthys zonatus* MRAC 89-043-P-2243-2245, 3 (alc).

Ariidae : *Arius hertzbergii* LFEM, 1 (alc). *Arius heudelotii* LFEM, 4 (alc). *Bagre marinus* LFEM, 1 (alc); LFEM, 1 (c&s). *Genidens genidens* LFEM, 2 (alc).

Aspredinidae : *Aspredo aspredo* USNM 226072, 1 (alc). *Aspredo sicuephorus* LFEM, 1 (alc). *Bunocephalus knerii* USNM 177206, 2 (alc). *Xyliphius magdalenae* USNM 120224, 1 (alc).

Astroblepidae : *Astroblepus phelpis* LFEM, 1 (alc); USNM 121127, 2 (alc).

Auchenipteridae : *Ageneiosus vittatus* USNM 257562, 1 (alc). *Auchenipterus dentatus* USNM 339222, 1 (alc). *Centromochlus hechelii* USNM 261397, 1 (alc).

Austroglanididae : *Austroglanis gilli* LFEM, 3 (alc); SAIAB 58416 (c&s). *Austroglanis sclateri* AMG, 1 (c&s); SAIAB 68917 (s).

Bagridae : *Bagrichthys macropterus* USNM 230275, 1 (alc). *Bagrus bayad* LFEM, 1 (alc); LFEM, 1 (c&s). *Bagrus docmak* MRAC 86-07-P-512, 1 (alc); MRAC 86-07-P-516, 1 (c&s). *Hemibagrus nemurus* USNM 317590, 1 (alc). *Rita chrysea* USNM 114948, 1 (alc).

Callichthyidae : *Callichthys callichthys* USNM 226210, 2 (alc). *Corydoras guianensis* LFEM, 2 (alc).

Cetopsidae : *Cetopsis coecutiens* USNM 265628, 2 (alc). *Helogenes marmuratus* USNM 264030, 1 (alc). *Hemicetopsis candiru* USNM 167854, 1 (alc).

Chacidae : *Chaca bankanensis* LFEM, 3 (alc). *Chaca burmensis* LFEM, 2 (alc). *Chaca chaca* LFEM, 2 (alc).

Clariidae : *Clarias anguillaris* LFEM, 2 (alc). *Clarias batrachus* LFEM, 2 (alc). *Clarias ebriensis* LFEM, 2 (alc). *Clarias gariepinus* MRAC 93-152-P-1356, 1 (alc), LFEM, 2 (alc). *Heterobranchus bidorsalis* LFEM, 2 (alc). *Heterobranchus longifilis* LFEM, 2 (alc). *Uegitglanis zammaronoi* MRAC P-15361, 1 (alc).

Claroteidae : *Auchenoglanis biscutatus* MRAC 73-015-P-999, 2 (alc). *Auchenoglanis occidentalis* LFEM, 2 (alc). *Chrysichthys auratus* UNB, 2 (alc); UNB, 2 (c&s). *Chrysichthys nigrodigitatus* UNB, 2 (alc); UNB, 2 (c&s). *Clarotes laticeps* MRAC 73-13-P-980, 2 (alc).

Cranoglanididae : *Cranoglanis bouderius* LFEM, 2 (alc).

Diplomystidae : *Diplomystes chilensis* LFEM, 3 (alc).

Doradidae : *Acanthodoras cataphractus* USNM 034433, 2 (alc). *Anadoras weddellii* USNM 317965, 2 (alc). *Doras brevis* LFEM, 2 (alc). *Doras punctatus* USNM 284575, 2 (alc). *Franciscodoras marmoratus* USNM 196712, 2 (alc).

Erethistidae : *Erethistes pusillus* USNM 044759, 2 (alc). *Hara filamentosa* USNM 288437, 1 (alc).

Heteropneustidae : *Heteropneustes fossilis* USNM 343564, 2 (alc); USNM 274063, 1 (alc); LFEM, 2 (alc).

Ictaluridae : *Amiurus nebolosus* USNM 246143, 1 (alc); USNM 73712, 1 (alc). *Ictalurus furcatus* LFEM, 2 (alc). *Ictalurus punctatus* USNM 244950, 2 (alc).

Loricariidae : *Hypoptopoma bilobatum* LFEM, 2 (alc). *Hypoptopoma inexspectata* LFEM, 2 (alc). *Lithoxus lithoides* LFEM, 2 (alc). *Loricaria cataphracta* LFEM, 1 (alc). *Loricaria loricaria* USNM 305366, 2 (alc); USNM 314311, 1 (alc).

Malapteruridae : *Malapterurus electricus* LFEM, 5 (alc).

Mochokidae : *Mochokus niloticus* MRAC P.119413, 1 (alc); MRAC P.119415, 1 (alc). *Synodontis clarias* USNM 229790, 1 (alc). *Synodontis schall* LFEM, 2 (alc). *Synodontis sorex* LFEM, 2 (alc).

Nematogenyidae : *Nematogenys inermis* USNM 084346, 2 (alc); LFEM, 2 (alc).

Pangasiidae : *Helicophagus leptorhynchus* USNM 355238, 1 (alc). *Pangasius larnaudii* USNM 288673, 1 (alc). *Pangasius sianensis* USNM 316837, 2 (alc).

Pimelodidae : *Calophysus macropterus* USNM 306962, 1 (alc). *Goeldiella eques* USNM 066180, 1 (alc). *Hepapterus mustelinus* USNM 287058, 2 (alc). *Hypophthalmus edentatus* USNM 226140, 1 (alc). *Microglanis cottoides* USNM 285838, 1 (alc). *Pimelodus blochii* LFEM, 2 (alc). *Pimelodus clarias* LFEM, 2 (alc); USNM 076925, 1 (alc). *Pseudopimelodus raninus* USNM 226136, 2 (alc). *Pseudoplatystoma fasciatum* USNM 284814, 1 (alc). *Rhamdia guatemalensis* USNM 114494, 1 (alc).

Plotosidae : *Cnidoglanis macrocephalus* USNM 219580, 2 (alc). *Neosilurus rendahli* USNM 173554, 2 (alc). *Paraplotosus albilabris* USNM 173554, 2 (alc). *Plotosus anguillaris* LFEM, 2(alc). *Plotosus lineatus* USNM 200226), 2 (alc).

Schilbidae : *Ailia colia* USNM 165080, 1 (alc). *Laides hexanema* USNM 316734, 1 (alc). *Pseudeutropius brachypopterus* USNM 230301, 1 (alc). *Schilbe intermedius* MRAC P.58661, 1 (alc). *Schilbe mystus* LFEM, 3 (alc). *Siluranodon auritus* USNM 061302, 2 (alc).

Scoloplacidae : *Scoloplax distolothrix* LFEM, 1 (alc); USNM 232408, 1 (alc).

Siluridae : *Silurus aristotelis* LFEM, 2(alc). *Silurus glanis* LFEM, 2 (alc). *Silurus asotus* USNM 130504, 2 (alc). *Wallago attu* USNM 304884, 1 (alc).

Sisoridae : *Bagarius yarreli* USNM 348830, 2 (alc); LFEM, 1 (c&s). *Gagata cenia* USNM 109610, 2 (alc). *Glyptosternon reticulatum* USNM 165114, 1 (alc). *Glyptothorax fukiensis* USNM 087613, 2 (alc).

Trichomycteridae : Hatcheria macraei LFEM, 2 (alc). Trichomycterus areolatus LFEM, 2 (alc). Trichomycterus banneaui LFEM, 2 (alc). Trichomycterus immaculatus USNM 301015, 2 (alc).

RESULTS

In the anatomical descriptions of *N. inermis* the nomenclature for the osteological structures of the cephalic region follows basically that of ARRATIA (1997). However, for the several reasons explained in detail in our recent papers (DIOGO et al., 2001a; DIOGO & CHARDON, 2003), with respect to the skeletal components of the suspensorium we follow DIOGO et al. (2001a). The myological nomenclature is based mainly on WINTERBOTTOM (1974), but for the different adductor mandibulae sections, DIOGO & CHARDON (2000a) is followed. In relation to the muscles associated with the mandibular barbels, which were not studied by WINTERBOTTOM (1974), DIOGO & CHARDON (2000b) is followed. Concerning the nomenclature of the pectoral girdle bones and muscles, DIOGO et al. (2001b) is followed.

Osteology

Os mesethmoideum. Unpaired bone situated on the antero-dorsal surface of the neurocranium (Figs 1, 2). Its anterior tip is forked with two slender branches. The antero-ventro-lateral margins of the bone are ligamentously connected to the premaxillae. The mesethmoid does not reach the anterior border of the anterior fontanel, which is exclusively surrounded by the frontals.

Os lateroethmoideum. The lateral-ethmoid (Fig. 1) presents a laterally directed articulatory facet for the autopalatine at its anterolateral margin. The anterolateral arms of the lateral-ethmoid extend laterally well beyond the lateral margins of the frontals (Fig. 2).

Os praevomerale. Large, unpaired T-shaped bone without a ventral tooth plate. Its anterior margin extends anteriorly, almost reaching the anterior margin of the mesethmoid (Fig. 2).

Os parasphenoideum. The unpaired parasphenoid is the longest bone of the cranium (Fig. 2), bearing a pair of ascending flanges that suture with the pterosphenoids and prootics.

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

Os pterosphenoideum. Posterior to the orbitosphenoid (Fig. 2), covering, together with this bone, the gap between the frontals and the parasphenoid. Together with the prootic and the orbitosphenoid, the pterosphenoid borders the large foramen of the trigemino-facial nerve complex, with a part of the bone being situated dorsally to this foramen and the other part being situated ventrally to it (Fig. 2).

Os frontale. The frontals (Figs 1, 2) are large rectangular bones that constitute a great part of the cranial roof. Posteriorly, they have a lateral extension, which sutures with the sphenotics. The frontals are largely separated by two median fontanels. The anterior median fontanel is exclusively surrounded by the frontals, while the posterior one is surrounded by both these bones and the parieto-supraoccipital.

Os sphenoticum. Smaller than the pterotic, constituting, together with this bone, an articulatory facet for the hyomandibulo-metapterygoid (Fig. 2). The sphenotic presents a prominent anterodorsolateral projection (Figs 1, 2) for the origin of the levator arcus palatini (Fig. 1).

Os pteroticum. Large bone (Figs 1, 2) contacting the sphenotic anteriorly, the parieto-supraoccipital laterally, and the posttemporo-supracleithrum posteriorly. In a ventral view of the neurocranium, it contacts the sphenotic and the prootic anteriorly, the exoccipital mesially, and the posttemporo-supracleithrum posteriorly (Fig. 2).

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

Os epioccipitale. The epioccipitals are small bones situated on the posterodorsal surface of the cranial roof. They are in contact with the parieto-supraoccipital, the posttemporo-supracleithra, the pterotics and the exoccipitals.

Os exoccipitale. The large exoccipitals are situated laterally to the basioccipital (Fig. 2). There is a very small foramen on the posteroventral margin of each exoccipital.

Os *basioccipitale.* Unpaired bone, forming the posteriormost part of the floor of the neurocranium (Fig. 2). It presents two long, thin, posteroventrolateral projections that are ligamentously connected to the thin ventromedial limbs of the posttemporo-supracleithra.

Os parieto-supraoccipitale. Large, unpaired bone constituting the postero-dorso-median surface of the cranial roof (Fig. 1). It presents a thin, somewhat triangular posterior process. As stated anteriorly, together with the frontals the parieto-supraoccipital borders the posterior median fontanel of the skull.

Os angulo-articulare. This bone (Figs 1, 3, 4), together with the dentary bone, the coronomeckelian bone, and the Meckel's cartilage, constitute the mandible. Postero-dorsally, the angulo-articular has an articulatory facet for the quadrato-symplectic. Postero-ventrally, it is ligamentously connected to both the interopercle and the posterior ceratohyal (Fig. 5).

Os dentale. The toothed dentary bone (Fig. 1) forms a great part of the lateral surface of the mandible. The postero-dorsal margin of this bone forms, together with the antero-dorsal margin of the angulo-articular, a broad dorsal process (processus coronoideus) (Figs 3, 4), the dorsal tip of which is curved medially and projects mesially somewhat beyond the main body of the mandible (Fig. 4).

Os coronomeckelium. This bone is lodged in the medial surface of the mandible (Fig. 3). Posterodorsally it bears a small crest for attachment of the adductor mandibulae A3'-d.

Os praemaxillare. The large premaxillae (Fig. 1) bear ventrally a tooth-plate with numerous large teeth having their tips slightly turned backward.

Os maxillare. The maxillary bone is connected to the coronoid process of the mandible by a strong, massive ligament (primordial ligament) (Fig. 1). As in most catfishes, the maxillary barbels are supported by the maxillary bones.

Os autopalatinum. The autopalatine (Figs 1, 2) is a large, somewhat flat bone with its posterior end capped by a small cartilage and its anterior end tipped by a large cartilage with two antero-lateral concavities that accept the two proximal heads of the maxilla. Dorsomedially, the autopalatine articulates, by means of a large, dorsomedially directed, articulatory surface, with the lateral ethmoid.

Os hyomandibulo-metapterygoideum. The homology, and, thus, the correct denomination, of this bone, as well as of the other suspensorium elements of catfish, has been the subject of endless controversies (McMurrich, 1884; De Beer, 1937; Hoedeman, 1960; Gosline, 1975; Howes, 1983ab, 1985; Arratia, 1990, 1992; Diogo el al., 2001a; Diogo & Chardon, 2003; etc.). As referred above, for the several reasons explained in detail in our recent papers (Diogo et al., 2001a; Diogo & Chardon, 2003), the nomenclature used here to describe these elements will follows strictly that presented by Diogo et al. (2001a). The hyomandibulo-metapterygoid (Figs 1, 2) is a large bone articulating dorsally with both the pterotic and the sphenotic and posteriorly with the opercular bone. Posteriorly to its cartilaginous articulation with the neurocranium, it presents a large posterodorsal extension, which is strongly connected, by means of connective tissue, with the ventrolateral margin of the pterotic, and from which originates a great part of the fibres of the levator operculi (Fig. 1).

Os sesamoideum 1. Small bone (Fig. 2) attached by means of a short but strong ligament to the anteromesial margin of the ento-ectopterygoid posteriorly and by means of a long and thick ligament to the ventrolateral margin of the prevomer anteriorly. The sesamoid bones 2 and 3 (see Diogo et al., 2001a) are absent.

Os entopterygoideo-ectopterygoideum. Large bone, its anteromesial and its anterolateral surfaces being connected to the sesamoid bone 1 of the suspensorium and to the autopalatine, respectively (Fig. 2). Posteriorly, the entopterygoideo-ectopterygoid is firmly associated with both the hyomandibulo-metapterygoid and the quadrato-symplectic.

Os quadrato-symplecticum. The quadrato-symplectic (Fig. 2) contacts the entopterygoideo-ectopterygoid anterodorsally, the hyomandibulo-metapterygoid posterodorsally, and the preopercle posteroventrally. It presents a large, anterior articulatory surface to articulate with the postero-dorsal surface of the angulo-articular.

Os praeoperculare. Long and thin bone (Fig. 1) firmly sutured to both the hyomandibulo-metapterygoid and the quadrato-symplectic. As is most other catfishes, the preopercle encloses a sensory canal, which exits the neurocranium and passes, via the preopercle, into the mandible.

Os operculare. The opercle (Figs 1, 2) is a large, roughly triangular bone attached ventrally, by means of connective tissue, to the interopercle. It presents a large, anterior articulatory surface for the hyomandibulo-metapterygoid. Anterodorsally, it presents a prominent, dorsally directed, roughly triangular process.

Os interoperculare. Its anterior surface is ligamentously connected to the postero-ventral margin of the mandible (Figs 1, 5). Medially, the interopercle is ligamentously connected to the lateral surface of the posterior ceratohyal.

Os ceratohyale anterior. Elongated bone that supports, together with the posterior ceratohyal, the eleven branchiostegal rays present on each side of the fish (Fig. 5). The anterior head of the branchiostegal ray 10 lies at the level of the cartilage situated between the anterior and the posterior ceratohyal, with the anterior head of the branchiostegal ray 11 being exclusively supported by the posterior ceratohyal and the remaining nine branchiostegal rays being exclusively supported by the anterior ceratohyal. The anterior ceratohyal presents a prominent posterodorsolateral projection, which borders, but is not sutured to, a significant part of the dorsolateral margin of the posterior ceratohyal (Fig. 5).

Os ceratohyale posterior. Somewhat triangular bone (Fig. 5) ligamentously connected to the postero-ventral edge of the mandible and to the medial surface of the interopercle. The interhyals are missing.

Os hypohyale ventrale. The ventral hypohyals (Fig. 5) are ligamentously connected to the antero-lateral edges of the parurohyal.

Os hypohyale dorsale. The dorsal hypohyals are small bones situated dorsally to the ventral hypoyals.

Os parurohyale. The parurohyal (Fig. 5) is a somewhat triangular, unpaired bone lying medially behind the ventromedial surfaces of the ventral hypohyals and being connected to these bones by means of two strong, thick ligaments.

Os posttemporo-supracleithrum. Large bone (Figs 1, 2), its dorso-medial limb being firmly associated to the epioccipital and the pterotic. Its ventro-medial limb is thin and ligamentously connected, by means of a thin and short ligament, to the basiocccipital. The posteromesial surface of the posttemporo-supracleithrum is attached, by means of connective tissue, to the parapophyses of the complex centrum, which encapsulate the reduced swimbladder.

Os cleithrum. The cleithra (Figs 6, 7) are large, well-ossified stout structures forming the greatest part of the pectoral girdle and the posterior boundary of the branchial chamber. They are attached in the antero-medial line via connective tissue. Each cleithrum bears a crescentic, medially faced groove that accommodates the proximal portion of the pectoral spine, which presents a somewhat rectangular dorsomedian process (Fig. 8 : pec-sp-dmp) for the insertion of the abductor profundus.

Os scapulo-coracoideum. Large bone (Fig. 7), it does not contact its counterpart in the anteromedial line. Postero-laterally, the scapulo-coracoid bears two condyles, which articulate, respectively, with the pectoral spine and

the complex radial (see MO, 1991). The mesocoracoid arch is present (Fig. 7).

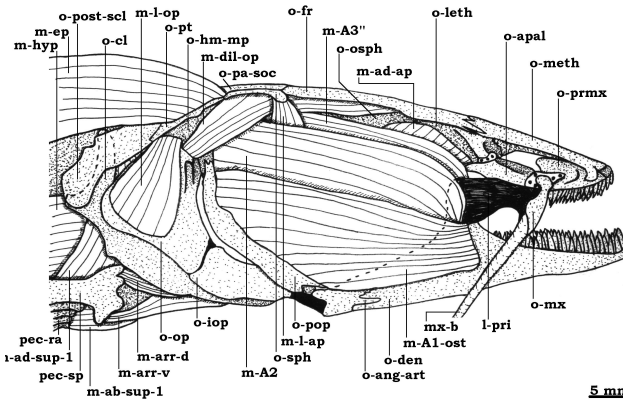

Fig. 1. – Lateral view of the cephalic musculature of *Nematogenys inermis*. *l-pri*, ligamentum primordium; *m-A1-ost, m-A2, m-A3'',* sections of musculus adductor mandibulae; *m-ad-ap*, musculus adductor arcus palatini; m-ab-sup-1, section 1 of musculus abductor superficialis; m-ad-sup-1, section 1 of musculus adductor superficialis; *m-arr-d*, musculus arrector dorsalis; *m-arr-v*, musculus arrector ventralis; *m-dil-op*, musculus dilatator operculi; *m-ep,* musculus epaxialis; *m-hyp,* musculus hypaxialis; *m-l-ap*, musculus levator arcus palatini; *m-l-op*, musculus levator operculi; *mx-b*, maxillary barbel; *o-ang-art*, os angulo-articulare; *o-apal*, os autopalatinum; *o-cl*, os cleithrum; *o-den*, os dentale; *o-fr*, os frontale; *o-hm-mp*, os hyomandibulo-metapterygoideum; *o-iop*, os interoperculare; *o-leth*, os latero-ethmoideum; *o-meth*, os mesethmoideum; *o-mx*, os maxillare; *o-op*, os operculare; *o-osph*, os orbitosphenoideum; *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-sph*, os sphenoticum; *pec-ra*, pectoral rays; *pec-sp*, pectoral spine.

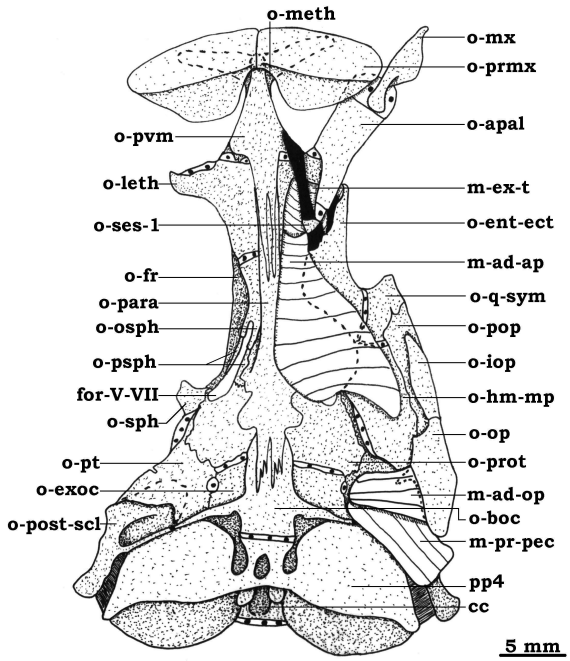

Fig. 2. – Ventral view of the neurocranium of *Nematogenys inermis*. On the right side the suspensorium, as well as the autopalatine, maxilla, adductor arcus palatini, extensor tentaculi, adductor operculi and protractor pectoralis, are also illustrated. Premaxillary teeth were removed. *cc*, complex centrum; *for-V-VII,* trigemino-facialis foramen; *m-ad-ap*, musculus adductor arcus palatini; *m-ad-op*, musculus adductor operculi; *m-ex-t*, musculus extensor tentaculi; *m-pr-pec*, musculus protractor pectoralis; *o-apal*, os autopalatinum; *o-boc*, os basioccipitale; *o-ent-ect*, os entopterygoideo-ectopterygoideum; *o-exoc*, os exoccipitale; *o-fr*, os frontale; *o-hm-mp*, os hyomandibulo-metapterygoideum; *o-iop,* os interoperculare; *o-leth*, os latero-ethmoideum; *o-meth*, os mesethmoideum; *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-sym*, os quadrato-symplecticum; *o-ses-1*, sesamoid bone 1 of the suspensorium; *o-sph*, os sphenoticum; *pp4*, parapophysis 4.

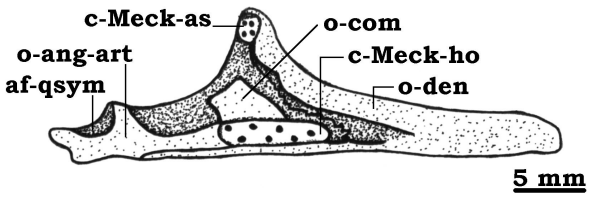

Fig. 3. – Medial view of the left mandible of *Nematogenys inermis*, with mandibular teeth removed. *af-qsym*, articulatory facet for os quadrato-symplecticum; *c-Meck-as, c-Meck-ho*, ascending and horizontal portions of cartilago Meckeli; *o-ang-art*, os angulo-articulare; *o-com*, os coronomeckelium; *o-den*, os dentale.

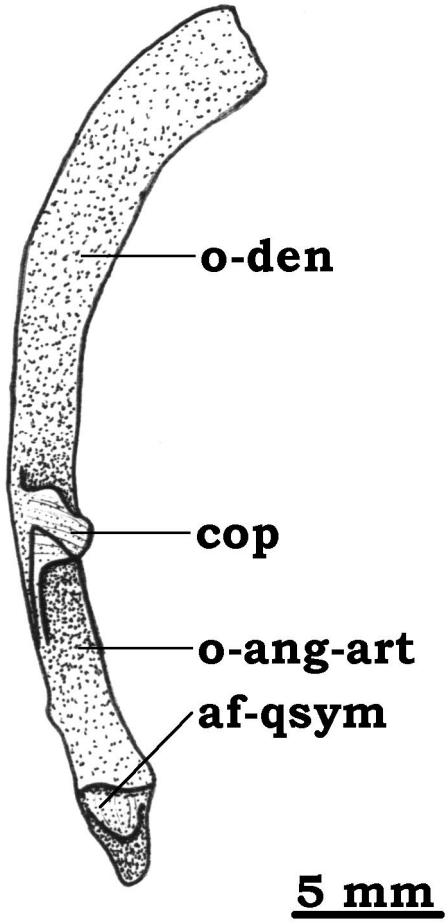

Fig. 4. – Dorsal view of the left mandible of *Nematogenys inermis*, with mandibular teeth removed. *af-qsym*, articulatory facet for os quadrato-symplecticum; *cop,* coronoid process of mandible; *o-ang-art*, os angulo-articulare; *o-den*, os dentale.

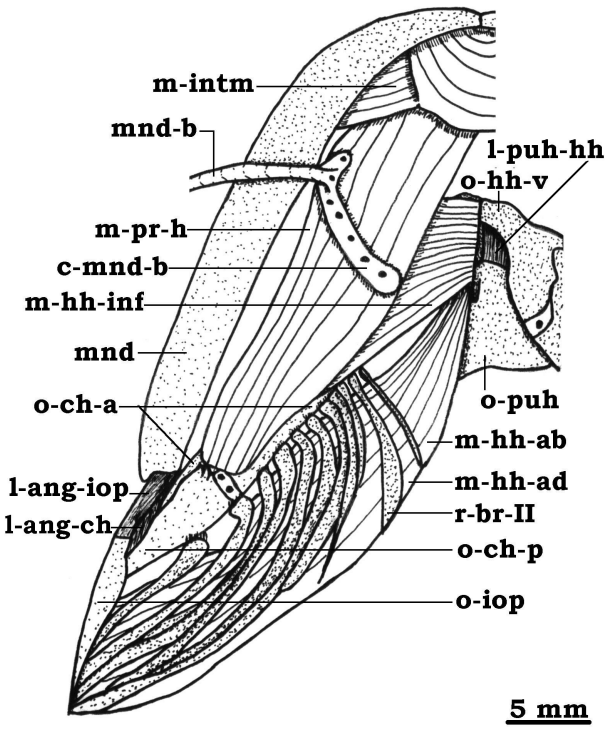

Fig. 5. – Ventral view of the cephalic musculature of *Nematogenys inermis*. On the right side, all the muscles, as well as the mandibular barbels and their associated cartilages, were removed. *c-mnd-b*, cartilago mandibularis tentaculi; *l-ang-ch,* ligamentum angulo-ceratohyale; *l-ang-iop,* ligamentum angulo-interoperculare; *l-puh-hh,* ligamentum parurohyalo-hypohyale; *mnd-b,* mandibular barbel; *m-hh-ab*, musculus hyohyoideus abductor; *m-hh-ad*, musculus hyohyoideus adductor; *m-hh-inf*, musculus hyohyoideus inferior; *m-intm*, musculus intermandibularis; *mnd,* mandible; *m-pr-h*, musculus protactor hyoideus; *o-ch-a*, os ceratohyale anterior; *o-ch-p*, os ceratohyale posterior; *o-hh-v,* os hypohyale ventrale; *o-iop*, os interoperculare; *o-puh*, os parurohyale; *r-br-II*, radius branchiostegus II.

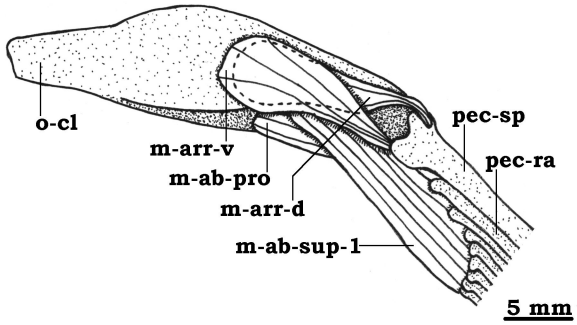

Fig. 6. – Ventral view of the pectoral girdle musculature of *Nematogenys inermis*. *m-ab-pro*, musculus abductor profundus; *m-ab-sup-1*, section 1 of musculus abductor superficialis; *m-arr-d*, musculus arrector dorsalis; *m-arr-v,* musculus arrector ventralis; *o-cl*, os cleithrum; *pec-ra*, pectoral rays; *pec-sp*, pectoral spine.

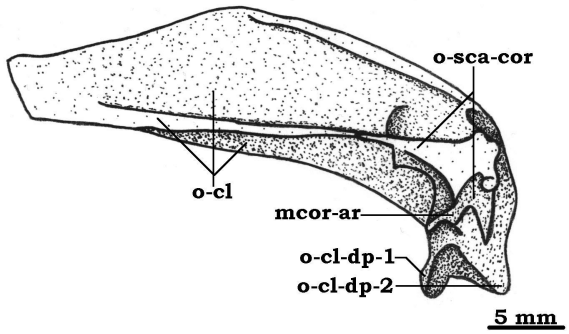

Fig. 7. – Ventral view of the pectoral girdle of *Nematogenys inermis*. The pectoral spine and pectoral rays, as well as the muscles associated with these structures, were removed. *mcor-ar*, mesocoracoid arch; *o-cl*, os cleithrum; *o-cl-dp-1, o-cl-dp-2*, dorsal process 1 and 2 of os cleithrum; *o-sca-cor*, os scapulo-coracoide.

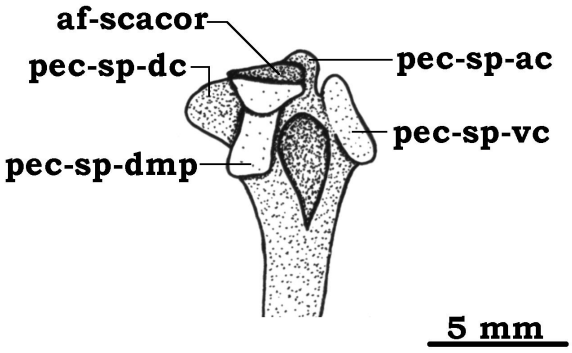

Fig. 8. – Medial view of the proximal portion of the left pectoral spine of *Nematogenys inermis*. *af-scacor*, articulatory facet for os scapulo-coracoid; *pec-sp-ac,* anterior condyle of pectoral spine; *pec-sp-dc,* dorsal condyle of pectoral spine; *pec-sp-dmp*, dorsomedial process of pectoral spine; *pec-sp-vc*, ventral condyle of pectoral spine.

Myology

Musculus adductor mandibulae. The adductor mandibulae A1-ost originates on the preopercle and the quadrato-symplectic and inserts on both the dorsolateral and the lateral surfaces of the angulo-articular and the dentary bone (Fig. 1). The A2 (Fig. 1), which lies dorso-mesially to the A1-ost, runs from the preopercle and hyomandibulo-metapterygoid to the medial surface of the dentary bone. The adductor mandibulae A3' is divided into a dorsal and a ventral part. The dorsal one (A3'-d), originates on the hyomandibulo-metapterygoid and inserts tendinously on the coronomeckelian bone, while the ventral one (A3'-v) originates on the quadrato-symplectic and inserts on the medial surface of the angulo-articular. The adductor mandibulae A3'' (Fig. 1), situated mesially to the A3' and to the levator arcus palatini, runs from the hyomandibulo-metapterygoid, sphenotic and entopterygoideo-ectopterygoid to the mesial surface of both the angulo-articular and the coronomeckelian bone. There is no adductor mandibulae Aω.

Musculus levator arcus palatini. The levator arcus palatini (Fig. 1) is situated medial to the adductor mandibulae A3'. It originates on the sphenotic and inserts on the lateral face of the hyomandibulo-metapterygoid.

Musculus adductor arcus palatini. This muscle (Figs 1, 2) runs from the parasphenoid, pterosphenoid, orbitosphenoid and lateral ethmoid to the hyomandibulo-metapterygoid, quadrate-symplectic and the ento-ectopterygoid.

Musculus levator operculi. It originates on both the ventro-lateral margin of the pterotic and the posterodorsal surface of the hyomandibulo-metapterygoid and inserts on a great part of the lateral surface of the opercle (Fig. 1).

Musculus adductor operculi. Situated medially to the levator operculi, it runs from the ventral surface of the pterotic to the dorso-medial surface of the opercle (Fig. 2). There is no adductor hyomandibularis (sensu DIOGO et al., 2002; DIOGO & VANDEWALLE, 2003).

Musculus dilatator operculi. The dilatator operculi (Fig. 1) is a large muscle originating on the pterotic, frontal and hyomandibulo-metapterygoid, passing laterally to the adductor mandibulae A2, and inserting on the antero-dorsal margin of the opercle.

Musculus extensor tentaculi. The extensor tentaculi 1 (Fig. 2) runs from the lateral ethmoid to the posteromedian surface of the autopalatine. Some fibres of this muscle, as well as some fibres of the adductor arcus palatini, are also associated with the sesamoid bone 1 of the suspensorium and its associated ligaments. There is no retractor tentaculi.

Musculus protractor hyoidei. This muscle (Fig. 5) is constituted by a single, voluminous mass of fibres, in which are lodged the cartilages associated with the mandibular barbels. It originates on the anterior ceratohyal and inserts anteriorly on the anteromesial surface of the dentary bone. There are no small, additional muscles (see DIOGO & CHARDON, 2000b) associated with the mandibular barbels.

Musculus intermandibularis. Large muscle joining the two mandibles (Fig. 5). It should be noted that the intermandibularis can somewhat be subdivided into three parts : in the mesial part, its fibers run rather rostrally, while in the parts situated laterally to this mesial part the fibers run rather laterally until they attach to the mesial surface of each mandible.

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

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

Musculus hyohyoideus adductor. Each hyohyoideus adductor (Fig. 5) interconnects the branchiostegal rays of the respective side, with the most lateral fibers of this muscle also attaching on the mesial surface of the opercular bone.

Musculus sternohyoideus. Large, roughly triangular muscle. It runs from the posterior portion of the parurohyal to the anterior portion of the cleithrum. The poster-

oventral fibers of the sternohyoideus cover ventrally the anterior portion of the hypoaxialis.

Musculus arrector ventralis. The arrector ventralis runs from the ventral surface of the cleithrum to the ventral condyle of the pectoral spine (Fig. 6).

Musculus arrector dorsalis. This muscle (Fig. 6), dorsal to the arrector ventralis, originates on the ventral surface of the cleithrum and inserts on the antero-lateral edge of the pectoral spine.

Musculus abductor superficialis. This muscle is differentiated into two sections. The larger section (Fig. 6 : m-ab-sup-1) runs from the ventral margin of both the scapulo-coracoid and the cleithrum to the antero-ventral margin of the ventral part of the pectoral fin rays. The smaller section (m-ab-sup-2), 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 abductor profundus. This small muscle originates on the posterior surface of the scapulo-coracoid and inserts on the prominent, somewhat rectangular, dorsomedial process of the pectoral spine (Fig. 8 : pec-sp-dmp).

Musculus adductor superficialis. Situated on the posterior margin of the pectoral girdle and 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 (m-ad-sup-2) runs from both the postero-ventrolateral 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. Thick muscle (Fig. 2) running from the ventral surface of the pterotic to the antero-dorsal surface of the cleithrum.

DISCUSSION

As referred to in the Introduction, although the nematogenyids are commonly grouped in a separate family, the Nematogenyidae, no unique, autapomorphic characters have been published previously to characterise these fishes and to distinguish them from the other siluriforms. In fact, as stressed by DE PINNA (1998 : 297), the most conspicuous and distinctive character of nematogenyids usually mentioned in the literature is the broad insertion of the levator operculi on the lateral surface of the opercle, but even this feature is present in other catfishes, namely in some pimelodids (e.g. DIOGO, 2005).

This lack of unique characters to distinguish the nematogenyids from other catfishes is probably due to their somewhat plesiomorphic general aspect (see ARRATIA & CHANG, 1975; ARRATIA & HUAQUIN, 1995), but also to the fact that, as stressed by DE PINNA (1998) and referred in the Introduction, their morphology was poorly studied so far. In fact, although our observations and comparisons confirmed the somewhat plesiomorphic condition of the nematogenyids, they have pointed out that these fishes are characterised by at least two unique, autapomorphic characters, which are described below.

Anterior ceratohyal with a prominent, posterodorsolateral projection bordering a significant part of the dorsolateral margin of the posterior ceratohyal. Plesiomorphically catfishes lack major posterior processes on the anterior ceratohyal (see, e.g., REGAN, 1911; EIGENMAN, 1925; ALEXANDER, 1965; HOWES, 1983a, 1985; MO, 1991; DE PINNA, 1996). In all specimens of *N. inermis* studied, and in no other catfish studied by us or described in the literature, there is a prominent posterodorsolateral projection of the anterior ceratohyal bordering, but not being sutured/interdigitated with, a great part of the dorsolateral margin of the posterior ceratohyal (see, e.g., Fig. 5).

Anterior portion of prevomer markedly extended anteriorly, with anterior tip of this bone being situated at about the same level as the anterior margin of the mesethmoid. Plesiomorphically in catfishes the anterior margin of the prevomer lies significantly posterior to the anterior margin of the mesethmoid (see, e.g., REGAN, 1911; EIGENMANN, 1925; DE BEER, 1937; ALEXANDER, 1965; GOSLINE, 1975; HOWES, 1983a, 1985; GHIOT et al., 1984; MO, 1991; DE PINNA, 1996; etc.). In all specimens of *N. inermis* examined the anterior portion of the prevomer is markedly extended anteriorly, with its anterior tip and the anterior margin of the mesethmoid being situated at about the same level (see, e.g., Fig. 2). The only catfishes examined where a somewhat similar condition is found are the trichomycterids, which are very likely the sister-group of the nematogenyids (see below), with this similarity thus constituting, eventually, an additional character to support this sister-group relationship. However, in the trichomycterids examined the anterior portion of the prevomer, although situated further anteriorly than in most siluriforms, does not extend at about the same level as the anterior margin of the mesethmoid, as is the case in nematogenyids. The condition found in these latter fishes constitutes, thus, a unique, autapomorphic feature within catfishes.

The exclusive presence of these two autapomorphic, unique features in the genus *Nematogenys* thus justifies, in our opinion, the placement of this genus in its own family, Nematogenyidae (see above). With respect to the phylogenetic relationships of the Nematogenyidae, our observations and comparisons pointed out two new, additional synapomorphies to support MO's (1991) and DE PINNA's (1992) studies, according to which this family and the Trichomycteridae form a monophyletic clade that is, in turn, the sister group of the clade Callichthyidae+(Scoloplacidae+(Astroblepidae+Loricariidae)). The two additional synapomorphies supporting the sister-group relationship between the nematogenyids and the trichomycterids are described below (for an overview of the other synapomorphies supporting this sister-group relationship, see DE PINNA, 1998; DIOGO, 2005).

Proximal portion of pectoral spine with prominent, somewhat rectangular, dorsomedian process for insertion of muscle abductor profundus. Plesiomorphically in catfishes the abductor profundus inserts on the medial surface of the dorsal condyle of the pectoral girdle, which lacks major processes on its medial surface for muscular insertion (see, e.g., ALEXANDER, 1965; DIOGO et al., 2001b). In all the nematogenyids and trichomycterids

examined, however, there is a prominent, somewhat rectangular, dorsomedian process on the proximal portion of the pectoral spine (see, e.g., Fig. 8 : pec-sp-dmp) for the insertion of the muscle abductor profundus. Such a dorsomedian process of the pectoral spine is also found, within the catfishes examined, in the cetopsids. As the Trichomycteridae and the Nematogenyidae do not seem to be closely related to the Cetopsidae (see, e.g., Mo, 1991 ; de Pinna, 1998 ; Diogo, 2003), this feature seems to constitute a synapomorphy of the clade formed by the two former families, with its presence in the Cetopsidae being due to an independent acquisition. In fact, it should be noted that this hypothesis was strongly supported by a phylogenetic comparison of 440 morphological characters, concerning the bones, muscles, cartilages and ligaments of both the cephalic region and the pectoral girdle, in 87 genera representing all the extant catfish families (Diogo, 2005).

Dorsal tip of coronoid process markedly curved mesially. Contrary to other catfishes, in the nematogenyids and the trichomycterids the coronoid process of the mandible is markedly curved medially, with its dorsal tip projecting medially beyond the main body of the mandible (see, e.g., Fig. 4). This feature is only found, within the catfishes studied by us or described in the literature, in the nematogenyids and the trichomycterids.

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Fidelity to nesting area of the European pond turtle, *Emys orbicularis* (Linnaeus, 1758)

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ABSTRACT. During a 16-year study (1987-2002) in the Borowiec Nature Reserve (central Poland), 118 nest sites of the turtle *Emys orbicularis* were marked : 115 nest sites of 23 different known females and 3 nest sites of unknown females. For seven females, 8 to 12 nest sites per individual are known from the studied period. For ten other females, 3 to 6 nest sites per individual are known. Two turtles presented long-term (>10 years) fidelity to the nesting area (defined arbitrary as : distances between nest sites were 20 m or less), and several other individuals did so for shorter periods (2-4 years consecutively). Other females did not display such behaviour. Thus, female turtles differed in their fidelity to the nesting area in the studied population.

KEY WORDS : long-term studies, nesting area fidelity, freshwater turtles.

INTRODUCTION

Turtles are considered long-living organisms (GIBBONS & SEMLITSCH, 1982; WILBUR & MORIN, 1988; CONGDON et al., 2001). Many freshwater turtle species can lay eggs every year, and sometimes several times a year (WILBUR & MORIN, 1988; IVERSON, 1992). The nest site can influence the hatchlings' sex ratio (VOGT & BULL, 1984; JANZEN & PAUKSTIS, 1991), the probability of nest predation (ESCALONA & FA, 1998; but see : BURKE et al., 1998), as well as the behaviour and survival of neonates (KOLBE & JANZEN, 2001). VOGT & BULL (1982) suggested that female turtles can return to the site of their own hatching success. However, females of some freshwater turtle species mature at the age of around ten years (IVERSON, 1992; GIRONDOT & PIEAU, 1993; SHINE & IVERSON, 1995), and the conditions of the natal nesting site can change in the interval between hatching and maturity.

Data about the locations of freshwater turtle nests in successive years are scarce. JOYAL et al. (2000) reported that some females of the Blanding's turtle (*Emydoidea blandingii*) deposited eggs at distances less than 50 m from their previous nests in consecutive years, while others could lay eggs over 1500 m away. In another study, 8 of 11 Blanding's turtles showed fidelity to a general nesting area (CONGDON et al., 1983). LINDEMAN (1992) found that painted turtles (*Chrysemys picta*) exhibited nest site fixity; he proposed a model in which the female turtle selects a nest site based on certain ecological characteristics that influence offspring survival, and then returns there on subsequent nesting forays as long as the site retains the features for which it was selected. Collecting data to verify such a hypothesis is very difficult, requiring information about many physical parameters, nest locations, and survival of eggs and hatchlings.

Most publications about nesting area fidelity concern marine turtles, and only provide information about placement patterns (distances from the nest to several landscape features, without precise nest site locations) (e.g., CHAVES et al., 1996; KAMEL & MROSOVSKY, 2004; NORDMOE et al., 2004). Publications about freshwater turtles are typically based on short-term studies and present few data (e.g., LINDEMAN, 1992; JOYAL et al., 2000). However, for species that live as long as some freshwater turtles do, long-term studies are important for understanding their natural history. The European pond turtle, *Emys orbicularis* (Linnaeus, 1758), is a long-living freshwater species (MITRUS & ZEMANEK, 2004). In this study I used nest location data recorded from 1987 to 2002 to determine whether these turtles exhibit fidelity to the nesting area.

MATERIAL AND METHODS

Fieldwork was conducted from 1987 to 2002 in the Borowiec Nature Reserve (BNR), situated in the Zwolenka River valley (central Poland, Radom district). Each year during the egg-laying period (mid May to mid June, depending on the weather), European pond turtle females, on their way to nesting areas or while nesting, were observed with binoculars. Some open areas were raked so that the tracks of females could be followed easily. Nests were marked by placing four pegs at the corners of a 50 cm square with the nest in the centre. All sites where eggs were deposited were marked as nest sites (abandoned digs were very rare during the study, and were not included in the analysis) : the egg-laying process was observed, hatchlings were taken for rearing as part of an active protection program (MITRUS, 2005), and/or pieces of eggshells from disturbed nests were observed.

Turtles were marked by notching the marginal scutes (PLUMMER, 1989), or (before 1991) numbers were engraved on the second vertical scute of the carapace. It was impossible to state the age of the observed females, except for E06, E85, and E101 (the "E" numbers are the identification numbers of the animals). Female E06 was marked when young in 1987 (about 6 years old as judged by the number of growth rings on scutes; M. ZEMANEK, pers. comm.), and was first observed during egg-laying in 1993. Individuals E85 and E101 had 11 growth rings in 2000 and 2001, respectively, when they were seen during nesting; previously they had not been spotted on land during the egg-laying period.

Nest sites were located on a 1 :5000 map of the study area, drawn on the basis of an aerial photo taken in 1997. Distances between the successive nest sites of each female were measured using the map. The map scale and precise descriptions in fieldwork notes made it possible to mark the nest sites on the map to an accuracy of 10 m. Based on the aerial photo and field observations, eight types of habitat were distinguished (see map symbol inset, Fig. 1). All the nest sites were classified as localized in one of the eight types of habitats.

Statistical analyses were done using Statistica ver. 5 (StatSoft Inc., 1999). For each female for which three or more nest sites were known, a distance matrix was calculated by cluster module analysis in Statistica. Average distances between nests for different intervals between nestings (1 year, 2 years, etc.) were calculated, and graphs of the average distances for the different intervals were drawn. Although statistically the average distances between nests of a single individual are not independent quantities, females that show nesting area fidelity are marked on the graph as points nearest the horizontal axis.

Arbitrarily, a distance of 20 m or less between consecutive nests of a given female was taken to indicate that the female displays nesting area fidelity.

RESULTS

Each year from 1987 to 2002, from 2 to 15 nest sites were marked in BNR. No multiple nesting by one female within the same season was observed. A total of 118 turtle nests were marked (Fig. 1) : 115 nest sites of 23 different known females and 3 nest sites of unknown females.

Seven females were found nesting a total of 65 times (range 8-12 per female) during the 16-year study. Ten other females were found nesting a total of 41 times (range 3-6 per female) during the same period.

Two females (E13 and E14) presented nesting area fidelity during the whole studied period (Fig. 2A, B). Some others presented nesting area fidelity during shorter periods, from two to four consecutive nestings. The rest did not show such behaviour.

The two highest measured distances between two nests of one female were about 840 m (female E54, nest sites in 1997 and 1999 vs. 2001) and about 690 m (female E11, nest sites in 1995 vs. 1999). The two highest distances between two nests of one female from two consecutive seasons was about 650 m (E54, nest sites in 2001 and 2002) and about 470 m (female E11, nest sites in 1998 and 1999).

Most clutches were deposited on xerothermic slopes and barrens (105 of 118 known nests; Fig. 1). Eggs were also deposited on agricultural fields that were in use or lying fallow (11 of 118), and on field roads and paths (2 of 118). Several more were deposited on barrens but less than 2 m from field roads).

Nest sites of probably young females are indicated in Fig. 1.

DISCUSSION

Most freshwater turtles lay eggs close to bodies of water, but some species or individuals can lay eggs even hundreds of meters from water (BURKE & GIBBONS, 1995; BURKE et al., 1998). Such behaviour was reported earlier for the European pond turtle (ROVERO & CHELAZZI, 1996; PAUL & ANDREAS, 1998) and observed during this study. In the studied population, however, most of the turtle nests were located less than 150 meters from water bodies (Fig. 1).

In studies of Blanding's turtles, only a proportion of individuals were found to present fidelity to the nesting area (CONGDON et al., 1983; JOYAL et al., 2000). The same was true in the present study. Very prolonged fidelity to the nesting area characterized only two of the seven European pond turtle females for which 8-12 nest sites per female were marked (females E13 and E14, Fig. 2A, B). Some females showed fidelity for shorter periods (2-4 consecutive nestings). Most known nests of each of the individuals were located not far from previous nesting sites. The shortest distance (measured in the field) between nest sites was about 1.2 m (for female E14, nests in years 2000 and 2001; S. MITRUS, unpublished data).

Some females presented fidelity to the nesting area in some seasons but later changed the area. My field observations suggest that usually this was because the area was shaded by growing trees or destroyed. As LINDEMAN (1992) proposed in his model, probably the females started to lay eggs at other nesting areas when ecological characteristics had changed and no longer were good for egg incubation. However, from 1989 to 1997 the eggs or hatchlings from all known clutches were taken for breeding as part of an active protection program (MITRUS, 2005), so the data about hatchling survivorship versus nesting area and versus individual female are fragmentary and cannot be generalized.

None of the six females for which only 3 to 6 nest sites per female are known presented fidelity to the nesting area as arbitrarily defined. Two of them laid eggs on both slopes of the river valley. However, during egg-laying time (or on the way to nesting areas) it is easier to locate a female that presents nesting area fidelity and whose nesting area is well known. Thus, large distances between nests from consecutive nestings could be more frequent than presented in this study.

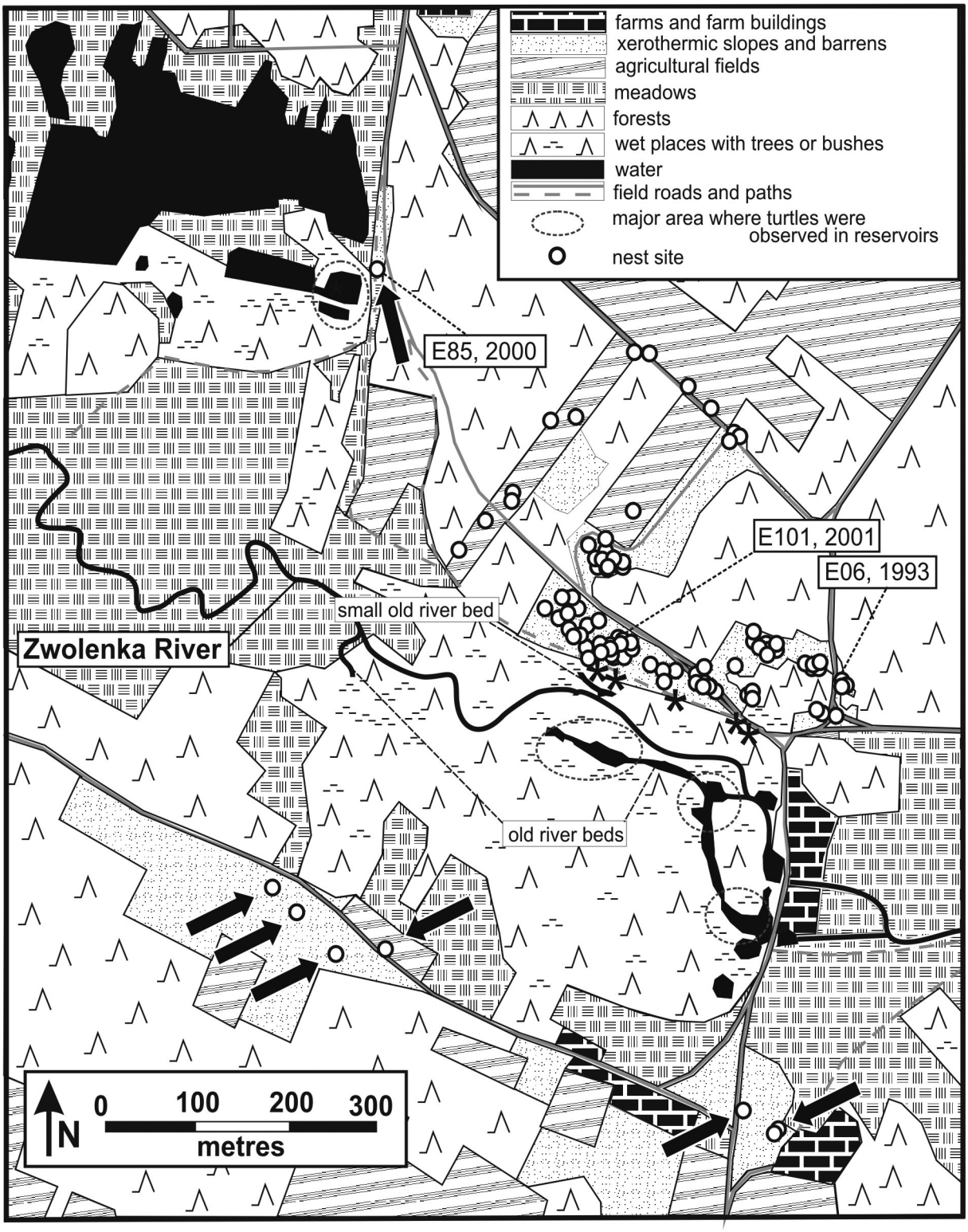

Fig. 1. – Distribution of nest sites of the turtle *Emys orbicularis* from 1987 to 2002 in the Borowiec Nature Reserve (central Poland). Each circle represents a nest site. Some circles are obscured in densely nested areas. Nests marked E06, E85 and E101 followed by year of oviposition probably belong to young females (see text for details). Arrows point to nest sites away from the main groups.
The map was drawn on the basis of a 1997 aerial photo. "E" numbers are the animal identification numbers. Asterisks show areas where females were typically observed on land at the beginning of nesting migrations.

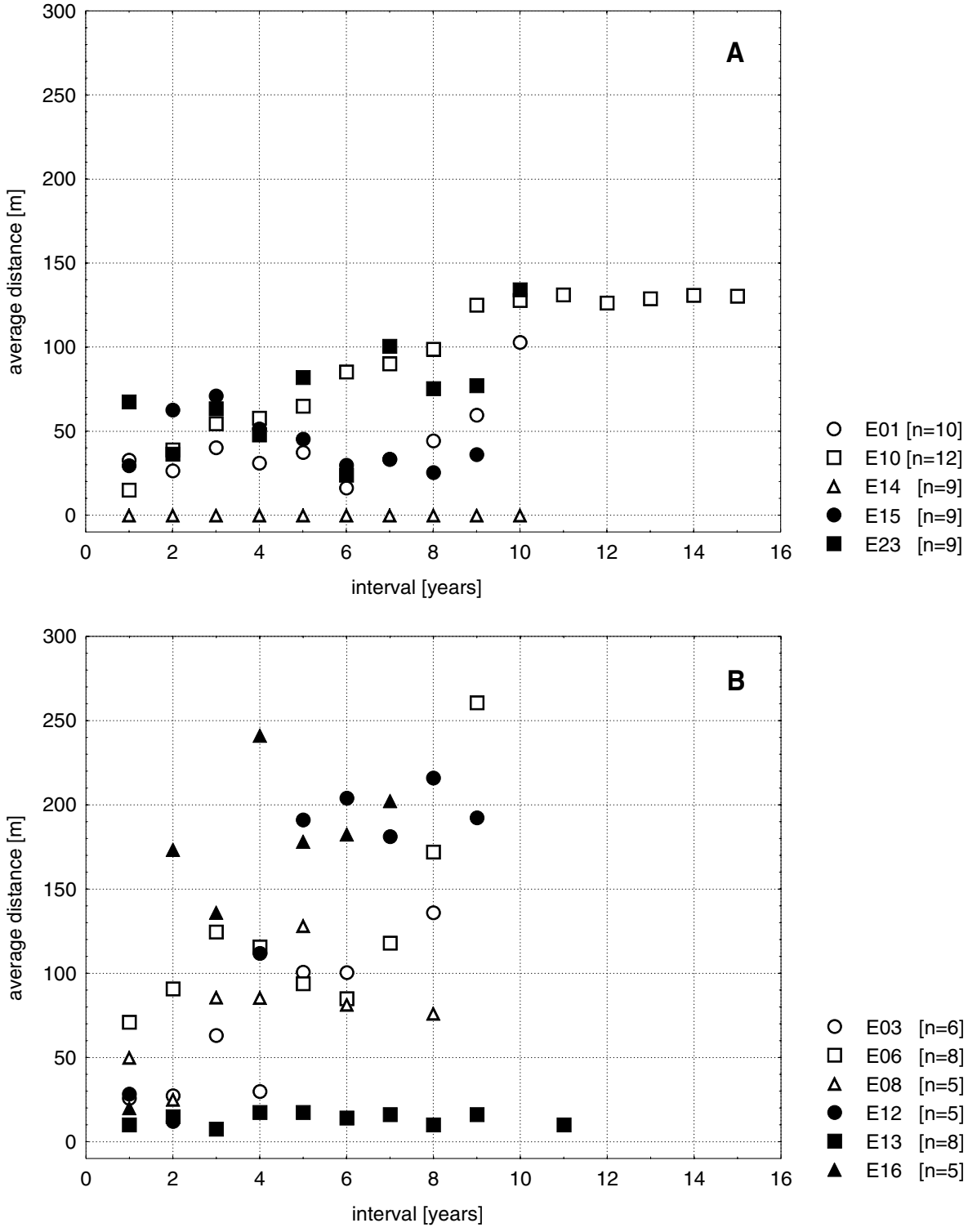

Fig. 2. – Average distance between nest sites at different intervals between nestings of the turtle *Emys orbicularis* from central Poland. Data is shown for females for which 5 or more nest sites are known. ”E” numbers are the animal identification numbers. Numbers in brackets are the numbers of known nest sites of each female from the 16-year study.

Another kind of fidelity is habitat fidelity : 105 of 118 known nests (89%, cf. Fig. 1) were made in areas described as xerothermic slopes and barrens. Most females laid eggs only in such areas, but 4 of the 5 known nest sites of female E16, for example, were laid on agricultural fields, and 1 of the 5 was on a field road (S. MITRUS, unpublished data). Apparently, turtles generally display habitat fidelity and some also exhibit nesting area fidelity within that habitat.

No data about the home ranges of turtles from BNR are available. During the summer, mature turtles are typically found in large old river beds and ponds (Fig. 1). Just before the nesting season, however, several can be seen in a small old river bed; on 14 and 15 May 2000, five mature females and one male were captured in the small old river bed. It is probably part of the migration route, but females starting from the same area could use different nesting areas (Fig. 1, and S. MITRUS, unpublished data). During nesting migrations, some individuals crossed areas used by other turtles for nesting and went on farther. It is impossible to say whether the turtles found the area unfavourable for egg development or else presented fidelity to other nesting areas (S. MITRUS, unpublished data).

Young females, perhaps laying for the first time, laid eggs rather close to water bodies (Fig. 1), but the paucity of information makes it impossible to characterize nest site selection by young females. Another problem is age estimation. For the turtle in Poland, the number of growth rings on scutes seems to be a good gauge of age up to 14 years (c.f. MITRUS & ZEMANEK, 2004), but the method can be fallible (cf. GERMANO & BURY, 1998), so the description of females as young cannot be unequivocal.

The indicator of fidelity to the nesting area was defined arbitrarily as a distance of 20 m or less between consecutive nest sites of the same female. For the turtle in Poland it seems a good indicator of such behaviour. For other populations of the species, or for other species, a different indicator might be more appropriate.

CONCLUSIONS

In the studied population, females of the European pond turtle differed in their fidelity to the nesting area. Some individuals observed in the study presented fidelity to the nesting area, and others did not. I believe that some turtles changed nesting areas apparently because the vegetation there grew and the former nesting area was shaded. Others changed nesting areas when there were no visible changes in the nesting area environment, and other females laid eggs in those abandoned areas (S. MITRUS, personal observations), indicating that the ecological parameters of the abandoned areas still favoured egg incubation. The reasons for such differences in behaviour are not known.

The turtle exhibits temperature-dependent sex determination (PIEAU, 1971; PIEAU & DORIZZI, 1981). Nest location can influence survival and behaviour (see : Introduction) as well as the hatchling sex ratio (e.g., VOGT & BULL, 1984; JANZEN & PAUKSTIS, 1991). Nests of females laying eggs in the same area could produce larger proportions of males in successive years as the vegetation grows and the nesting area becomes more shaded (cf. VOGT & BULL, 1982). Thus, females that do not exhibit nesting area fidelity could have a larger influence on the offspring sex ratio. Such behaviour might also be a useful strategy if predators can learn where turtle nests are located and return to them in succeeding years.

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Révision d'*Ichthyotringa africana*, poisson marin (Teleostei, Aulopiformes) du Crétacé supérieur de la Mésogée eurafricaine. Considérations sur les relations phylogénétiques du genre *Ichthyotringa*

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RÉSUMÉ. L'ostéologie d'*Ichthyotringa africana* du Cénomano-Turonien marin de la Mésogée eurafricaine est étudiée et la position systématique d'*Ichthyotringa* discutée. Quatre apomorphies attestent que ce genre appartient aux Aulopiformes : la présence de processus postéro-médians sur les os pelviens, la série épipleurale qui commence dès la première vertèbre, les premiers épipleuraux implantés au niveau du septum horizontal et la neurépine préurale 1 raccourcie et spatulée. L'allongement du museau, l'importance de l'ouverture buccale, le développement de la dentition palatine et ptérygoïdienne et la bouche bordée par le prémaxillaire et le maxillaire sont quatre caractères qui rapportent *Ichthyotringa* aux Enchodontoidei. Le grand nombre de plésiomorphies conservées par ce poisson et ses rares apomorphies indiquent qu'il est l'un des genres les plus primitifs du sous-ordre.

MOTS CLEFS : *Ichthyotringa*, Aulopiformes, Enchodontoidei, Teleostei, Crétacé supérieur marin, Mésogée eurafricaine, ostéologie, relations.

Revision of *Ichthyotringa africana*, marine fish (Teleostei, Aulopiformes) from the Upper Cretaceous of the Eurafrican Mesogea. Considerations on the phylogenetic relationships of the genus *Ichthyotringa*

ABSTRACT. The osteology of *Ichthyotringa africana* from the marine Upper Cretaceous of the Eurafrican Mesogea is studied and the systematic position of *Ichthyotringa* discussed. Four apomorphies prove that this genus belongs to the Aulopiformes : the presence of postero-medial processes on the pelvic bones, the epipleural series beginning from the first vertebra, the first epipleurals located at the level of the horizontal septum, and the preural neural spine 1 reduced and spatulate. The lengthening of the snout, the very large buccal cavity, the development of the palatine and pterygoid dentition, and the premaxilla and maxilla bordering the gape are four characters which refer *Ichthyotringa* to the Enchodontoidei. The numerous plesiomorphies kept by this fish and its rare apomorphies show that it is one of the most primitive genera within the suborder.

KEY WORDS : *Ichthyotringa*, Aulopiformes, Enchodontoidei, Teleostei, marine Upper Cretaceous, Eurafrican Mesogea, osteology, relationships.

INTRODUCTION

Le genre *Ichthyotringa* Cope, 1878 a remplacé *Rhinellus* Agassiz, 1844 préoccupé. Il regroupe plusieurs espèces d'un petit téléostéen marin du Crétacé supérieur dont les plus grands exemplaires n'excèdent pas une trentaine de cm de longueur, la taille des plus petits ne dépassant pas quelques cm. La tête s'allonge en un rostre très proéminent où les deux mâchoires sont de longueur égale. Le corps peu élevé reste court par rapport à la tête, le nombre des vertèbres n'excédant jamais la quarantaine.

L'espèce-type *I. tenuirostris* Cope, 1878, du Crétacé supérieur du Dakota et du Nebraska, est le seul représentant nord-américain du genre (Cope, 1878 ; Hay, 1903a). Les autres espèces appartiennent aux pays du pourtour de la Mésogée eurafricaine. On trouve ainsi *Ichthyotringa furcata* (Agassiz, 1844) dans le Santonien du Liban et d'Allemagne, *I. ferox* (Davis, 1887) et *I. damoni* (Davis,

1887) dans le Santonien du Liban, *I. delicata* (Hay, 1903) dans le Cénomanien du Liban et *I. africana* (Arambourg, 1954) dans le Cénomano-Turonien du Maroc et d'Italie (Agassiz, 1844 ; Pictet, 1850 ; Pictet & Humbert, 1866 ; Von Der Marck, 1873 ; Davis, 1887 ; Hay, 1903b ; Arambourg, 1954 ; Leonardi, 1966 ; Sorbini, 1976 ; Kriwet & Gloy, 1995).

Malgré les descriptions d'Arambourg (1954 : 121-124) et de Goody (1969 : 7-17), l'anatomie d'*Ichthyotringa* demeure imparfaitement connue. Le présent article a pour but de préciser l'ostéologie de l'espèce *I. africana* et de définir sur de meilleures bases anatomiques la position phylogénétique du genre. Mon travail s'inscrit également dans la révision générale des téléostéens non-acanthoptérygiens du Cénomanien du Djebel Tselfat (Maroc) que je poursuis depuis de nombreuses années (Taverne, 1976, 1977, 1983, 1985, 1986, 1987, 1992, 1993, 1994, 1995ab, 1996), les Acanthomorpha, quant à eux, ayant été revus par Gaudant (1978).

MATÉRIEL ET MÉTHODES

Le matériel étudié ci-après provient des collections du Muséum national d'Histoire naturelle de Paris (MNHN), du Museo Civico di Storia Naturale de Vérone (MCSN) et du Natural History Museum de Londres (NHM).

Ichthyotringa africana

MNHN T. 225 (holotype), T. 226 (paratype), T. 227 D et G (paratype), T. 18 (dans la cavité digestive d'un autre poisson) et T. 228 D et G [ce matériel provient du Cénomanien du Djebel Tselfat, Maroc].

MCSN IG 37470, 37515, 37516, 37517/18, 37519, 37520, 37521, 37522, 37547 [ce matériel provient du Cénomano-Turonien de Cinto Euganeo, Italie].

Ichthyotringa furcata

NHM P. 48081, 48089a et b, 48091, 48092, 48096, 48144, 48155 et 49544 [ce matériel provient du Santonien de Sahel Alma, Liban].

Le matériel a été examiné à l'aide d'un stéréomicroscope Wild M 5 et les dessins exécutés par l'auteur grâce à une chambre claire. L'observation de certains détails ostéologiques a été facilitée par une immersion dans l'éthanol.

J'ai pu étudier le matériel du Djebel Tselfat au Muséum de Paris à deux reprises, une première fois à la fin des années 1960 et durant les années 1970 lorsqu'il était entreposé dans l'ancien conservatoire et une seconde fois après son transfert dans de nouvelles armoires de rangement à la fin des années 1980 et pendant les années 1990. Durant la période qui a précédé ce déménagement, les pièces concernées ont été mises en réserve dans des boîtes et elles ont souffert de ce traitement. J'ai constaté que des détails ostéologiques autrefois clairement visibles sur plusieurs de ces poissons fossiles étaient aujourd'hui en partie estompés. Les spécimens d'*I. africana* sont malheureusement dans ce cas.

Sorbini (1976) a décrit le matériel de Cinto Euganeo sous le nom de *Rhinellus* sp. Pour ma part, je n'ai pas observé de différences ostéologiques entre les fossiles du Maroc et ceux d'Italie. Je range donc ces derniers également dans l'espèce *Ichthyotringa africana*.

ABRÉVIATIONS SUR LES FIGURES

AN : angulaire (= angulo-splénial)
ART : articulaire
BO : basioccipital
BSPH : basisphénoïde
C : côte
DN : dentaire (= dento-splénial)
ECPT : ectoptérygoïde
ENPT : entoptérygoïde (= endoptérygoïde, mésoptérygoïde)
EP1-2 : épuraux 1 et 2
EPI : épiotique (= épioccipital)
EPINE : épineuraux
EPIPL : épipleuraux
FR : frontal
HEM : arc hémal (= hémarcual)
HEMAP : hémapophyse (= parapophyse)
HEMEP : hémépine (= hémacanthe)
HY1-6 : hypuraux 1 à 6
HYOM : hyomandibulaire
METH : mésethmoïde
MPT : métaptérygoïde
MX : maxillaire
NEUR : arc neural (= neurarcual)
NEUREP : neurépine (= neuracanthe)
NP PU1 : neurépine de la vertèbre préurale 1
NP PU2 : neurépine de la vertèbre préurale 2
OP : operculaire
OSPH : orbitosphénoïde
PA : pariétal
PAL : palatin
PHY : parhypural
PMX : prémaxillaire
POP : préoperculaire
PS : parasphénoïde
PTE : ptérotique
PU1-4 : vertèbres préurales 1 à 4
RART : rétroarticulaire
QU : carré (= quadratique)
SOC : supraoccipital
SOP : sous-operculaire
SPH : sphénotique (= autosphénotique)
ST : supratemporal (= extrascapulaire)
SY : symplectique
U1-2 : vertèbres urales 1 et 2
V : corps vertébral
VO : vomer (= prévomer)
c. ext. : commissure sensorielle extrascapulaire
c. l. l. : canal sensoriel de la ligne latérale
c. ot. : canal sensoriel otique (= postorbitaire)
c. p. : commissure sensorielle pariétale
c. sorb. : canal sensoriel supraorbitaire
d. : droit
g. : gauche

ÉTUDE SYSTÉMATIQUE

Classe **TELEOSTEI**
Cohorte **CLUPEOCEPHALA**
Sous-cohorte **EUTELEOSTEI**
Ordre **AULOPIFORMES**
Sous-ordre **ENCHODONTOIDEI**
Famille **Ichthyotringidae**
Genre ***Ichthyotringa*** Cope, 1878
Espèce ***Ichthyotringa africana*** Arambourg, 1954

Morphologie (Fig. 1)

La tête est très allongée, suite à la présence d'un très long rostre. Le corps est modérément allongé et peu élevé. Le spécimen MNHN T. 228 D et G est presque complet. Il mesure environ 97 mm de longueur totale dont 22 mm pour la tête mais il lui manque le bout du museau, la mandibule et la plus grande partie de la nageoire caudale. On peut penser qu'il mesurait aux environs de 120 mm quand il n'était pas amputé de ses deux extrémités. La tête seule, quand le rostre était complet, devait représenter près du tiers de la longueur du poisson. Le crâne isolé MCSN IG 37522, qui est le plus grand mais auquel manque l'extrémité de la région occipitale, est long de 47 mm. Toutes proportions gardées, on peut donc estimer que les plus grands spécimens d'*Ichthyotringa africana* pouvaient approcher une trentaine de cm de longueur totale. Aucun exemplaire n'est suffisamment complet ni bien conservé pour permettre une description morphométrique détaillée.

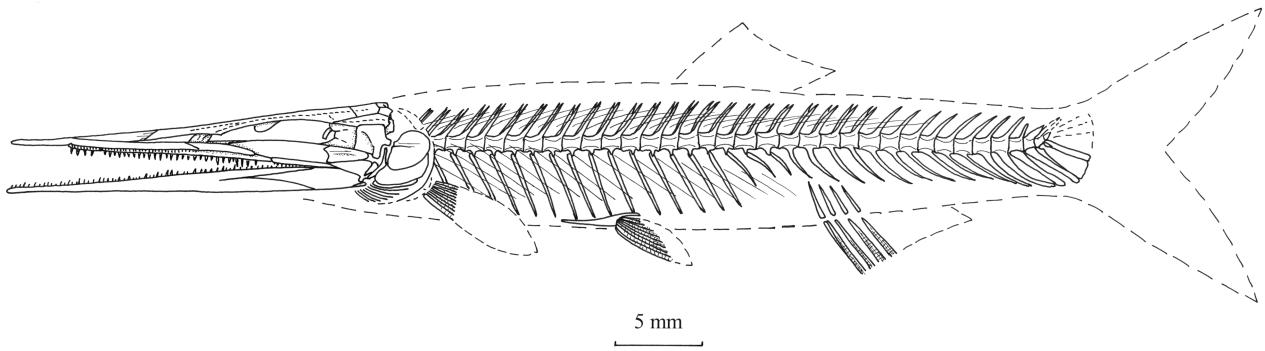

Fig. 1. – *Ichthyotringa africana* (Arambourg, 1954). Reconstitution générale d'après l'holotype MNHN T. 225 (auquel se rapporte l'échelle), les paratypes MNHN T. 226, T. 227 D et G et les spécimens MNHN T. 228 D et G, MCSN IG 37517/37518, IG 37522 et IG 37547.

Le crâne (Figs 2-4)

Plusieurs os participent au très long rostre du poisson : les frontaux, le mésethmoïde, les prémaxillaires, le vomer, le parasphénoïde, les palatins et les dentaires. Les parties maxillaire et mandibulaire du rostre sont d'égale longueur.

Le mésethmoïde est allongé, fin et pointu vers l'avant, un peu plus large à l'arrière et taillé en un coin triangulaire qui s'insère entre les deux frontaux. Les ethmoïdes latéraux sont massifs, situés très en avant de l'orbite, entre les frontaux et le parasphénoïde. Ils ne présentent pas de contact direct avec le mésethmoïde. Un massif ethmoïdien cartilagineux réunissait probablement ces trois os sur le poisson vivant. Le vomer, bien visible sur le paratype MNHN T. 227 D, est une très longue et très fine baguette osseuse qui porte quatre grandes dents (dont seules les vastes alvéoles d'implantation sont préservées) vers son extrémité antérieure. Je n'ai pas observé les nasaux que signale ARAMBOURG (1954 : 122).

Les frontaux forment la plus grande partie du toit crânien. Ils sont très allongés mais demeurent assez étroits, y compris dans leur partie postérieure. Les pariétaux sont grands et en contact l'un avec l'autre, déterminant un crâne de type médio-pariétal. Les sphénotiques forment de chaque côté un petit nodule osseux qui dépasse légèrement du frontal en vue dorsale. Les ptérotiques sont longs et fins. Ils encadrent les pariétaux ainsi que l'extrémité postérieure des frontaux depuis le niveau des sphénotiques. Le ptérotique couvre et ferme dorsalement la fosse temporale (= posttemporale). C'est sur lui aussi que se creuse la fossette articulaire pour l'hyomandibulaire. En revanche, on n'observe pas de *dilatator fossa* sur le ptérotique. Le supraoccipital est petit et porte une crête médiane peu marquée. Sur le paratype MNHN T. 227 D et G, on observe que le supraoccipital émet un long et fin processus antéro-médian qui passe sous les pariétaux et rejoint les frontaux. Les épiotiques sont bien développés et encadrent le supraoccipital. Le canal sensoriel supraorbitaire s'étire tout au long du frontal et s'élargit considérablement vers l'avant. Vers l'arrière, il se poursuit par une longue commissure pariétale qui se prolonge jusque sur le pariétal ainsi qu'on peut le voir sur le paratype MNHN T. 227 D et G. A hauteur du sphénotique, le canal supraorbitaire émet une branche latérale qui se poursuit sur le ptérotique pour y former le canal otique (= postorbitaire). Le spécimen MNHN T. 228 D et G montre le supratemporal gauche. C'est un os assez grand mais qui ne rejoint pas son homologue sur la ligne médiane du crâne. On y distingue le début du canal sensoriel de la ligne latérale ainsi qu'une courte commissure extrascapulaire.

Le paratype MNHN T. 227 D montre juste en avant de l'orbite l'empreinte d'un os assez massif qui doit être l'orbitosphénoïde car il occupe une situation trop postérieure pour être un ethmoïde latéral. Les pleurosphénoïdes ne sont clairement visibles chez aucun exemplaire. L'empreinte du bélophragme du basisphénoïde est profondément imprimée dans le substrat juste en dessous du sphénotique et devant le prootique chez le spécimen MNHN T. 226. Le parasphénoïde est une longue et épaisse tige osseuse, édentée, dépourvue de processus basiptérygoïde mais creusée vers l'avant d'une longue gouttière où se loge le vomer.

Les prootiques, partiellement visibles sur quelques exemplaires, ne sont jamais suffisamment bien conservés pour que l'on puisse observer la chambre trigémino-faciale et la *pars jugularis*. Les exoccipitaux ne sont pas connus ni les éventuels intercalaires. Le contour du basioccipital se voit sur l'holotype MNHN T. 225. Il semble qu'il forme seul le condyle articulaire pour le squelette axial.

Aucun exemplaire ne montre de restes clairement identifiables des os de la série orbitaire. Soit ils sont absents chez *Ichthyotringa africana*, soit ils sont trop minces ou trop fragiles pour se conserver. Les os orbitaires existent chez *I. furcata* ainsi que chez *I. delicata* et, chez cette dernière espèce, les trois infraorbitaires postérieurs sont même de grande taille (obs. pers. ; GOODY, 1969 : 16-17).

Le palatin est un os très allongé, élevé, qui s'étire sur une grande partie du rostre depuis le niveau du vomer et du mésethmoïde jusqu'à celui du début de l'orbite. Le bord ventral du palatin porte une série de grandes dents très fines et très pointues. En arrière du palatin, on observe un ectoptérygoïde assez court, étroit et qui porte une rangée de petites dents pointues. L'entoptérygoïde est long, large et édenté. Le métaptérygoïde, plus long que haut, surplombe le carré. Celui-ci est triangulaire, se renfle ventralement en un condyle articulaire pour la mandibulaire et porte un processus quadratojugal le long de son bord postérieur.

La mâchoire supérieure est constituée dans chacune de ses moitiés d'un prémaxillaire et d'un maxillaire. Il n'y a pas de supramaxillaire. Les prémaxillaires sont étroits, extrêmement allongés et dentés. Ils encadrent le mésethmoïde et la partie la plus antérieure des frontaux. Leurs dents pointues sont plus petites que celles du palatin. En avant du mésethmoïde, les deux prémaxillaires fusionnent en une fine pointe osseuse qui forme l'extrémité antérieure de la moitié dorsale du rostre. Le maxillaire est édenté, peu allongé, nettement plus court que le palatin, mais il participe complètement à la formation du bord buccal. Chaque hémi-mandibule comporte un dentaire, un angulo-articulaire et un rétroarticulaire. La présence d'un éventuel corono-meckélien n'a pu être décelée. L'articulation de la mandibule avec le carré est située en arrière de l'orbite. Le dentaire est très allongé et il forme avec son homologue la moitié ventrale du rostre, laquelle est aussi longue que la moitié dorsale. Le bord oral du dentaire est orné de dents pointues petites et grandes. Le rétroarticulaire est exclu de la formation de la fossette articulaire pour le carré, toute entière creusée dans l'angulo-articulaire. Le canal sensoriel mandibulaire n'est clairement visible chez aucun exemplaire.

Les os de la série operculaire sont petits par rapport à la taille de la tête. Le préoperculaire est minuscule, avec une branche dorsale courte et très étroite et une branche ventrale un peu plus large et orientée obliquement vers l'avant. Un fin canal sensoriel préoperculaire s'observe sur la branche ventrale de l'os. Il n'y a pas d'interoperculaire. L'operculaire est arrondi. Le sous-operculaire est proportionnellement plus développé que les autres os de la série. Il est plus allongé que l'operculaire mais moins élevé et son extrémité antérieure glisse sous le préoperculaire, occupant ainsi en partie la place laissée vacante suite à l'absence d'interoperculaire. Il y a au moins huit fins rayons branchiostèges.

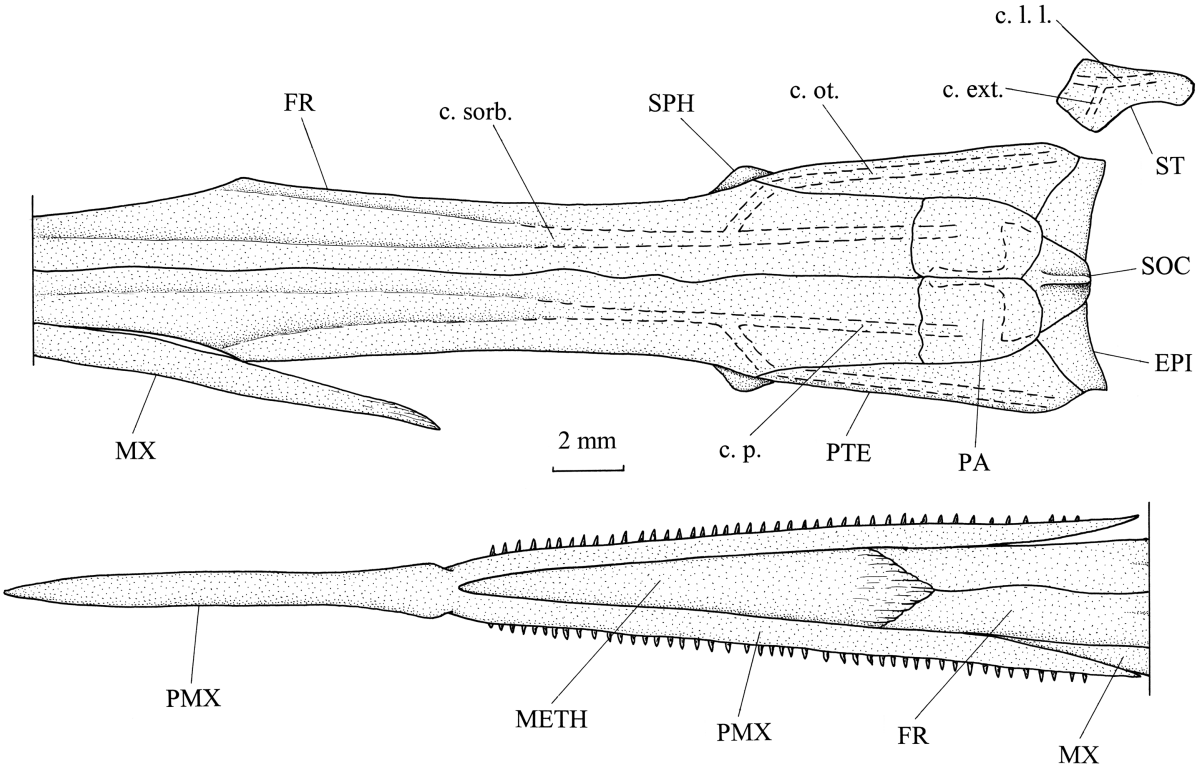

Fig. 2. – *Ichthyotringa africana* (Arambourg, 1954). Reconstitution du crâne en vue dorsale d'après l'holotype MNHN T. 225, les paratypes MNHN T. 226, T. 227 D et G (auquel se rapporte l'échelle) et les spécimens MNHN T. 228 D et G, MCSN IG 37522 et IG 37547.

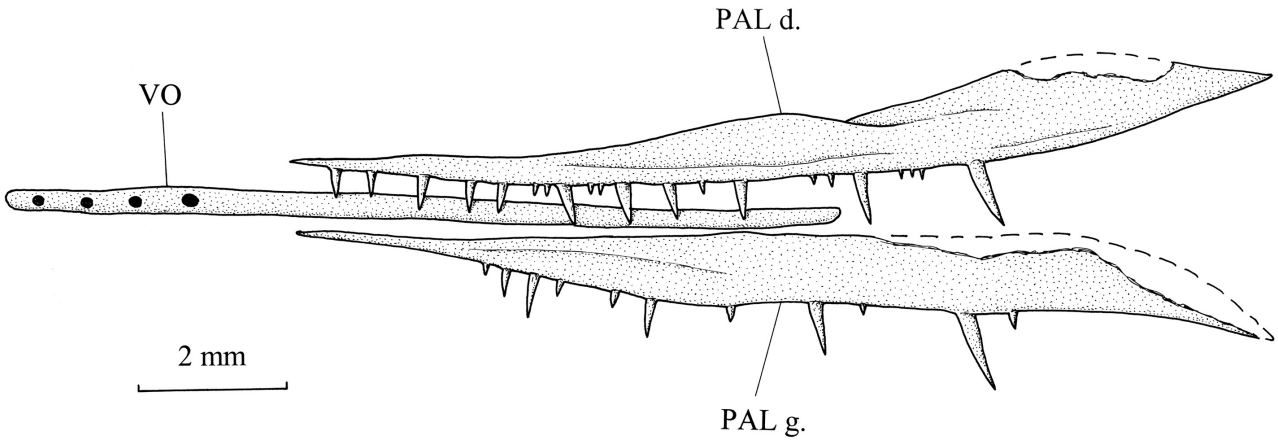

Fig. 4. – *Ichthyotringa africana* (Arambourg, 1954). La région voméro-palatine du paratype MNHN T. 227 D. Le vomer est conservé en vue ventrale, tandis que les deux palatins sont vus par leur face latérale suite aux aléas de la fossilisation.

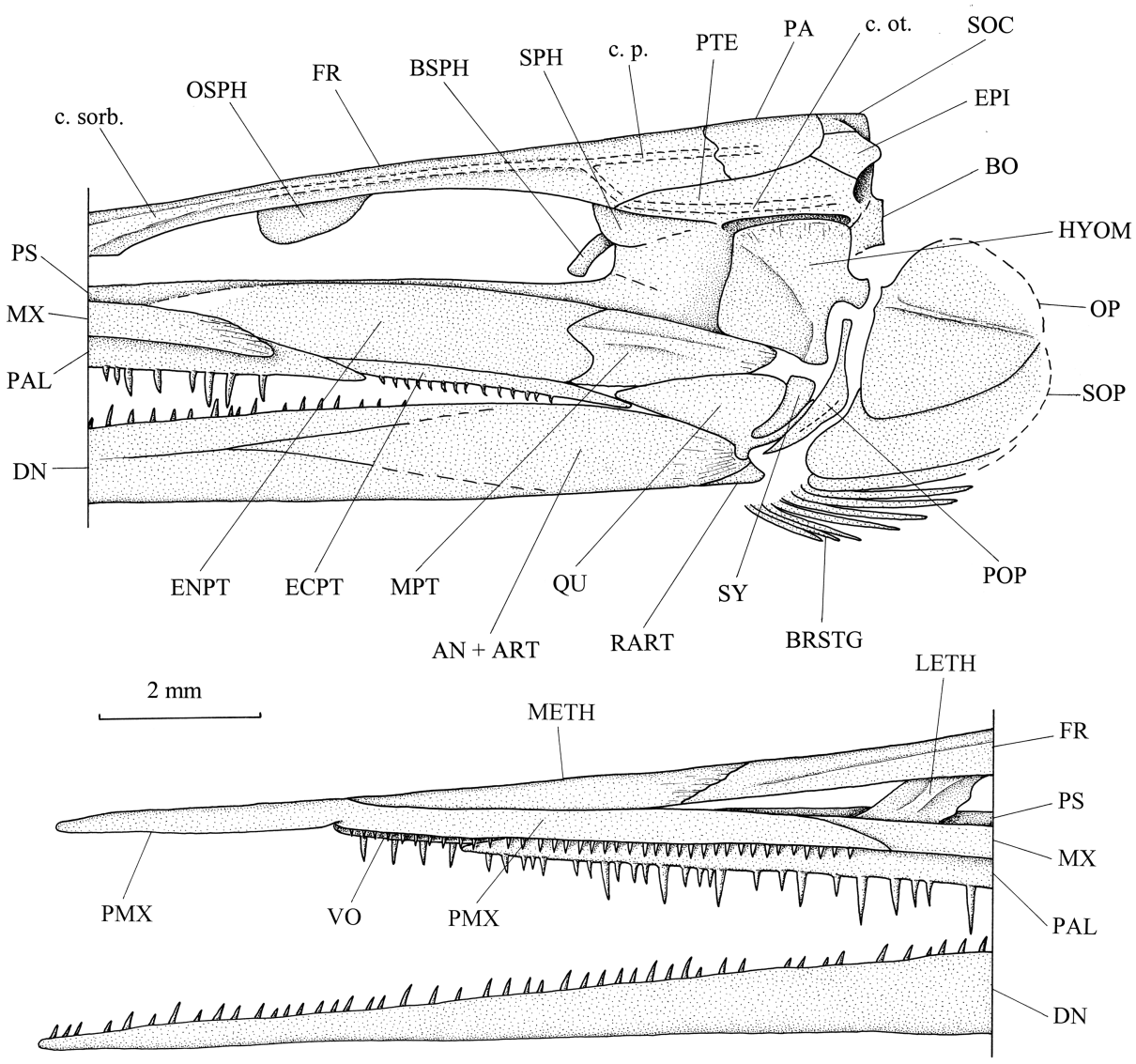

Fig. 3. – *Ichthyotringa africana* (Arambourg, 1954). Reconstitution du crâne en vue latérale gauche d'après l'holotype MNHN T. 225 (auquel se rapporte l'échelle), les paratypes MNHN T. 226, T. 227 D et G et les spécimens MNHN T. 228 D et G, MCSN IG 37522 et IG 37547.

Le squelette hyoideo-branchial (Fig. 3)

L'hyomandibulaire, visible sur l'holotype MNHN T. 225, est court et large. Sa tête articulaire est allongée et composée d'un seul long condyle. Le *processus opercularis*, bien développé mais court, émerge à mi-hauteur du bord postérieur de l'os. La branche ventrale est courte et étroite. Une grande aile osseuse occupe la partie antérieure de l'os et surplombe l'arrière du métaptérygoïde. Le symplectique est bien développé, coincé entre le carré et son processus quadratojugal. Le spécimen MNHN T. 226 et le paratype MNHN T. 227 D et G montrent dans la région operculaire les traces de plusieurs os fins et allongés qu' ARAMBOURG (1954 : 123) interprète comme des branchiospines. Cependant, ces os me paraissent trop longs et trop clairsemés pour être des branchiospines. J'y vois plutôt des restes des cératobranchiaux et épibranchiaux.

Les ceintures (Fig. 1)

La ceinture scapulaire est très mal conservée. Quelques spécimens montrent des restes d'un cleithrum très étroit. La nageoire pectorale est courte, insérée bas sur les flancs, mais trop mal préservée pour qu'on puisse en dénombrer les rayons.

L'exemplaire MCSN IG 37517/37518 est le seul à montrer la ceinture pelvienne mais, là aussi, l'état de conservation est pauvre. Les nageoires ventrales, qui comptent près d'une quinzaine de rayons, sont insérées au niveau de la douzième vertèbre. On remarque que les os pelviens, accolés l'un à l'autre, se prolongent quelque peu en arrière du niveau de la base des nageoires ventrales, ce qui indique la présence d'une paire de processus médio-postérieurs.

Le squelette axial (Fig. 1, 5)

Le squelette axial comporte 36 vertèbres, y compris le centre ural 2, dont 20 abdominales et 16 caudales chez l'exemplaire MNHN T. 228 D et G et approche les 40 vertèbres chez l'exemplaire MCSN IG 37517/37518 où le compte précis est plus difficile à établir. Les vertèbres abdominales sont plus longues que hautes, en forme de sablier, mais les vertèbres caudales se raccourcissent quelque peu. Là où l'état de conservation est suffisant, on observe que les faces latérales des vertèbres sont ornées de fines stries. Les arcs neuraux s'allongent sur les corps vertébraux auxquels ils sont soudés. Ils portent des neurépines étroites, dirigées vers l'arrière et modérément allongées. Jusque et y compris la vingt-quatrième vertèbre, les neurépines sont bifides. Au-delà, elles sont simples. Les hémapophyses de la région abdominale sont réduites à de courtes expansions osseuses pointues soudées latéralement au milieu du bord ventral des corps vertébraux. Dans la région caudale, l'arc hémal, soudé à la vertèbre à l'exception de ceux des vertèbres préurales 1 et 2 qui demeurent autogènes, se referme sur lui-même et porte une fine hémépine dirigée vers l'arrière. On n'observe pas de supraneuraux. Chez l'exemplaire MNHN T. 228 D et G, il y a 18 paires de côtes insérées depuis la troisième jusqu'à la vingtième vertèbre.

Les épineuraux sont présents dès le début du squelette axial et leur série se prolonge jusqu'aux premières vertèbres caudales. Ils sont situés au-dessus des centra, au niveau des neurépines. Il y a de longs épipleuraux dans la région abdominale. Le premier de ceux-ci est situé au niveau de la première vertèbre. Les huit premiers épipleuraux ont leurs extrémités supérieures qui remontent jusqu'au niveau des faces latérales des vertèbres, ce qui indique que ces fines arêtes étaient insérées dans le septum horizontal.

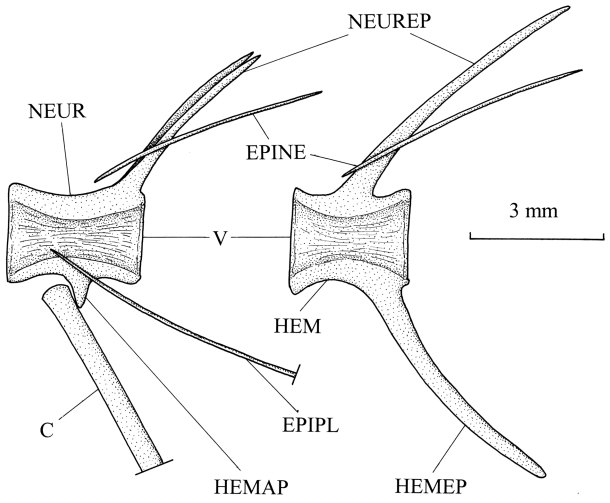

Fig. 5. – *Ichthyotringa africana* (Arambourg, 1954). Reconstitution d'une des premières vertèbres abdominales (à gauche) et d'une des premières vertèbres caudales (à droite) d'après l'holotype MNHN T. 225 et le spécimen MNHN T. 228 D et G (auquel s'applique l'échelle).

Les nageoires dorsale et anale (Fig. 1)

Aucun spécimen ne montre la nageoire dorsale. La nageoire anale est visible sur l'exemplaire MCSN IG 37517/37518 mais l'état de conservation est mauvais et ne permet pas d'en compter les rayons ni même d'en estimer l'étendue complète. L'origine de cette nageoire anale se situe au niveau de la dix-neuvième vertèbre, ce qui paraît être le début de la région caudale chez ce spécimen.

Le squelette caudal (Figs 6, 7)

L'exemplaire MNHN T. 228 D et G est le seul qui montre un complexe urophore suffisamment bien conservé pour être interprété. Les dernières vertèbres caudales deviennent de plus en petites mais les centres préural 1, ural 1 et ural 2 sont bien individualisés et indépendants les uns des autres. La neurépine préurale 2 est un peu plus courte que les précédentes mais elle reste fine. La neurépine préurale 1 est raccourcie et élargie en forme de spatule. Les dernières hémépines sont allongées. Le parhypural est étroit; sa tête est articulée et non pas soudée à la vertèbre préurale 1. Il semble que l'hémépine préurale 2 soit, elle aussi, articulée à la vertèbre correspondante mais le mauvais état de conservation rend cette observation incertaine. Seuls les deux hypuraux ventraux sont préservés. Ils sont accolés mais demeurent indépendants l'un de l'autre. Tous deux sont articulés avec la vertèbre urale 1. Le premier hypural est large, tandis que le deuxième reste étroit. Un petit morceau du premier uroneural est visible à

côté de la neurépine préurale 1. Les hypuraux dorsaux manquent.

Les parties préservées de ce squelette caudal correspondent parfaitement avec celui mieux connu d'*Ichthyotringa furcata* (Fig. 7). Chez cette espèce, on observe que les vertèbres préurale 1, urale 1 et urale 2 demeurent indépendantes et qu'il y a deux épuraux et six hypuraux autogènes. Aucun diastème ne sépare le deuxième hypural du troisième et ce troisième est très large, faisant ainsi le pendant dorsal du premier hypural. La neurépine préurale 1 est réduite et spatulée. La neurépine préurale 2 est légèrement raccourcie par rapport à celles qui la précèdent. Dans un cas (NHM P. 48089a, b), la neurépine préurale 2 est également raccourcie et spatulée. Le parhypural peut, selon les cas, être articulé (NHM P. 49544) ou soudé (NHM P. 48092) à la vertèbre préurale 1. Il en va de même des deux hémépines précédentes qui peuvent se souder à leurs vertèbres respectives ou s'y articuler. Il y a deux uroneuraux allongés. Le premier uroneural peut rester autogène (NHM P. 48081, 48092, 48144) mais il peut aussi se souder à la face latérale de la vertèbre préurale 1 et former alors une sorte de pleurostyle (NHM P. 48089a, b, 49544).

GOODY (1969 : fig. 4) signale chez *I. furcata* un arc neural ural 1 sous forme d'une aile osseuse accolée au premier uroneural. Cette observation est erronée. Un tel arc n'existe pas. Je pense que l'auteur a été induit en erreur par l'exemplaire NHM P. 48092. La partie basse de la neurépine préurale 1 spatulée y est en grande partie perdue suite aux aléas de la fossilisation. Seule sa partie haute est bien conservée et elle peut donner la fausse impression d'être un arc neural ural 1 jouxtant le premier uroneural (Fig. 7A).

La nageoire caudale n'est pas connue chez *Ichthyotringa africana*. Chez *I. furcata*, il y a 19 rayons caudaux principaux segmentés dont 17 branchus ainsi qu'un écusson dorsal et ventral en avant de la nageoire (obs. pers.; GOODY, 1969 : 13).

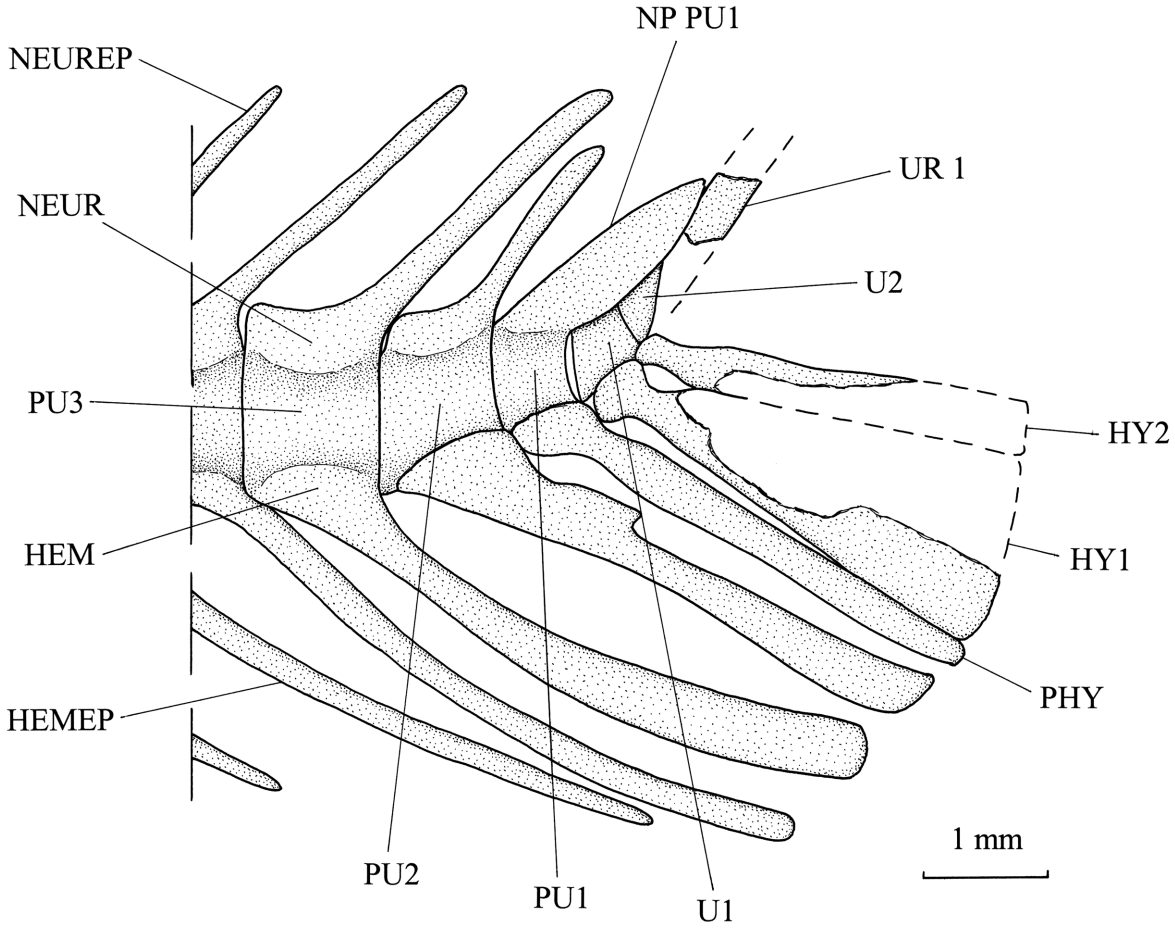

Fig. 6. – *Ichthyotringa africana* (Arambourg, 1954). Le squelette caudal du spécimen MNHN T. 228 D.

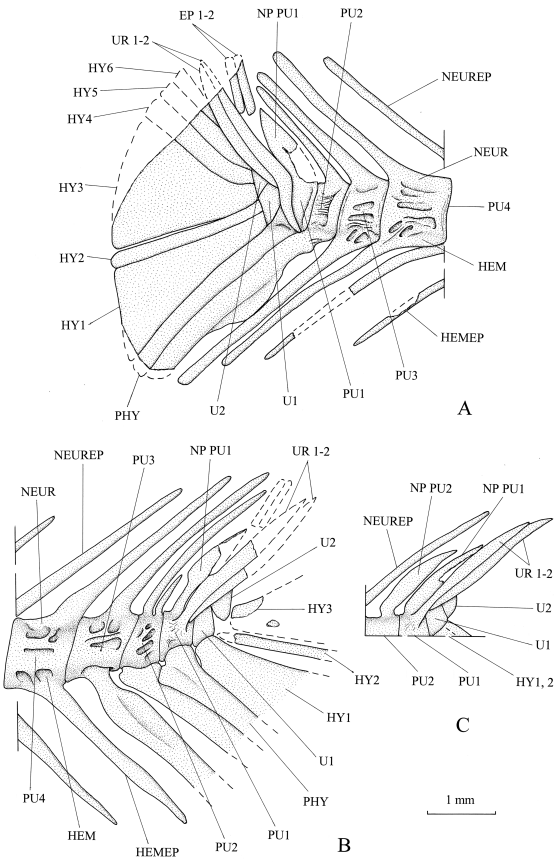

Fig. 7. – *Ichthyotringa furcata* (Agassiz, 1844). Les squelettes caudaux des exemplaires MNH P. 48092 (A), P. 48089a (B) et P. 49544 (C).

L'écaillure

Les écailles ne sont pas conservées. Chez les espèces où l'écaillure est connue, comme *Ichthyotringa furcata*, les écailles sont petites, fines, cycloïdes et elles couvrent tout le corps du poisson, tandis que celles de la ligne latérale deviennent losangiques et s'ornent d'une fine carène horizontale (obs. pers.; GOODY, 1969 : 13).

DISCUSSION

Ichthyotringa *au sein des téléostéens*

Les rares auteurs du 19ème siècle qui ont essayé de situer *Ichthyotringa* au sein des téléostéens (PICTET & HUMBERT, 1866; DAVIS, 1887) ont placé ce genre dans un groupe des Halecoidei sensé inclure les Clupeidae et les Salmonidae mais dans lequel on rangeait également des formes aussi variées que l'albuliforme *Osmeroides* Agassiz, 1844, l'ichthyodectidé *Chirocentrites* Heckel, 1849 ou l'osméroïde *Spaniodon* Pictet, 1850.

WOODWARD (1901) est le premier, au 20ème siècle, à avoir placé *Ichthyotringa* dans la famille des Scopelidae (= Myctophidae) et son avis fut suivi par ARAMBOURG (1954), LEONARDI (1966) et SORBINI (1976). En revanche, GOODY (1969), considéra *Ichthyotringa* comme un salmoniforme et non pas comme un myctophiforme. ROSEN (1973) classa *Ichthyotringa* et les divers enchodontoïdes parmi les Aulopiformes et plus particulièrement dans le sous-groupe qu'il appela «the Cretaceous alepisauroids», soulignant les affinités qui liaient ces formes fossiles aux Alepisauroidei modernes. Cet auteur ne traita cependant pas de façon détaillée le cas *d'Ichthyotringa*. NELSON (1994) entérina les idées de ROSEN (1973) et rapporta lui aussi ces poissons crétacés aux Aulopiformes, tout en les rangeant dans un sous-ordre particulier des Enchodontoidei, pour bien marquer que les Alepisauroidei récents se démarquent d'eux par plusieurs caractères spécialisés.

La plupart des apomorphies par lesquelles on définit aujourd'hui l'ordre des Aulopiformes (REGAN, 1911; GOSLINE et al., 1966; ROSEN, 1973; SULAK, 1977; STIASSNY, 1986; HARTEL & STIASSNY, 1986; JOHNSON et al., 1996; BALDWIN & JOHNSON, 1996) concernent principalement les tissus mous et certains détails ostéologiques qui ne sont guère accessibles chez *Ichthyotringa*. Néanmoins, quatre caractères spécialisés de ce genre confirment qu'il appartient effectivement aux Aulopiformes :

(1) Les os pelviens présentent des processus postéromédians articulés entre eux et qui dépassent vers l'arrière le niveau d'insertion des nageoires ventrales (REGAN, 1911 : 11; PARR, 1929 : fig. 3D; STIASSNY & MOORE, 1992 : fig. 2A; JOHNSON et al., 1996 : fig. 19; BALDWIN & JOHNSON, 1996 : fig. 4, 5).

(2) La série épipleurale débute dès le niveau de la première vertèbre (PATTERSON & JOHNSON, 1995 : 28).

(3) Les premiers épipleuraux sont situés au niveau du septum horizontal (ibid. : 29-30).

(4) La neurépine préurale 1 est réduite et spatulée (GOODY, 1969 : fig. 4, 42, 48, 64, 69; ROSEN, 1973 : fig. 44, 47-53; etc.).

Parmi les Aulopiformes, l'allongement du museau, l'importance de l'ouverture buccale et le grand développement des dents sur l'arcade palatine rapprochent davantage *Ichthyotringa* des Alepisauroidei que des Aulopoidei. Toutefois, *Ichthyotringa*, comme tous les enchodontoïdes du Crétacé, présente deux caractères plésiomorphes déjà disparus chez les Alepisauroidei, la pleine participation du maxillaire au bord buccal et la conservation de dents sur l'ectoptérygoïde. Cela détermine pour eux l'appartenance à un sous-ordre particulier des Enchodontoidei. Les Aulopiformes récents, quant à eux, montrent un maxillaire rejeté du bord buccal au profit du prémaxillaire et les Alepisauroidei modernes possèdent une dentition palatine limitée au seul palatin et n'intéressant donc pas l'ectoptérygoïde (REGAN, 1911 : fig. 2, 6; PARR, 1929 : fig. 2, 5C, 6, 10, 11, 14; GOODY, 1969 : fig. 78, 79, 90; SULAK, 1977 : fig. 3, 5, 7, 11, 13, 20; STIASSNY, 1986 : 5; HARTEL & STIASSNY, 1986 : fig. 2; JOHNSON et al., 1996 : fig. 6).

Ichthyotringa *et les Ichthyotringoidei*

GOODY (1969) crée une famille des Apateopholidae pour l'unique genre *Apateopholis* Woodward, 1891 du Cénomanien du Liban. Il place cette famille aux côtés des Ichthyotringidae dans un nouveau sous-ordre des Ichthyotringoidei qu'il range parmi les Salmoniformes.

Pourtant, les caractères sur lesquels l'auteur fonde ce sous-ordre sont plésiomorphes chez les Enchodontoidei (corps court, rayons branchiostèges nombreux, post-

cleithra présents, deux vertèbres urales indépendantes, etc.). Par contre, les différences entre *Ichthyotringa* et *Apateopholis* sont très nombreuses au niveau des caractères spécialisés. *Apateopholis* a perdu l'orbitosphénoïde, le basisphénoïde et le supratemporal. Ses os dermiques sont ornés de tubercules. Son crâne est latéro-pariétal. Sa fosse temporale est ouverte dorsalement. Son préoperculaire porte une forte épine. Le condyle articulaire de son hyomandibulaire est dédoublé. Les écailles sont perdues sauf celles de la ligne latérale.

Le seul réel caractère apomorphe commun à ces deux genres est l'existence d'un très long rostre mais ce caractère est extrêmement homoplasique chez les Aulopiformes enchodontoïdes et se manifeste dans diverses familles du groupe.

Il ne paraît donc pas y avoir de raison valable de maintenir un sous-ordre des Ichthyotringoidei qui grouperait les Ichthyotringidae et les Apateopholidae, alors que le rapprochement de ces deux taxons n'est pas soutenu.

Ichthyotringa *et Apateodus*

GOODY (1969) range, mais avec doute, *Apateodus* Woodward, 1901, un genre du Turonien d'Angleterre connu uniquement par des restes crâniens, aux côtés d'*Ichthyotringa* dans la famille des Ichthyotringidae.

Il est clair cependant que les caractères anatomiques que GOODY (1969) invoque sont tous plésiomorphes chez les Enchodontoidei (os dermiques peu ou pas ornementés, crâne médio-pariétal, fosse temporale couverte dorsalement, orbitosphénoïde et basisphénoïde conservés, vastes infraorbitaires postérieurs, absence d'épines sur le préoperculaire, etc.). Quant aux caractères spécialisés d'*Apateodus* (hypertrophie du sphénotique, développement d'énormes crocs sur le palatin et le dentaire, perte de la branche ventrale du préoperculaire, etc.), elles ne se retrouvent pas chez *Ichthyotringa*. De plus, *Apateodus* conserve encore l'interoperculaire et des dents sur l'entoptérygoïde mais ne possède pas de dents sur le vomer. En revanche, *Ichthyotringa* a déjà perdu l'interoperculaire et les dents de l'entoptérygoïde mais conserve celles du vomer. Aucun caractère spécialisé ne semble donc militer pour unir ces deux genres au sein d'une même famille.

Dans ces conditions, il est préférable d'exclure *Apateodus* des Ichthyotringidae.

Ichthyotringa *et les Dercetidae*

Tout récemment, FIGUEIREDO & GALLO (sous presse) ont associé *Ichthyotringa* aux Dercetidae, une autre famille d'Aulopiformes enchodontoïdes à long rostre.

Toutefois, à part l'existence d'un rostre et de quelques caractères primitifs en commun, l'ostéologie comparée montre qu'*Ichthyotringa* ne partage pas les principaux traits spécialisés qui caractérisent les Dercetidae (GOODY, 1969; TAVERNE, 1987, 1991), à savoir l'allongement très important du corps, l'augmentation concomitante du nombre des vertèbres, la perte des écailles, le gain d'écussons dermiques qui couvrent partiellement ou totalement le corps, la perte de l'orbitosphénoïde, l'hypertrophie des hémapophyses, la diminution du nombre des hypuraux et la soudure du petit centre ural 2 avec l'un ou l'autre des hypuraux dorsaux, ces deux dernières apomorphies étant déjà réalisées chez les formes les plus primitives de la famille, comme *Dercetis triqueter* Pictet, 1850 du Santonien du Liban (obs. pers.).

Il n'y a donc pas lieu d'intégrer *Ichthyotringa* aux Dercetidae.

Ichthyotringa *au sein des Enchodontoidei*

Ichthyotringa ne présente pas les caractères par lesquels GOODY (1969) définit les principales familles d'Aulopiformes enchodontoïdes : les Enchodontidae, les Eurypholidae, les Cimolichthyidae, les Halecidae et les Prionolepididae. Il n'offre pas non plus les apomorphies de *Rharbichthys* Arambourg, 1954, de *Yabrudichthys* Chalifa, 1989, de *Nardorex* Taverne, 2004, ni des Serrilepidae, quelques Enchodontoidei dont GOODY (1969) n'a pas traité (ARAMBOURG, 1954; TAVERNE, 1985, 2004; CHALIFA, 1989; FIGUEIREDO et al., 2001). Nous avons déjà vu qu'*Ichthyotringa* diffère également d'*Apateodus*, des Apateopholidae et des Dercetidae.

Les caractères spécialisés d'*Ichthyotringa* sont peu nombreux et concernent presque tous son long rostre. Il y a l'allongement des os qui participent à ce rostre, le mésethmoïde qui acquiert une extrémité antérieure pointue ainsi que les prémaxillaires qui se soudent en avant du mésethmoïde pour former la pointe dorsale de ce rostre. On peut encore ajouter la perte du supramaxillaire et de l'interoperculaire. Or, ces apomorphies sont homoplasiques chez les Aulopiformes enchodontoïdes et ne permettent guère de situer un genre au sein du groupe.

D'autre part, *Ichthyotringa* a conservé plus de plésiomorphies qu'aucun autre genre du sous-ordre. Son corps est court et couvert d'écailles cycloïdes. Les vertèbres sont peu nombreuses. Les os dermiques sont peu ou pas ornementés. Le crâne est médio-pariétal. La fosse temporale est couverte dorsalement. La commissure sensorielle pariétale est longue et se poursuit jusque sur le pariétal. Il y a conservation des dents vomeriennes, de l'orbitosphénoïde, du basisphénoïde, des grands infraorbitaires postérieurs, du supratemporal, de la branche ventrale du préoperculaire, de nombreux rayons branchiostèges, de deux postcleithra, de deux uroneuraux et de six hypuraux autogènes. Les vertèbres préurale 1, urale 1 et urale 2 sont autogènes.

Ce grand nombre de plésiomorphies et ces rares apomorphies présentes chez *Ichthyotringa*, incitent à penser que ce genre est l'un des plus primitifs, si ce n'est le plus primitif du groupe, ce qui était d'ailleurs déjà l'avis de GOODY (1969).

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Contribution to the study of the post-embryonic development and reproduction of the African millipede *Epibolus pulchripes* (Gerstäcker, 1873) (Diplopoda, Pachybolidae)

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ABSTRACT. A collection of 215 specimens of different stadia of the African millipede *Epibolus pulchripes* allowed studying its post-embryonic development, with special attention drawn to (i) the number of segments and ocelli at each stadium and (ii) the development of the copulatory organs of males and females. Laboratory rearing of adult *E. pulchripes* from Kenya revealed some aspects of reproduction, from the structure of the adult reproductive organs to mating behaviour and oviposition, as well as the first stadia of post-embryonic development. Based on histological observations and scanning electron microscopy, the position of the copulatory organs during mating and the function of the different parts of the reproductive organs are hypothesized.

KEY WORDS : *Epibolus pulchripes*, reproduction, development, morphology

INTRODUCTION

The post-embryonic development and life histories of Diplopoda have already been studied in Glomerida (JUBERTHIE-JUPEAU, 1967; IATROU & STAMOU, 1988), Spirobolida and Spirostreptida (DEMANGE & GASC, 1972; BERCOVITZ & WARBURG, 1988), Polydesmida (BHAKAT, 1987), Chordeumatida (BLOWER, 1987), Penicillata (KARAMAOUNA, 1990), Colobognatha (DAVID & COURET, 1983) and a lot of Julida (see references in SAHLI 1974). A relevant synopsis dealing with these topics has also appeared (ENGHOFF et al., 1993). Apart from the ecological features, much of the attention in these studies has been drawn to ocular and segmental formulae, mostly culminating in a cone-shaped presentation of all the possible segmental formulae revealed for each stadium. In some papers, the development of the copulatory organs is considered (*e.g.* BERNS, 1968). However, knowledge of the ontogeny is a very important step in the understanding of the evolution and functions of the different parts of the reproductive system.

Only a few studies on the life history (reproduction and development) of pachybolid millipedes have hitherto been made (e.g. : VACHON, 1947; DEMANGE, 1972; AOUTI, 1980). None of them concerns the millipede *Epibolus pulchripes* Gerstäcker, 1873, even though this species is of significant ecological importance and potential use in biotope rehabilitation (HALLER & BAER, 1994).

In this paper, the most important features of the different stadia of the post-embryonic development are described including external morphology, segmental and ocular formulae and the development of the genitalia. The main objective is to describe the reproductive organs and to formulate a hypothesis of the function of the different parts during copulation.

MATERIALS AND METHODS

Epibolus pulchripes is quite common along the coastal line of Kenya. A total of 215 specimens were collected by hand at Arabuko Sokoke, a mixed *Afzelia* forest near Malindi. Adults were kept alive for rearing in the Royal Museum for Central Africa at Tervuren, Belgium (MRAC). The rest was stored in 70% ethanol. Millipedes were fed with fresh cucumbers and fish food (Tetramin) on a substrate of wet soil (Terrau Universel) in a terrarium at 18–20°C. One male with one female were accommodated in small mating arenas for observation of copulation and oviposition. Additional males were occasionally added to test male competition in *E. pulchripes*.

Developmental stadia were distinguished based on DEMANGE'S (1972) observations that each moult is followed by adding one row of ocelli. In spirobolidans, stadium I has one ocellus, stadium II has 3 ocelli in 2 rows, stadium III with 6 in 3 rows, and so forth. Thus, stadium number and the number of rows of ocelli are equal.

The study of the male genitalia was made using specimens previously anaesthetized with Nembutal and injected with Bouin's fixative to cause an extension of the penis and gonopods. Gonopodal and vulval nomenclature follows BERNS (1968) and ENGHOFF (1977), respectively.

For histological observations, the anterior body rings were removed from anaesthetized individuals (with Nembutal). They were fixed in Bouin's fixative, decalcified, embedded in paraplast, cut in 5-µm sections, and stained

with Masson's trichrome (GANTER & JOLLÈS, 1969–1970).

For scanning electron microscopy (SEM), samples were dried, mounted on aluminium stubs, coated with gold and observed with a JEOL JSM–5400LV microscope.

All the material studied here is deposited in the Royal Museum for Central Africa (MRAC)

RESULTS

External morphology of the adults

Epibolus pulchripes is a black millipede with red legs, commonly called the "Mombasa train". It can reach 9–12 cm in length, while the head and anal valves are bright red in colour. Starting from ring 6, brown dots can be found on the sides, the so-called ozopores, the openings of the defensive glands. When mature, the *E. pulchripes* male can easily be distinguished from the female by its bright black body and white adhesive pads (Ad) on the tarsus (Ta) (Fig. 1). Females are generally larger and duller black in appearance, without adhesive structures on their legs.

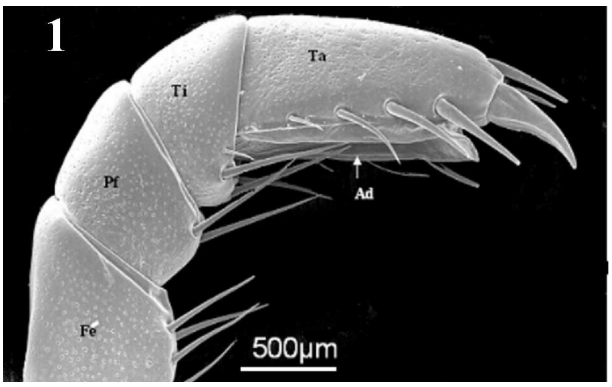


Fig. 1. – Posterior view of a male leg with a detail presentation of an adhesive pad : Ad : Adhesive pad; Fe : Femur; Pf : Postfemur; Ta : Tarsus; Ti : Tibia.

Structure of adult genitalia

Male genitalia : The male has two essential structures for copulation : the so-called penis (P) and the gonopods.

The penis : The penis is the differentiated end of a ladder-shaped testis. As the penis in *E. pulchripes* takes no part in direct sperm transfer, this structure cannot properly be termed as penis. To emphasize its double structure due to fusion of two leg anlages, the penis in Diplopoda is often referred to in plural, as penes. Like the gonopores in any Diplopoda, it lies on ring 2 and is only everted during copulation. When extended, the penis reaches up to body ring 6 (10–15 mm) and shows a typical bifurcated apical end. The two apical tips bend back to the front, thus shaping like an anchor (Fig. 2).

Histological observations reveal that both spermal ducts (Sd) form a single channel when entering the first part of the penis. Towards the end, this channel splits again into two apical tips. A longitudinal section of a retracted penis shows the anterior membrane as being more rugose than the posterior one. A cross-section reveals retractor muscles (Rm) at the base of the penis (Fig. 5).

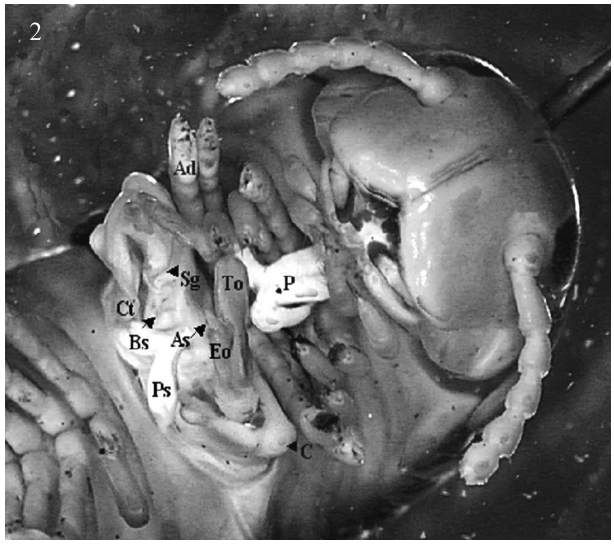


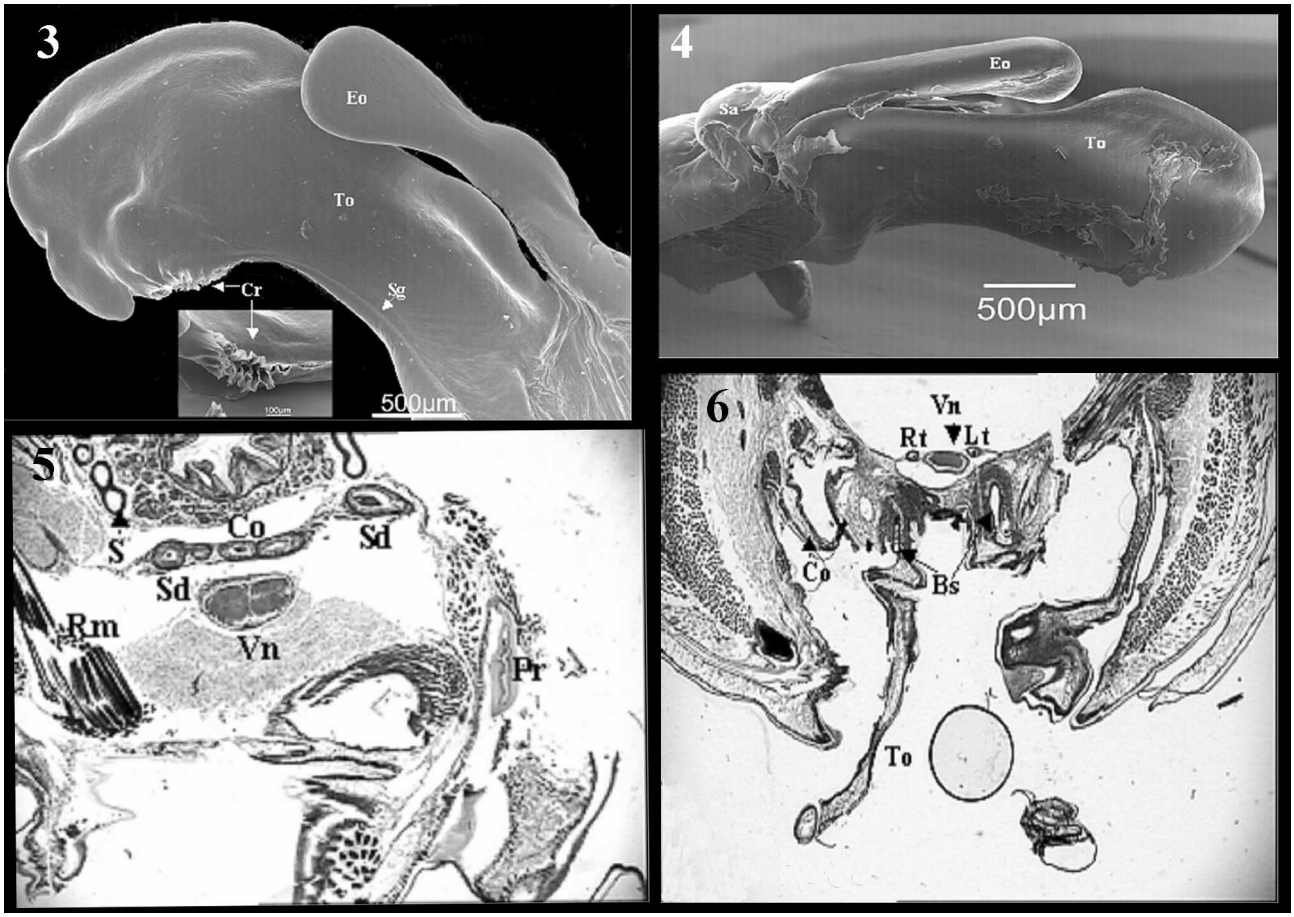
Fig. 2. – Photo of the anterior segments of a male with artificially extended reproductive organs, ventral view :
Ad : Adhesive pad; As : Anterior sternum; Bs : Bursae seminalis; C : Coxa; Ct : Coxal telepodite; Eo : Endite of opisthomere; P : Penis; Ps : Posterior sternum; To : Telopodite of opisthomere.

The gonopods *:* The gonopods are modified legs used for the transfer of sperm; they consist of two main parts : the anterior gonopod (Ag), or coxoid, and the posterior gonopod (Pg), or opisthomere. Functionally, the gonopods are not a penis either, instead they serve as spermatopositors. The Ag includes a coxa (C), a coxal bar, a coxal endite and a telopodite (Fig. 2). The Ag protects the Pg, which consist of a pair of telopodites (To) and its spatuliform endites (Eo) (Fig. 3). This endite is attached to the base of the telopodite in a spring-like way and bends back when moved aside. The Pg telopodite shows two small excavations at its base and a groove (Sg) that runs from these excavations to a membranous crown (Cr) at the tip (Figs 2 & 4). In cross-section, the groove appears to be a tube formed by the integument of the anterior side overlapping that of the posterior side.

Histological observations show that the two excavations at the base of the telopodites are in fact two small receptacles (Bs) that are connected to the groove (Fig. 6). They also reveal retractor muscles at the base of each gonopod (Fig. 6).

Female genitalia : The female genitalia consist of two vulvae, each located in a vulval sac (Vs) on the posterior side of the coxae of the second pair of legs.

Each vulva consists of four sclerites : a caudal valve (Vca), a rostral valve (Vr), a ventral ridge (R) in-between and a median operculum (O) (Fig. 7). The ridge can be seen from outside as a structure that is folded between the two valves; in the middle of the vulva it disappears between these valves. On the opposite side of the operculum, an apical cluster of setae (S) can be found on the valves.



Figs. 3-6. – SEM and histological images of male reproductive system :
3 : Posterior gonopod or opisthomere with details of membranous crown at the end of seminal groove; 4 : Details of a spring-like attachment of the endite to the telepodite of opisthomere; 5 : Section through the second segment with a retracted penis; 6 : Section through the seventh segment with retracted gonopods.
As : Anterior sternum; Bs : Bursae seminalis; C : Coxa; Co : Connection; Cr : (membranous) Crown; Ct : Coxal telepodite; Eo : Endite of opisthomere; Lt : Left testis; P : Penis;; Pr : Penis : right part; Ps : Posterior sternum; Rm : Retractor muscle; Rt : Right testis; S : Salivary gland; Sa : Spring-like attachment; Sd : Spermal duct; Sg : Seminal groove; To : Telepodite of opisthomere; Vn : Ventral nerve chord.

Histological observations show that the single ovary splits into two oviducts (Od). Each oviduct enters a vulva dorsally through the bottom of the vulval sac and between the two valves.

The bottom of the ridge is extremely digitised and shows two peculiar glands (Bg) just beneath the setae on the valves (Figs 8 & 9). They are formed of club-shaped ciliated cells which bear an apical cilium. Between both the glands, a long cilium is located. The glands are flanked by two bands of muscles (M). One band connects the outer epidermis of the valves with the epidermis of the ridge, while the other band is attached to the two outer epidermis layers of the two valves.

Reproduction and egg-laying

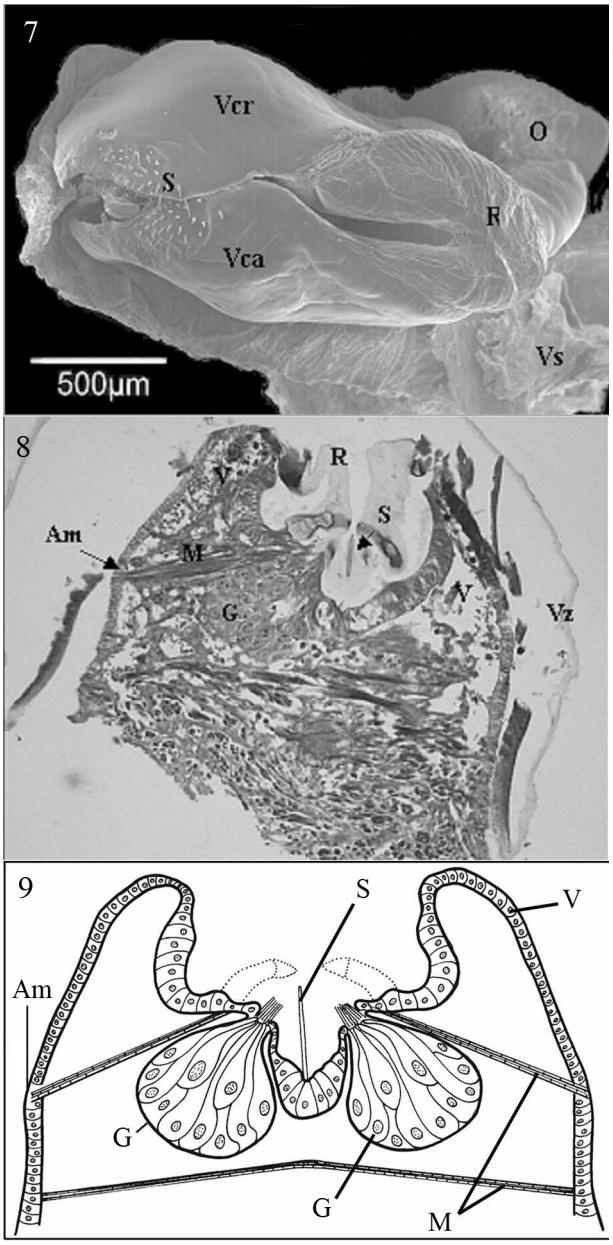
Reproduction : Several copulations were observed in captivity. Once a male finds an adult female, he mounts her back using the adhesive structures on his legs. If he succeeds to climb entirely onto her back, he taps his legs against her flanks in an undulating fashion to stimulate her. This process can last hours, resulting in the female carrying the male around over considerable distances. When the female is ready for copulation, she raises her head and the male entwines his body around hers until their copulatory organs come in contact. He then inserts his posterior gonopods placed inside the seventh ring into the vulvae of the female which are positioned inside the second ring. Then sperm transfer takes place.

When further males were placed inside the mating arena, they did try to compete and interfere with this male by climbing on top of the mating pair.

Egg-laying : The female can lay eggs several times following one copulation. In captivity, the first egg-laying event was observed two weeks after copulation, followed by a second egg-laying event one week later.

For egg-laying, the female makes a small depression in the soil. She then rolls up and starts depositing the eggs one by one. As the eggs come out of the vulvae, she picks

them up by her front legs to coat them with a mixture of soil and saliva, making them look exactly like faeces. Each time she produces 10 to 15 eggs, depositing them while defecating.



Figs. 7-9. – Female reproductive system :
7 : SEM image of vulva and second pair of legs; 8 : Section through bursal glands (place of section indicated by arrows in Fig. 7); 9 : Scheme of the section given in Fig. 8.
Am : Attachment of muscle; G : Bursal gland; M : Muscle; O : Operculum; R : Ridge; S : Setae; V : Valve; Vca : Caudal valve; Vr : Rostral valve; Vs : Vulval sac.

Development

Eleven stadia have been recognized during the development, the most important features of the different stadia being summarized in Table 1.

Stadium I : After hatching, the pupoid moults into the first true larval stadium. This was not observed during our study.

Stadium II : Seven juveniles of stadium II were recovered from the nursery. They were slightly transparent and measured 0.5 cm in length. They can easily be recognized by their unique segmental formula consisting in a collum, 6 podous and 14 apodous rings, as well as a telson (6+14+T).

Since the fifth ring is the first diplosegment like in all Spirobolida, seven pairs of legs can be found. The number of ocelli is 3, they are arranged in two rows : 1+2 ocelli. At this stadium the first defensive gland is visible as a black dot through the transparent cuticle.

Stadium III : Slightly longer than stadium II, juveniles of stadium III are 0.7–0.8 cm long. They show 20 podous and 6 apodous rings (20+6+T), as well as three rows of ocelli, bringing the total up to six ocelli (1+2+3); 15 defensive glands are visible through the white cuticle on each side of the body.

Stadium IV : At stadium IV, juveniles measure about 1 cm in length and have a black body with white legs. Each specimen shows 26 podous and 6 apodous rings (26+6+T), as well as four rows of ocelli (1+2+3+4). At this stadium, male and female can be distinguished; in males, the legs of the seventh ring have disappeared, being replaced by two pairs of gonopod primordia (Table 1, IV a, c).

Stadium V : At stadium V, the millipedes are 1.5–2.0 cm long and show the typical adult colour pattern of a black body with red legs. The defensive glands are visible as small brown circles on the cuticle around the pores. All 21 males and 27 females observed have a segmental formula of 32+6+T, as well as five rows of ocelli (1+2+3+4+5) (Table 1, Va).

Although the development of the vulvae in females has not started yet, the anterior gonopod (Ag) and posterior gonopod (Pg) primordia in males are clearly distinguishable. The Pg primordia have migrated between the two primordia of the Ag, both pairs, especially the Pg, being elevated above a sclerotized sternum (Table 1, Vc).

Stadium VI **:** At stadium VI, the juveniles measure 2.5 ± 0.2 cm in length. Only one segmental formula has been found : 38+6+T. All 52 millipedes observed had six rows of ocelli bringing the number up to a total of 1+2+3+4+5+6 = 21 ocelli (Table 1, VIa).

The gonopods start forming distinctive structures. Each Ag is divided into two pieces : a future coxa (C) and telepodite (Ct). Each Pg has now clearly sunk into the sternum to start showing the first curve which will be best recognized in an adult Pg telopodite (Table 1, VIc).

This is also the first stadium where the primordia of the vulvae can be seen behind the coxae of the second pair of legs. Three small protuberances represent the future valves and the ridge (R) of the vulva. They are placed in a triangular way, the valves forming the two longest sides, the ridge making out the top of the triangle (Table 1, VIb).

Stadium VII : At stadium VII, the millipedes are 3.9 ± 0.4 cm in length. This is the first stadium where two different segmental formulae have been observed : 43+5+T and 44+5+T. The total number of ocelli is no longer con-

stant, as the rows that are added vary in the number of ocelli. However, the number of rows, here seven, can still be used to determine the stadium (Table 1, VIIa).

The gonopods now have a triangular anterior sternum, while the Pg coxa and telopodite are placed around the Pg (Table 1, VIIc). The vulvae are a little larger, the only difference from the preceding stadium laying in that the ridge starts bending (Table 1, VIIb).

Stadium VIII : At this stadium the millipedes are 5.0 ± 0.5 cm long. Two different segmental formulae have likewise been found : 49+3+T and 48+3+T. The ocular field has also gained one row of ocelli, resulting in 8 rows (Table 1, VIIIa). The gonopods are more compact, the Pg fitting into the cavity formed by the Ag. Only the Pg telopodite has grown significantly (Table 1, VIIIc).

The valves of the vulvae are now almost fully separated from the coxae; the ridge shows its first curve, being folded between the two valves. A primordial operculum (O) can be distinguished on the opposite side of the vulva. The posterior valve is significantly larger, but narrower than the anterior one (Table 1, VIIIb).

Stadium IX : At stadium IX, the millipedes measure 7.1 ± 0.9 cm in length and show 9 rows of ocelli; only one segmental formula has been revealed (52+0+T; Table 1, IXa).

The gonopods are very similar to those of an adult, but they are not functional yet, because each Pg is not completely developed, being very small and still showing its typical curved shape absent (Table 1, IXc).

Being larger, the vulvae differ from the preceding stadium only by the occurrence of a dozen setae (S) on each valve just above the ridge. The operculum is shell-shaped (Table 1, IXb).

Stadium X : At stadium X, specimens are 8.3 ± 0.7 cm in length and also count 52 podous rings and a telson. Only the addition of an extra row of ocelli (10 rows) indicates that the millipedes are at stadium X (Table 1, Xa).

The gonopods are now almost completely developed. The Ag are completely developed in shape, but still are a little smaller, while the apical part of the Pg is different from the adult stadium : the membranous crown is not yet formed and only wrinkles of the integument around the end of the seminal groove can be seen (Fig. 10) (Table 1, Xc).

The valves of the vulvae are now shaped like those of a functional vulva and 20–30 setae occur on the valves on the opposite side of the operculum. The ridge is very small and still located on the side opposite to the operculum of the vulva (Table 1, Xb).

Stadium XI : Both males and females are now sexually mature and have eleven rows of ocelli. During the last moult towards sexual maturity in males, only the telopodite of the Pg undergoes a last change, i.e. formation of the membranous crown around the apical pore (Figs 1 & 4). The vulvae undergo one last big change as well; this is a migration of the ridge lying between the two valves to the other side of the vulva, thus creating an opening between the two valves and the folded ridge (Fig. 7).

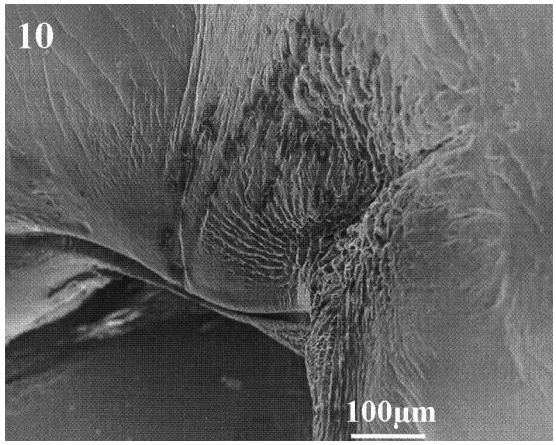


Fig. 10. – Details of membranous crown of opisthomere at stage X.

DISCUSSION

Development patterns

Like in the spirobolid *Narceus annularis* (Rafinescue, 1820), maturity of *E. pulchripes* is attained at stadium XI. The eggs of *E. puchripes* are laid individually in earthen capsules carefully manufactured by the female. Stadium I remains inside the capsule and is likely to have only one ocellus, as all other spirobolidans (pachybolids) studied (Vachon, 1947; Demange, 1972; Aouti, 1980). Based on the literature (Nguyen Duy-Jacquemin, 1992; Enghoff et al., 1993), the first stadium probably has three podous and four apodous rings with a telson. This hexapodous larva moults into the first free larval stadium, which is also the first stadium with one diplosegment. Up to stadium IX, the post-embryonic development seems to be anamorphotic, meaning that each moult results in an addition of podous and apodous rings. The presence of immature specimens without apodous rings at stadia IX and X suggests that anamorphosis is followed by epimorphosis. Thus, as in most species of Spirobolida, the post-embryonic development of *E. pulchripes* appears to be hemianamorphotic.

Like in *Pelmatojulus ligulatus* (Voges, 1880) (see Enghoff et al., 1993), the development of *E. pulchripes* is remarkable for its very modest variability in segmental formulae. Up to stadium VI (38+6+T), all show a single segmental formula for each stadium. Looking at stadium VII, however (44+5+T and 43+5+T), other possible options might exist at stadium VI as well : 37+6+T or 38+5+T. Such a variation has already been well documented (e.g. Sahli, 1969; Enghoff et al., 1993) that, from a certain stadium on, two possible segmental formulae are evident that can give rise to two new possible formulae, and so on. Thus, a cone-shaped scheme can be constructed with most of the possible segmental formulae observed at the last stadia before adulthood. No coniform presentation could be obtained in our study, because we have apparently failed to discover all segmental formulae. Although in almost all pachybolid millipedes the variation in ring number is known to be somewhat more modest than in the other millipedes (Enghoff, 1977), *E. pul-*

chripes studied here shows an extremely low variation in segmental formulae in the course of development. This might be due to the fact that all material was collected in the same place and represented the same population. SAHLI (1969) showed that variation within a single population is usually smaller than that between two separated populations, while ENGHOFF (1977), in his revision of the genus *Epibolus* Cook, 1897, found a higher degree of variation in the adult number of rings of *E. pulchripes* between populations stemming from different geographical regions (51+0+T up to 54+0+T).

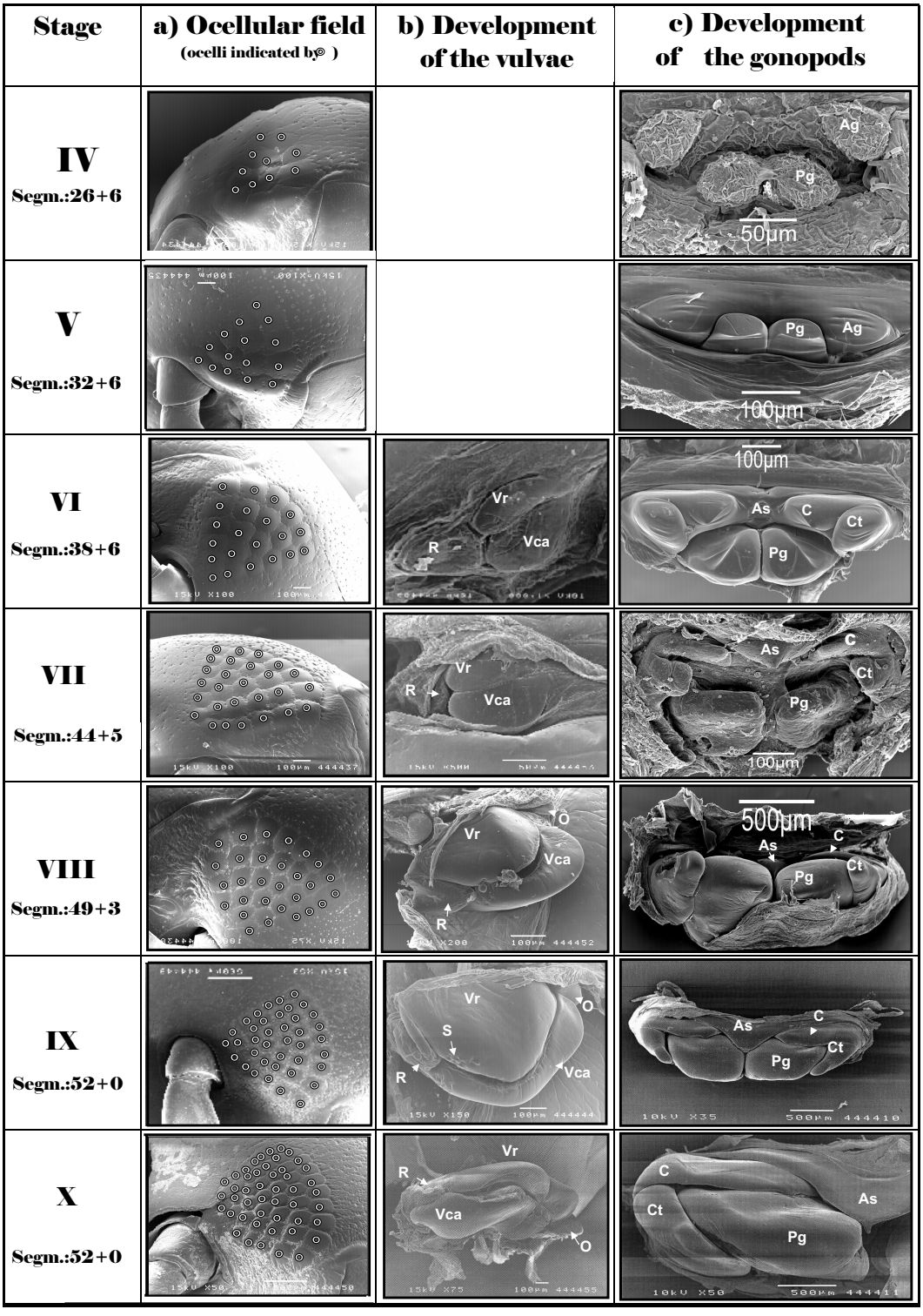


Table 1. – **Most important features of different stadia of the post-embryonic development**. **Ag :** Anterior gonopod; **As :** Anterior sternum; **C :** Coxa; **Ct :** Coxal telepodite; **O :** operculum; **Pg :** Posterior gonopod; **R :** Ridge; **S :** Setae; **Vca :** Caudal valve; **Vr :** Rostral valve

The gonopods of spirobolidan millipedes have long been assumed to represent modified appendages of the seventh body ring (e.g. KEETON, 1960). BERNS (1968) was the first to study the development of the copulatory organs in the spirobolid *Narceus annularis*. He showed that the gonopods actually originate from the elliptical primordia that arise when the legs of the seventh ring fall off during the stadium IV to V transition. In *E. pulchripes*, the legs on the seventh body ring also fall off, but this occurs during the moult of stadium III to IV. This looks like an evolutionary imperfection, when considering entropy, since the development of the legs on the seventh body ring during the earlier stadia seems to be unnecessary. Based on the present observations, it seems impossible to determine the evolutionary advantages of this process.

BRÖLEMANN (1922) was the first to study the structure of the vulva in millipedes. According to him, the vulva consists of four sclerites : a ridge, an operculum, a caudal valve and an apical valve. Although ENGHOFF (1977) mentions that, in *Epibolus*, the apical valve is divided into two different sclerites, we agree with Brölemann, as in our study of the ontogeny of the vulva only four sclerites could be distinguished. On the other hand, in adult females one of the two valves is partly divided, creating the illusion that it consists of two parts instead of one. In their histological study of the vulvae of several diplopods, BRÖLEMANN & LICHTENSTEIN (1919) described the structure of two glands called 'glandes de la bourse' in the vulvae of *Archispirostreptus tumuliporus sudanicus* (Brölemann, 1905) (Spirostreptidae, Spirostreptida). The histological observations we made of the vulva of *E. pulchripes* also reveal the presence of two glands at the lateral end of the vulva. The location and structure of these glands are very similar to the "glandes de la bourse" as described by BRÖLEMANN & LICHTENSTEIN (1919). According to these authors, the position of the excretion pore indicates that the secretion is to be spread at the lateral end at the bottom of the ridge, which acts as a receptaculum seminis (Rs). The glands can be emptied by contraction of the muscles after some stimulation by the setae. The secretion could then cover the sperm at the bottom to preserve it until the eggs are laid.

Mating

Earlier observations show that the first rains after a dry season initiate a mass activity during which the millipedes seek a mate. The adhesive structures on the tarsus of the legs of males are used to obtain a better contact during copulation. These structures have been suggested to have evolved as a result of male competition, if males can be seen trying to take over the female from copulating pairs (TORNHILL & ALCOOK, 1983; EBERHARD, 1985). TADLER (1996) could not support this hypothesis by his study of the reproduction in Julida, since he never observed a male trying to steal a female from another male during copulation. In *E. pulchripes*, however, this behaviour has been observed during this study, adding more support to the male competition hypothesis.

Before mating, the male must evert his penis and gonopods in order to inseminate the female. The julid species *Unciger foetidus* (C. L. Koch, 1838) and *Cylindroiulus boleti* (C. L. Koch, 1847) (see VERHOEFF, 1928; TADLER, 1996) possess a "gonopodal sac" which forms a conspicuous eversible hydraulic structure with intersegmental membranes for a protrusion of the gonopods. Such an elaborate hydraulic system exists neither in *Nemasoma varicorne* C. L. Koch, 1847 (Nematomatidae, Julida), nor *Brachyiulus lusitanus* (Verhoeff, 1898) (Julidae, Julida) (see TADLER, 1996), nor *E. pulchripes* (present paper). Several obsrervations suggest that, in *E. pulchripes*, the reproductive structures of the male are only everted due to an increased inner body pressure : (i) both penis and gonopods can be extended artificially when an anaesthetized animal is injected with a fluid at its rear end; (ii) during copulation and an artificial extension of the gonopods, a white inflated membrane can be seen at their base; and (iii) on the histological slides, only retractor muscles are found for both penis and gonopods.

When the penis is everted, and even when artificially extended, it bends back toward the gonopods. This can be accounted for by the structure of the penis which has the anterior wall longer than the posterior one, constraining to fold it automatically when extended.

As soon as the penis is everted, the millipede bends its head down and the two apical ends of the penis, which are shaped like an anchor, reach exactly the posterobasal parts of the Pg telopodites, where two excavations are observed. These two pouch-shaped structures are in connection with the grooves that run up to the apical membranous crowns of these telopodites. This strongly suggests that these structures are in fact two so-called bursae seminales. Before or during copulation, the bursae seminales are charged with sperm through the two tips of the penis. As observed by ENGHOFF (unpublished), a male can charge his gonopods several times during one copulation. The sperm will then be conducted from the bursae seminales via the seminal grooves towards the membranous crown, through which it will be delivered inside the vulvae of the female.

Although copulation has been observed in captivity, the position of the copulatory organs during insemination has not been monitored clearly enough. So the following hypothesis of the position of the copulatory organs during copulation (Fig. 11) is mainly based on detailed observations of both male and female genitalia.

The telopodite of the posterior gonopod is inserted into the vulvae through the ridge, while the endite bends over the overlapping valves. This way the endite contacts the setae at the apical end of the two valves and thus might stimulate contraction of the muscles that flank the bursal glands. The contraction of the gland is to initiate the secretion and release of a product that might fix and protect the sperm that is being deposited. When the posterior gonopod is drawn back, the endite would bend back into its original position. In this hypothesis, the endites are essential for the stimulation of females. So egg fertilization depends also on stimulation quality.

Since copulations have been observed neither during the period between the first copulation and the first oviposition nor between two ovipositions, it is apparent that the female is capable of carrying about fertile sperm for several weeks. When the eggs leave the vulvae, they might be fertilized by sperm stored in the ridge.

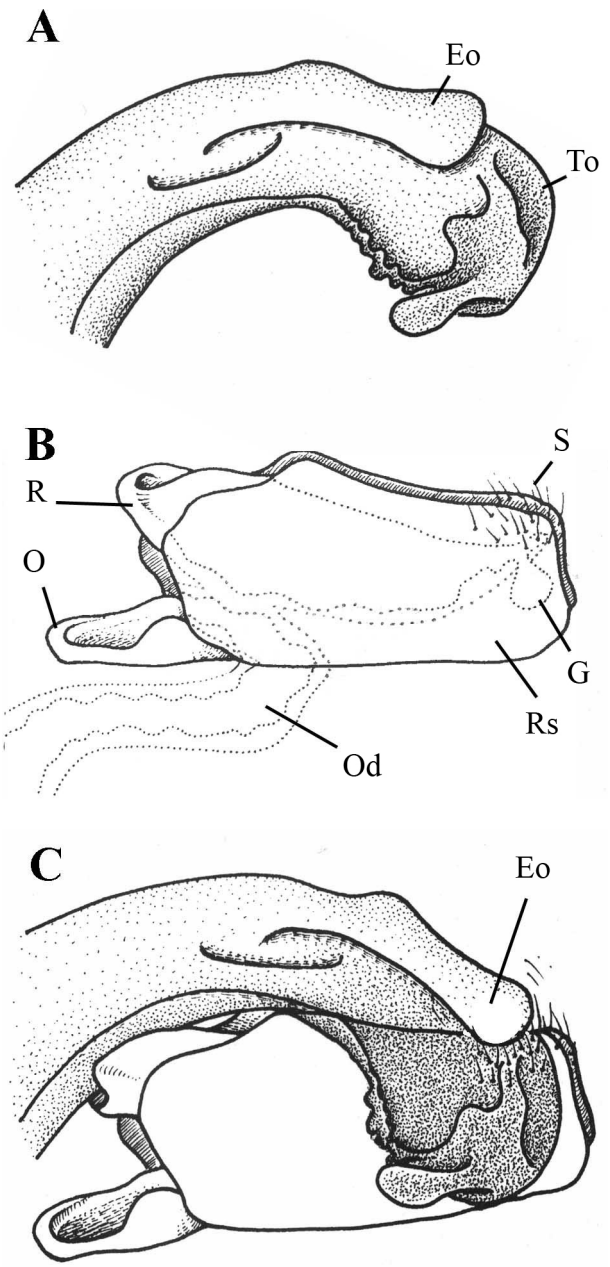


Fig. 11. – **Position of reproductive organs during copulation** : A : Posterior gonopod; B : Female reproductive system; C : Position of genitalia during copulation.
Eo : Endite of opisthomere; **G** : Bursal gland; **M** : Muscle; **O** : Operculum; **Od** : Oviduct; **R** : Ridge; **Rs** : Receptaculum seminis; **S** : Setae; **To** : Telepodite of opisthomere.

Several mechanisms in the process of oviposition can be interpreted as adaptations in order to ensure the best survival of the offspring : by coating the eggs with a mixture of soil particles and saliva the female not only protects the eggs against desiccation, mould and other hazards, but she also camouflages them, since they can hardly be distinguished from the faeces. Upon a single copulation, a female lays 10–15 eggs every two weeks. This way she spreads her offspring in time and space, an evident adaptation against predation.

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Effects of Cypermethrin on Total Body Weight, Glycogen, Protein, and Lipid Contents of *Pimpla turionellae* (L.) (Hymenoptera : Ichneumonidae)

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ABSTRACT. We investigated the changes in total body weight, glycogen, protein, and lipid contents of the endoparasitoid *Pimpla turionellae* L. (Hymenoptera : Ichneumonidae) reared on *Galleria mellonella* L. (Lepidoptera : Pyralidae) exposed to various sublethal doses of cypermethrin added to the food of host larvae. Cypermethrin affected the total body weight of larvae, pupae, and adult females but not males. Results revealed that the levels of glycogen, protein, and lipid in all stages and sexes of the wasp tended to decline with respect to controls. Females showed the most striking decrease in glycogen content whereas larvae were more susceptible to cypermethrin than pupae and adults in terms of decrease in protein and lipid contents.

KEY WORDS : *Pimpla turionellae*, cypermethrin, body weight, lipid, protein, glycogen.

INTRODUCTION

Pesticide research and development has brought a large number of chemicals in protecting the crop against insect pests. However, these chemicals have posed a grave environmental problem because of their indiscriminate usage in fields (TILLMAN & MULROONEY, 2000). Insecticides are also toxic to many nontarget organisms (NATH et al., 1997; SUH et al., 2000) and their usage can disrupt the balance between a host and its natural enemy, resulting in an increase of pest numbers (VAN DRIESCHE & BELLOWS, 1996; TOMBERLIN et al., 2002). Studies have reported that insecticides cause numerous sublethal effects, including increases and decreases in fecundity (TAKADA et al., 2001) and developmental rate (WILLRICH & BOETHEL, 2001), changes in sex ratio, diapause, and morphology by direct interaction with parasitoids and indirectly through host physiology (CROFT, 1990). Pyrethroids are currently among the major insecticides used against pests (USMANI & KNOWLES, 2001). Although pyrethroids are effective at low rates and relatively inexpensive, it has been also shown that they are harmful to beneficial insects (NOWAK et al., 2001; XU et al., 2001). Cypermethrin ((±) α-cyano-3-phenoxybenzyl (±) cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) is a pyrethroid insecticide that is used to control insect pests (Cox, 1996). Like all pyrethroids, it kills insects by disrupting normal functioning of the nervous system (VIJVERBERG & BERCKEN, 1990).

Pimpla turionellae L. (Hymenoptera : Ichneumonidae) is a poliphagous endoparasitoid that spends its immature stages in pupae of various lepidopterous species. It serves as a potential biological control agent of many lepidopterous pests (KANSU & UĞUR, 1984; FISHER, 1987). Adult wasps feed on plant nectar and host pupae in nature. Because host species of this parasitoid feed on plants during larval stages, the accumulation of insecticides in host pupae is likely to occur. Therefore, it is possible for *P. turionellae* to be exposed to insecticides by way of nutrients. Biochemical parameters seem quite promising to assess and predict the effects of toxicants on beneficial species. The assessment of the potential effects that insecticides have on the total body weight and biochemical milieu of parasitoids is of great interest for success in biological control applications. This research was aimed at showing how cypermethrin that is likely to be accumulated in the host affects total body weight, glycogen, protein, and lipid contents of parasitoid during development.

MATERIAL AND METHODS

Insect Rearing

The solitary pupal endoparasitoid *P. turionellae* were reared on the pupae of the greater wax moth, *Galleria mellonella* L. (Lepidoptera : Pyralidae) at 25 ± 1 °C, 60 ± 5% RH and a photoperiod of 12 :12 h (L : D). Adult parasitoids were fed 30% (vol :vol) honey solution and provided with host pupae (4 pupae for 10 wasps) once every three days to meet their protein requirement (UÇKAN et al., 2004). Host colony was maintained by feeding the insects with a diet described by BRONSKILL (1961). The diet was modified by increasing the bran ratio by 50% to decrease the humidity of the composition in the diet. A piece of honeycomb was added for egg deposition and feeding of the newly hatched larvae.

Bioassays

Cypermethrin (Imperator, 250 g/liter EC, Zeneca Ltd., İzmir, Turkey) was used in all bioassays as water source

and prepared in distilled water as parts per million of active ingredient. Nine serial dilutions of cypermethrin (5; 50; 100; 150; 200; 300; 400; 500; and 1000 ppm) were used in determining PD_{50} (the median pupation dose) of *G. mellonella* larvae. Different doses of cypermethrin were added in 10 g of the diet in each 80 ml jar. Last instars of *G. mellonella* (n = 10; average weight = 0,16 ± 0,01 g) were exposed to selected doses of cypermethrin for 7 days. The jars were maintained in another laboratory under the same conditions mentioned for the stock cultures. Larvae were removed from the jars and the pupation rate for each dose was observed for 30 days. Experiments were repeated four times. Mortality data were derived from pupation rates, and PD_{50} with 95% confidence limits was calculated by using probit analysis (PriProbit, PriProbitNM (C) 1998-2000 Masayuki Sakuma, Kyoto University, Kyoto, Japan) after Abbott's correction (ABBOTT, 1925) for natural mortality. The 30-d PD_{50} (95% CI) of cypermethrin was 207,3 (181,7 – 235,1) ppm. Therefore, we applied doses less than PD_{50} value to host instars to evaluate the dose-dependent effect of cypermethrin on total body weight, glycogen, protein, and lipid contents of *P. turionellae* stages/sexes (defining larvae, pupae, adult males and females).

G. mellonella larvae were exposed to four different doses (20; 50; 100; and 150 ppm) below PD_{50} value to evaluate the effects of the insecticide on total body weight, glycogen, protein, and lipid contents of *P. turionellae* last instars, pupae, and adults. Batches of 50 host larvae (0,16 ± 0,01 g) were exposed to 50 g of the diet including the selected doses of the cypermethrin for 7 days. Larvae were removed from the diet and those pupated were parasitized by *P. turionellae* females. Parasitoid larvae, pupae, and 0 to 24-h-old adult males and females maintained from host pupae were used in analyses. Groups of 10 insects were sampled at each stage/sex and fresh-weighed. The method developed by ROE et al. (1961) was conducted for glycogen extraction. An anthrone test developed by CARROL et al. (1956) was used for the detection of glycogen obtained from larvae, pupae, and adults using glucose (Merck, Darmstadt, Germany) as standard. Protein extraction was also made with the method developed by ROE et al. (1961) and total protein content of the same samples was estimated by the method of LOWRY et al. (1951) using bovine serum albumin (BSA) (Merck) as standard. The lipid fractions in larvae, pupae, and adults were extracted and total lipid contents were determined using the method described by FOLCH et al. (1957). Total wet weight of each group was calculated, and insects were kept in 5 ml chloroform – methanol (2 :1 vol :vol) at –20 °C until extractions. Samples taken in chloroform – methanol solution were homogenized at 26.000 *X g* for 5 min. After filtering through Whattman paper No : 41, the extracts were placed in hexane solution and washed through distilled water 5 times. Total lipid content as a percentage of wet weight was calculated graviometrically. Groups of 10 individuals of each stage/sex were repeated three times for glycogen-protein and lipid analyses. The effect of cypermethrin on total body weight of *P. turionellae* was determined by pooling the results of mean weight estimations of insects used in glycogen-protein and lipid analyses (6 replicates with 10 individuals per replicate). The amount of average total glycogen, protein, and lipid was estimated as a percentage of wet weight of insects.

Statistics

Data for total body weight and glycogen, protein, and lipid contents were subjected to two-way analysis of variance (ANOVA) (SPSS, 1999) to determine the main effects of cypermethrin doses and stage/sex on total weight and glycogen, protein, and lipid contents, respectively. Differences due to cypermethrin doses in total weight of each stage/sex were inferred using one-way ANOVA (SPSS, 1999). The relationship between cypermethrin doses and percent amount of glycogen, protein, and lipid contents was also compared with one-way ANOVA for each stage/sex. Means were separated using the Tukey's HSD posthoc test (SPSS, 1999). An arcsine square-root transformation was performed on percentage values of glycogen, protein, and lipid contents before analyses. Results were considered statistically significant when $P<0,05$.

RESULTS

The percentage of pupation did not differ from that of the control group when *G. mellonella* larvae exposed to 5 ppm of cypermethrin and the rate was 100% in both cases. The decrease in pupation rate was dose-wise in the order; 92,5; 80; 72,5; 57,5; 35; 20; and 5% when larvae exposed to increasing doses from 50 to 500 ppm. Finally, none of the larvae pupated at 1000 ppm. The effect of cypermethrin on total body weight was dose ($P<0,001$) and stage/sex ($P<0,001$) dependent, and the relationship between doses and weight was significantly influenced by stage/sex ($P<0,05$) (Table 1). Changes in the total weight of cypermethrin treated stages/sexes are presented in Table 2. Total body weights of larvae, pupae and adult females were considerably affected by cypermethrin treatment. However, males did not exhibit a significant weight loss due to cypermethrin treatment (larva : $F= 4,62$; $df= 4, 25$; $P<0,01$, pupa : $F= 7,47$; $df= 4, 25$; $P<0,001$, male : $F= 1,62$; $df= 4, 25$; $P>0,05$ and female : $F= 5,50$; $df= 4, 25$; $P<0,01$). Cypermethrin treatment at 20 ppm induced a slight increase in weight for pupae and adult females (Table 2).

Two-way ANOVAs indicated that the effects of cypermethrin doses and stages/sexes on total glycogen, protein, and lipid contents of *P. turionellae* were significant. Cypermethrin dose – stage/sex interactions were not significant ($P>0,05$) for glycogen and protein, indicating that variation as a result of dose was consistent among stages/sexes. However, stage/sex significantly influenced the relationship between cypermethrin dose and total lipid content (Table 1). Glycogen level of all stages/sexes tended to decrease on exposure to cypermethrin, but did not differ significantly among experimental groups (Fig. 1). The content did not also differ significantly between controls and cypermethrin treated groups in larvae ($F= 0,11$; $df= 4$; 10; $P>0,05$), pupae ($F= 3,18$; $df= 4$; 10; $P>0,05$), and males ($F= 0,10$; $df= 4$; 10; $P>0,05$). However, there was a significant decrease in experimental groups with respect to control group in terms of glycogen level of females ($F= 20,42$; $df= 4$; 10; $P<0,001$). Total

protein in each stage/sex also tended to decrease on exposure to different doses of cypermethrin (Fig. 2). Protein content of cypermethrin treated groups was significantly lower than that of the control group for larvae (F= 12,92; df= 4; 10; P<0,001). There was also a decrease in total protein for pupae (F= 6,46; df= 4; 10; P<0,01) with respect to the control group, but this decline was only significant at 20 ppm. Cypermethrin treated adults had also lower percentage of protein than the control group. However, the decline was not significant (males : F= 1,52; df= 4; 10; P>0,05, females : F= 0,45; df= 4; 10; P>0,05). The effect of cypermethrin on lipid content was not dose dependent and did not decrease all the time with increasing cypermethrin doses (Fig. 3). The difference in decrease between control and experimental groups was only significant in larvae (F= 8,52; df= 4; 10; P<0,01). Lipid contents of cypermethrin treated pupae (F= 2,78; df= 4; 10; P>0,05), males (F= 3,47; df= 4; 10; P>0,05), and females (F= 2,63; df= 4; 10; P>0,05) were not significantly different from those of their controls.

TABLE 1

ANOVAs of the effects of cypermethrin dose, stage/sex, and their interactions on total body weight and glycogen, protein, and lipid contents of *P. turionellae*.

	Source	df	MS	F	P	r^2
Total body weight	Cypermethrin dose	4	240,037	13,191	0,000	0,80
	Stage/sex	3	1894,876	104,131	0,000	
	Cypermethrin* Stage/sex	12	42,675	2,345	0,011	
	Error	100	18,197			
Glycogen	Cypermethrin dose	4	$2,091 \times 10^{-4}$	5,906	0,001	0,85
	Stage/sex	3	$2,130 \times 10^{-3}$	60,15	0,000	
	Cypermethrin* Stage/sex	12	$6,859 \times 10^{-5}$	1,937	0,059	
	Error	40	$3,541 \times 10^{-5}$			
Protein	Cypermethrin dose	4	$1,419 \times 10^{-3}$	7,519	0,000	0,55
	Stage/sex	3	$8,602 \times 10^{-4}$	4,559	0,008	
	Cypermethrin* Stage/sex	12	$9,468 \times 10^{-5}$	0,502	0,901	
	Error	40	$1,887 \times 10^{-4}$			
Lipid	Cypermethrin dose	4	$3,336 \times 10^{-3}$	8,658	0,000	0,93
	Stage/sex	3	$5,913 \times 10^{-2}$	153,486	0,000	
	Cypermethrin* Stage/sex	12	$1,545 \times 10^{-3}$	4,011	0,000	
	Error	40	$3,853 \times 10^{-4}$			

TABLE 2

Cypermethrin-related changes in total fresh weight (mg) of *P. turionellae*.[a]

	CYP	Last Instars $\bar{x}$ ± SE	Pupae $\bar{x}$ ± SE	Males $\bar{x}$ ± SE	Females $\bar{x}$ ± SE
Weight*	C	45,29 ± 3,77a	35,39 ± 1,57ab	20,04 ± 0,52a	26,13 ± 0,97ab
	20	40,99 ± 1,66ab	37,94 ± 2,06a	18,69 ± 0,66a	28,39 ± 1,10a
	50	33,75 ± 3,87ab	29,48 ± 1,26bc	18,97 ± 1,37a	25,48 ± 0,89ab
	100	32,21 ± 2,39b	27,64 ± 0,97c	18,40 ± 0,79a	23,39 ± 0,86b
	150	31,62 ± 1,45b	30,13 ± 1,85bc	16,76 ± 1,08a	23,47 ± 0,50b

a. Numbers in column followed by the same letter are not significantly different (P>0,05; Tukey's HSD test).
CYP : Cypermethrin doses (ppm), C : Control group.
* Six replicates with 10 individuals per replicate.

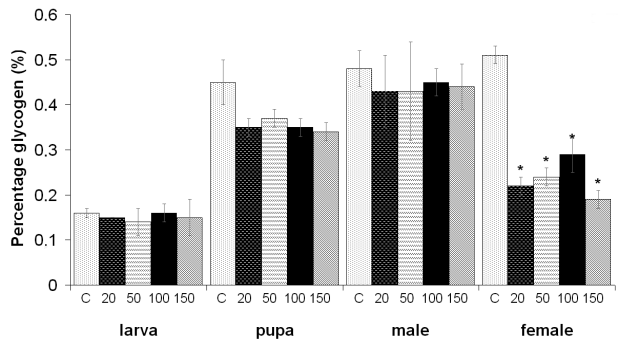

Fig. 1. – Glycogen levels (% of fresh weight) in successive developmental stages of *P. turionellae* exposed to different doses of cypermethrin.
Each bar represents mean ± SE of three replicates with 10 individuals per replicate.
Significant differences are indicated by * (P<0,05).

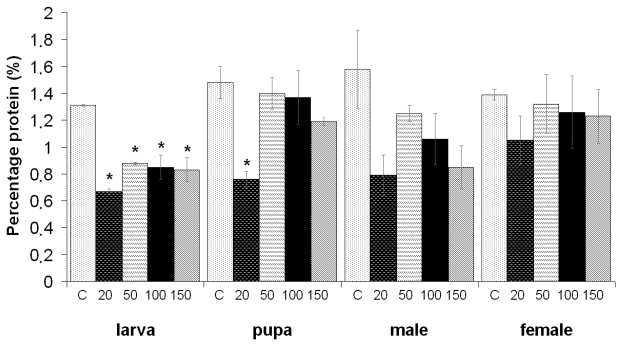

Fig. 2. – Protein levels (% of fresh weight) in successive developmental stages of *P. turionellae* exposed to different doses of cypermethrin.
Each bar represents mean ± SE of three replicates with 10 individuals per replicate.
Significant differences are indicated by * (P<0,05).

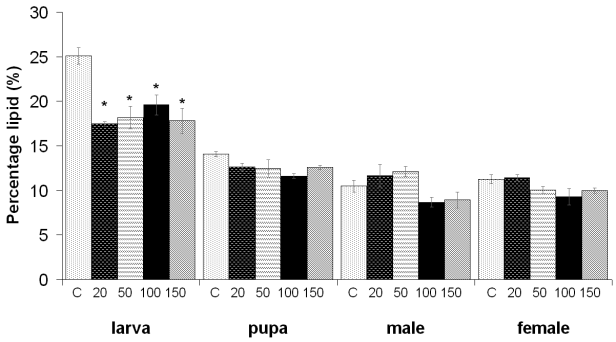

Fig. 3. – Lipid levels (% of fresh weight) in successive developmental stages of *P. turionellae* exposed to different doses of cypermethrin.
Each bar represents mean ± SE of three replicates with 10 individuals per replicate.
Significant differences are indicated by * (P<0,05).

DISCUSSION

Insects tended to lose weight in all cypermethrin treated groups except for pupae and females at 20 ppm. It has been reported that low dosages of toxicants may have beneficial effects on organisms (ORTEL, 1996). Thus, it can be speculated that the increase at 20 ppm relative to control group might also prove this assumption. However, the effect at 20 ppm was not significant in both cases, therefore from the statistical point of view; this can not be demonstrated to be the same hormesis effect reported by TOMBERLIN et al. (2002). Significant decreases were mostly apparent at higher doses of cypermethrin except for males. Studies on *G. mellonella* (MATHOVA, 1990) and *Lymantria dispar* L. (Lepidoptera : Lymantriidae) (ORTEL, 1996) larvae feeding on heavy metal contaminated food revealed that larvae lose weight at only high doses of heavy metals. The weight loss of *P. turionellae* may be attributed to the interference of sufficient food supply from host due to the antifeeding effect of cypermethrin on host.

Data on glycogen decrease were not significant for larvae, pupae and males. Only female glycogen level decreased significantly with respect to controls, but the reduction did not seem to be dose-dependent; thus suggesting that the effect is probably related to damage of reproductive system. This situation may also be of importance for the continuity of the generation, because the effect of cypermethrin is much likely to be transferred to the eggs. The eggs may have received less glycogen during oogenesis due to cypermethrin exposure. Accordingly, the glycogen amount in eggs laid by *P. turionellae* females exposed to 2,4-D and maleic hydrazine displayed significant decrease (ÖZKAN & YANIKOĞLU, 1999). It appeared that the adverse effect of cypermethrin application on glycogen content of *P. turionellae* increased through development. There was a sharp increase in glycogen level at larva to pupa transition in control and cypermethrin treated groups. The sharp increase in glycogen reserve at pupal stage was also noted during metamorphosis of *Ceratitis capitata* Wiedman (Diptera : Tephritidae) (TOLMASKY et al., 2001; NESTEL et al., 2003). However, the ratio of glycogen increase in our cypermethrin treated groups was lower than that of control groups at larva to pupa transition. This result might be associated with the increased effect of cypermethrin on pupae relative to larvae. There was no difference in glycogen level between male and female wasps in controls. However, glycogen levels of males in all cypermethrin treated groups were higher than those of females. In other studies, sexual difference in susceptibility to pesticides has also been noted in some parasitoids with males being generally more susceptible than females (SCHOONEES & GILLIOMEE, 1982; SCOTT & RUTZ, 1988; RATHMAN et al., 1992) or vice versa (SPOLLEN & HOY, 1992). The differences may be partly related to variation in size and physiology between sexes (BAKER et al., 1995; CROFT, 1990). Glycogen depletion in *P. turionellae* may also appear as a result of cypermethrin-induced effects on glycolytic pathway.

P. turionellae larvae were more susceptible to cypermethrin treatment than pupae and adults in terms of decrease in protein level. This result suggests that insecticide application affected the larval stage more than pupal and adult stages. It has been reported that organophosphorous insecticides cause a significant depletion in total protein content in the haemolymph and fat body of the silkworm, *Bombyx mori* L. (Lepidoptera : Bombycidae) (NATH et al., 1997). USMANI & KNOWLES (2001) also stated that larvae of different species were more susceptible to insecticides than adults. Our results revealed that larvae had the lowest level of protein both in cypermethrin treated and control groups. Protein level increased at larva to pupa transition. However, the increase in total protein level at larva to pupa transition at 50, 100, and 150 ppm was much more than the increase at 20 ppm and in control group. This may be partially based on the lower protein level of larvae in cypermethrin treated groups compared to controls. It has been reported that total protein increases at larva to pupa transition, but decreases in adults when larvae of *Spodoptera litura* Fabr. (Lepidoptera : Noctuidae) exposed to various insecticides (VIJAYARAGHAVAN & CHITRA, 2002). The protein level of males was higher than females in control group, but it was lower in cypermethrin treated groups. This situation indicates that cypermethrin affects the protein level of males much more than females. A hidden damage that would further affect population density might have occurred when insects were exposed to sublethal doses of insecticides at larval stage (DAVIS et al., 1988).

P. turionellae larvae displayed a striking decrease in lipid content when exposed to cypermethrin whereas no significant alterations were observed in the lipid content of pupae and adults. Similar to the protein results, insecticide treatment mostly affected the larval stages in all experimental groups. Glycogen content of *P. turionellae* larvae was not affected by cypermethrin application, but lipid content declined drastically. This could point to a shift in energy metabolism to lipid catabolism due to insecticidal stress. Adults had the lowest and larvae had the highest level of lipid in all cypermethrin treatments and controls. LOHAR & WRIGHT (1993) also verified lipid depletion in haemolymph, fat body, and oocyctes in *Tenebrio molitor* L. (Coleoptera : Tenebrionidae) females exposed to malathion. They stated that depletion of lipid

might have been due to the effect of insecticide on the adipokinetic hormone that controls lipid metabolism. Lipid content displayed a drastic decrease at larva to pupa transition in control and experimental groups. Lipids have usually been thought of as the predominant source of energy during this nonfeeding stage of insects (BEENAKKERS et al., 1981). It has been assumed that a large proportion of carbohydrates digested during larval development are converted to lipids (CANDY, 1985). The accumulation of lipids during larval stage and the main consumption of lipids during the adult stage suggest that lipids may have been secured for this last highly energy-demanding phase of metamorphosis. However, lipid depletion in our cypermethrin treated groups was lower than that of in controls during larva to pupa transition. This indicates that lipid content of larvae is more susceptible to insecticide treatment than that of pupae.

Our results may provide an overall picture of glycogen, protein, and lipid metabolism during metamorphosis under insecticidal stress. Depletion of glycogen and lipid content may be due to utilization of these reserves for energy generation as a result of insecticide-induced stress (SANCHO et al., 1998; RAMBABU & RAO, 1994). The decrease in protein content may also indicate a physiological adaptability to compensate for insecticidal stress (RIBEIRO et al., 2001). Animals require high energy under stress conditions and the energy demand may have led to the stimulation of protein catabolism. The decrease in protein content might also be due to a mechanism of lipoprotein formation, which will be used to repair damaged cell and tissue organelles (SANCHO et al., 1998; RAMBABU & RAO, 1994). Protein depletion in tissues may also constitute a physiological mechanism by retaining free amino acid content in haemolymph, to compensate for osmoregulatory problems encountered due to the leakage of ions and other essential molecules during the insecticidal stress (SRIVINAS, 1986). In the present study, it is clearly evident that cypermethrin has a toxic impact on parasitoid metabolic pathway. Whatever the reasons are, the decrease in the level of glycogen, protein, and lipid contents will adversely affect the growth, development, and reproduction of parasitoid species. This fact, in turn, may disrupt the effectiveness of parasitoid species in biological control programs. The assessment of potential effects that insecticides have on natural enemies of pests will also contribute to success in IPM programs. The authors are currently evaluating the effect of insecticide on the biological parameters of *P. turionellae*.

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C-Heterochromatin and chiasma terminalization in the jerboas *Allactaga* and *Jaculus* (Rodentia : Dipodidae)

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ABSTRACT. C-heterochromatin and chiasma distribution patterns of meiotic chromosomes were studied in the dipodids *Allactaga tetradactyla*, *Jaculus jaculus jaculus* and *Jaculus orientalis* and chiasma terminalization was discussed. Darkly stained C-band located at the centromeric region of some bivalents was observed in all species at diakinesis and metaphase I. Of the occurrence of C-heterochromatin in the 24 bivalents, *J. orientalis* exhibited the highest percentage of absence of the C-blocks, where it was 68.8%, compared to 18.8% in *A. tetradactyla* and 14.6% in *J. j. jaculus*. Analysis of variance of the means of relative distances of interstitial and terminal chiasmata from the centromere in the larger bivalents no. 1 and 2 of each species at diakinesis and metaphase I showed that the interstitial chiasmata are stable in their positions at the two stages and do not move to the chromosome termini, while the terminal chiasmata scored at diakinesis are in fact achiasmatic terminal associations and some of them gradually terminalize at metaphase I. Therefore, it is concluded that C-heterochromatin pattern of meiotic chromosomes is quite similar to that of mitotic chromosomes except the pair no. 1 in *A. tetradactyla*, stability of interstitial chiasmata at the two stages might negate the idea of chiasma terminalization and C-heterochromatin has not any role in the distribution of chiasmata.

KEY WORDS : C-heterochromatin, chiasma terminalization, Jerboas, Dipodidae, *Allactaga tetradactyla, Jaculus* spec.

INTRODUCTION

At the cytological level, heterochromatin is far known to vary in its amount, position, and type (for recent reviews, see CRAIG & BICKMORE, 1993; CARVALHO et al., 2001; EISSENBERG & ELGIN, 2000; PARK & KURODA, 2001). Closely related species may differ not only in the amount of heterochromatin in their genomes, but also in the number of heterochromatic bands, their location and their staining properties (SUMNER, 1990; ARTONI & BERTOLLO, 2001; KAVALCO et al., 2004). This variation has been attributed either to transformation of euchromatin into heterochromatin or *vice versa* (KING, 1980, 1991; CUEVAS & FORMAS, 2003; SHAHIN & ATA, 2004), duplication or deletion of heterochromatic segments (WHITE, 1973; SHAHIN & ATA, 2004), variation of euchromatin content and its correlation with the chromosome size and rearrangement of heterochromatin (SHAHIN & ATA, 2004); euchromatic translocation (WARCHAŁOWSKA-ŚLIWA et al., 1994) or to tandem translocation (HSU et al., 1975).

Since the first finding of chiasma by JANSSENS (1909, 1924), the chiasma frequency per cell and its position on bivalents have been considered two useful cytological parameters for the analysis of crossing-over and ultimately of genetic recombination in a population. Chiasma frequency per cell reveals the frequency of gene shuffling, while its distribution on bivalents provides information on the location of crossing-over points on chromosomes (for review, see JOHN, 1990; WADA & IMAI, 1995; IMAI et al., 1999). IMAI et al. (1999) assumed that interstitial chiasmata at diakinesis are distributed randomly and almost uniformly along bivalents except for the centromere and telomer regions and the size of these chiasma blank regions is consistently 0.8% of the total length of the haploid autosomes in all chromosomes, while terminal chiasmata are mostly telomere-telomere associations. Moreover, they added that variation of chiasma frequency among species is linearly proportional to the haploid chromosome number and would be evolution-adaptive because gene shuffling is dependent upon chromosome numbers.

Centromeric heterochromatin, the common pattern of heterochromatin in many organisms, is suggested to play a role in sister chromatid cohesion and proper segregation of chromosomes during cell division (for more details, see BERNARD et al., 2001). However, its role as a triggering factor of chiasma formation and terminalization remains doubtful and needs further investigations. JOHN & MIKLOS (1979) found that chiasma formation is inhibited in centromeric heterochromatin. NAVAS-CASTILLO et al. (1985) pointed out that heterochromatin variation among homologue chromosomes, which an effect of loss or addition of heterochromatin, leads to chiasma redistribution within the affected bivalents, independently of its position. However, SUJA et al. (1994) reported that chiasma redistribution within the bivalents is independent on the nature of the segment (heterochromatic/euchromatic ones). Moreover, BIDAU (1993) and TORREZAN & POGLIARINI (1995) demonstrated that chiasma frequency and terminalization might be affected by many factors, such as the heterochromatin regions, structural rearrangement, mutagens, etc. TORREZAN & POGLIARINI (1995) mentioned that there is an increase in chiasma frequency,

with a predominance of interstitial chiasmata, in euchromatin during diplotene stage, while there is a reduction in chiasma frequency in euchromatin during diakinesis as a result of consequent increase in the amount of heterochromatin. Further, they suggested that the chiasmata localized in euchromatin appear to move to the region of heterochromatin. The heterochromatin, however, seems to serve as a barrier against terminalization since terminalization is not observed beyond the heterochromatin bands in any heteromorphic bivalent.

On the other hand, it has been established on the basis of karyotypic analysis (SHAHIN & ATA, 2001) that all the dipodid species occurring in Egypt have a diploid number of 2n=48 chromosomes and a fundamental number (FN) of 95 in males and 96 in females. Of the 48 chromosomes, the X chromosome is submetacentric, while the Y is telocentric (acrocentric) in all species. In addition, *A. tetradactyla* has six subtelocentrics (pairs no. 2, 4, 5, 6, 7, and 9), while the remainders are submetacentrics, except the pairs no. 10, 11, 18, and 19, which are metacentrics. Nevertheless, *J. orientalis*, and *J. j. jaculus* have only three subtelocentrics (pairs no. 2, 21, and 22) and the others are submetacentrics, except the pair no. 18 in *J. orientalis* and 20 in *J. j. jaculus*, which are metacentrics. These morphological variations amongst karyotypes of the three species have been interpreted by ATA & SHAHIN (1999) on the basis of G-banding analysis as pericentric inversions occurred during karyotype evolution. However, they attributed the G-banding heterogeneity in the similar chromosomes to variations in the heterochromatin content and its correlation with the chromosome size.

Following this approach, the present study was basically undertaken after the findings of ATA (2000) and ATA et al. (2001) and the postulation of chiasma graph analysis by WADA & IMAI (1995) and the assumption that the so-called terminal chiasmata should be excluded when chiasma frequency is estimated. This assumption is basically assumed because terminal chiasmata are in fact mostly achiasmatic terminal associations (telomere-telomere associations) and are cytologically functional for ensuring the normal disjunction of bivalents at anaphase, but genetically non-functional for gene shuffling that is primarily the main function of interstitial chiasmata in addition to binding of bivalents (ATA, 2000; ATA et al., 2001; WADA & IMAI, 1995; IMAI et al., 1999).

The main aims of this study were 1) to describe and compare the distribution of heterochromatin in meiotic chromosomes with that of the somatic metaphase chromosomes (SHAHIN & ATA, 2004), 2) to analyze the chiasmata distribution and terminalization in the light of principles of the chiasma graph method, and 3) to examine the effect of C-heterochromatin distribution on chiasma frequency and terminalization.

MATERIAL AND METHODS

Three males of each of the dipodids *Allactaga tetradactyla* Lichtenstein, 1823, *Jaculus jaculus jaculus* Linnaeus, 1758, and *Jaculus orientalis* Erxleben, 1777 were captured in Egypt from Al Salum, Abu Rawash, and Mersa Matruh, respectively, either with the use of butterfly nets or with live traps placed in tracks made in sand. The animals were intraperitoneally injected with 0.05% colchicine solution (0.01 ml/g body weight) and one and half hour later killed with chloroform. The testes were excised and dissected gently into small pieces in 0.9% sodium chloride solution. The cell suspension was then centrifuged at 1000 r.p.m. for five min at room temperature. Subsequently, the cell pellet was treated with a hypotonic solution of 1% sodium citrate for 20 min at 37°C. Then, the suspension was recentrifuged as mentioned above and the cell pellet was fixed in 3 :1 ethanol :acetic acid at room temperature. Meiotic chromosome spreads were prepared by the flame drying technique using the method of EVANS et al. (1964), with a slight modification (unpublished data)[1]. C-bands were obtained according to the standard protocol of SUMNER (1972), with major modifications (SHAHIN & ATA, 2004). Chromosome spreads from each animal were examined and good spreads (about 10-15) from each species were scored and photographed using Olympus BX 51 microscope with a C-4040 zoom digital camera. Chromosomes were classified according to the method of LEVAN et al. (1964), with some modifications (SHAHIN & ATA, 2001).

Chiasmata were nomenclated following WHITE (1973) and as described by ATA et al. (2001), while the different bivalents were classified and identified at diakinesis and metaphase I according to the procedure of IMAI & MORIWAKI (1982) and as described by ATA (2000) and ATA et al. (2001). Chiasma distribution, both interstitial and terminal, of a total of 200 chromosome arms of the larger bivalents no. 1 and 2 obtained from 20-30 cells at both of diakinesis and metaphase I was scored in each species. Then, using C-heterochromatin as a marker, the distance of interstitial and of terminal chiasma from the centromere in both of the short and long arms of each bivalent pair in each cell was calculated at each stage using the Soft Imaging System (SIS) analysis program (version 3.0) edited in 1999 by Soft Imaging System GmbH, Germany. Afterwards, the mean value and relative distance of each chiasma from the centromere in relation to the arm length of each bivalent pair were calculated. Data were tested for normality using Anderson-Darling test prior to further statistical analysis. In case of normally distributed data, two-sample T-test was used to compare between the position of both the interstitial and distal chiasmata at diakinesis and metaphase I, while Mann-Whitney *U* test, as a non-parametric test, was used in case of not normally distributed data. All of these statistical analyses were carried out using the MINITAB software, version 13.13 (MINITAB, State College, Pennsylvania Area, USA, 2002).

Bivalents no. 1 and 2 were only chosen herein for accurate and perfect analysis of chiasmata distribution amongst the three species because of their relatively large size in comparison to other bivalents sizes and their large amount of centromeric heterochromatin. The remainder bivalents, however, were either relatively smaller that they cannot be accurately measured or some of them having no centromeric heterochromatin in one or more species.

[1] ATA, A.M., (1988). *Cytological studies on rodents*. M.Sc. Thesis, Minia Univ., Egypt.

RESULTS

C-heterochromatin variation : The karyotype of the dipodid species examined consists of a total of 24 normal bivalents, which are characterized by the presence of a relatively small amount of constitutive heterochromatin located at the centromeric region of some bivalents in all species at diakinesis and metaphase I (Figs 1-3). The bivalents are identified herein according to the karyotype previously cited by SHAHIN & ATA (2001). Of the 24 bivalents, only five pairs and the Y chromosome of the XY bivalent (22.9%) possess C-bands in all taxa, 18 (75%) pairs also have C-bands, but exhibit intertaxon variation, and only one chromosome (2.1%), the X of the XY bivalent, is entirely devoid of C-bands. *J. orientalis*, in this regard, has the highest percentage of absence of C-blocks, where it is 68.8%, compared to 18.8% in *A. tetradactyla* and 14.6% in *J. j. jaculus*. The following is a detailed description of the C-heterochromatin distribution among bivalents of each species at diakinesis and metaphase I.

Allactaga tetradactyla :. All bivalents at diakinesis and metaphase I have C-bands except the pairs no. 4, 5, 6, 8, and the X chromosome of the XY bivalent, which are nearly devoid of heterochromatin. Of these C-banding stained bivalents, a large centromeric C-band is observed only in the bivalent no. 1, while the remainder pairs and the Y chromosome of the XY bivalent possess a small centromeric C-band (Figs 1a, b).

***Jaculus jaculus jaculus* :** Three bivalents no. 9, 16, and 23 and the X chromosome of the XY bivalent have no C-bands at both diakinesis and metaphase I, while the other bivalents and the Y chromosome of the XY bivalent have a dark centromeric C-band (Figs 2a, b).

***Jaculus orientalis* :** The bivalents of this species exhibited a species-specific C-banding pattern at both diakinesis and metaphase I, which is quite different from the other two species. Sixteen bivalents and the X chromosome of the XY bivalent are completely devoid of C-bands; however, centromeric C-bands are found in the remainder seven pairs and in the Y chromosome of the XY bivalent (Figs 3a, b).

Chiasma distribution patterns : The data on the distribution of interstitial and terminal chiasmata in both of the short and long arms of each of the bivalents no. 1 and 2 in each species at both diakinesis and metaphase I are given in Tables 1 and 2. A summary of the results is as follows :

Interstitial chiasmata : In the three species, as a rule, the interstitial chiasmata distribute as a whole randomly and almost uniformly on the arms of each bivalent type at both diakinesis and metaphase I except at the centromere and telomere regions in which chiasma formation is suppressed. Generally in all species, the short arms of the bivalents no 1 and 2 exhibit a rather fewer number of interstitial chiasmata per cell than the long arms (Figs 1-3). Hence, the analysis employed herein includes only the chiasmata of the long arms (Table 1).

In *A. tetradactyla*, it is noteworthy to mention that no interstitial chiasmata are recorded at all in the short arms of the bivalents no. 1 and 2 neither at diakinesis nor at metaphase I (Figs 1a, b). In the long arms, however, the mean relative distances of interstitial chiasmata from the centromere at diakinesis and metaphase I are 1.65 ± 0.08 (range = 1.13-2.08) and 1.51 ± 0.10 (range from 1.08 to 2.36) in the bivalent no. 1, compared to means of 1.59 ± 0.22 (range = 0.85-2.36) and 1.86 ± 0.16 (range = 1.29-2.89) in the bivalent no. 2. Analysis of variance between the matrices of relative distance means of chiasmata in each bivalent at both stages using T-test showed no significant differences between their positions at diakinesis and metaphase I ($T = 1.01$, $P = 0.324$, $df = 20$, $P > 0.05$ for bivalent no. 1 and $T = -1.00$, $P = 0.331$, $df = 16$, $P > 0.05$ for bivalent no. 2; Table 1).

In *J. jaculus*, the mean relative distance of interstitial chiasmata from the centromere in the bivalent no. 1 is 1.71 ± 0.07 (range = 1.64-1.77) at diakinesis and 1.84 ± 0.09 (range = 1.75-1.92) at metaphase I, while it is 1.01 ± 0.47 (range = 1.53-1.84) and 1.69 ± 0.08 (range = 1.53-1.78) at the two stages in the bivalent no. 2. Likewise in *A. tetradactyla*, no significant differences are found between the chiasmata positions at the two stages ($T = -1.21$, $P = 0.438$, $df = 22$, $P > 0.05$ for bivalent no. 1 and $T = 1.11$, $P = 0.468$, $df = 20$, $P > 0.05$ for bivalent no. 2; Table 1).

In *J. orientalis*, the means are 1.47 ± 0.15 (range = 0.94-2.30) at diakinesis and 1.55 ± 0.10 (range = 1.02-2.14) at metaphase I in the bivalent no. 1, compared to mean values of 1.58 ± 0.08 (range = 1.24-2.02) and 1.40 ± 0.28 (range 1.18-2.57) at the two stages in the bivalent no. 2. Likewise in *A. tetradactyla* and *J. jaculus*, there are no significant differences between the localizations of chiasmata at the two stages ($T = -.0.43$, $P = 0.67$, $df = 15$, $P > 0.05$ for bivalent no. 1 and $T = 0.59$, $P = 0.567$, $df = 20$, $P > 0.05$ for bivalent no. 2; Table 1).

Terminal chiasmata : Terminal chiasmata also distribute randomly on the arms of each bivalent, but they richly distribute on the long arms than on the short arms and their number is higher in *J. orientalis* than in the other two species (Figs 1-3). Terminal chiasmata exhibit significant differences in their distribution on the short arms of the bivalents no. 1 in both of *J. jaculus* and *J. orientalis* and no. 2 in *A. tetradactyla* and on the long arms of the bivalents no. 1 in both of *A. tetradactyla* and *J. orientalis*. Nevertheless, non-significant differences in the distribution of terminal chiasmata are observed at diakinesis and metaphase I in the short arms of the bivalent no. 1 in *A. tetradactyla* and no. 2 in *J. orientalis* and on the long arms of the bivalents no. 1 and 2 in *J. jaculus* and the bivalent no. 2 in both of *A. tetradactyla* and *J. orientalis* (for more details, see Table 1).

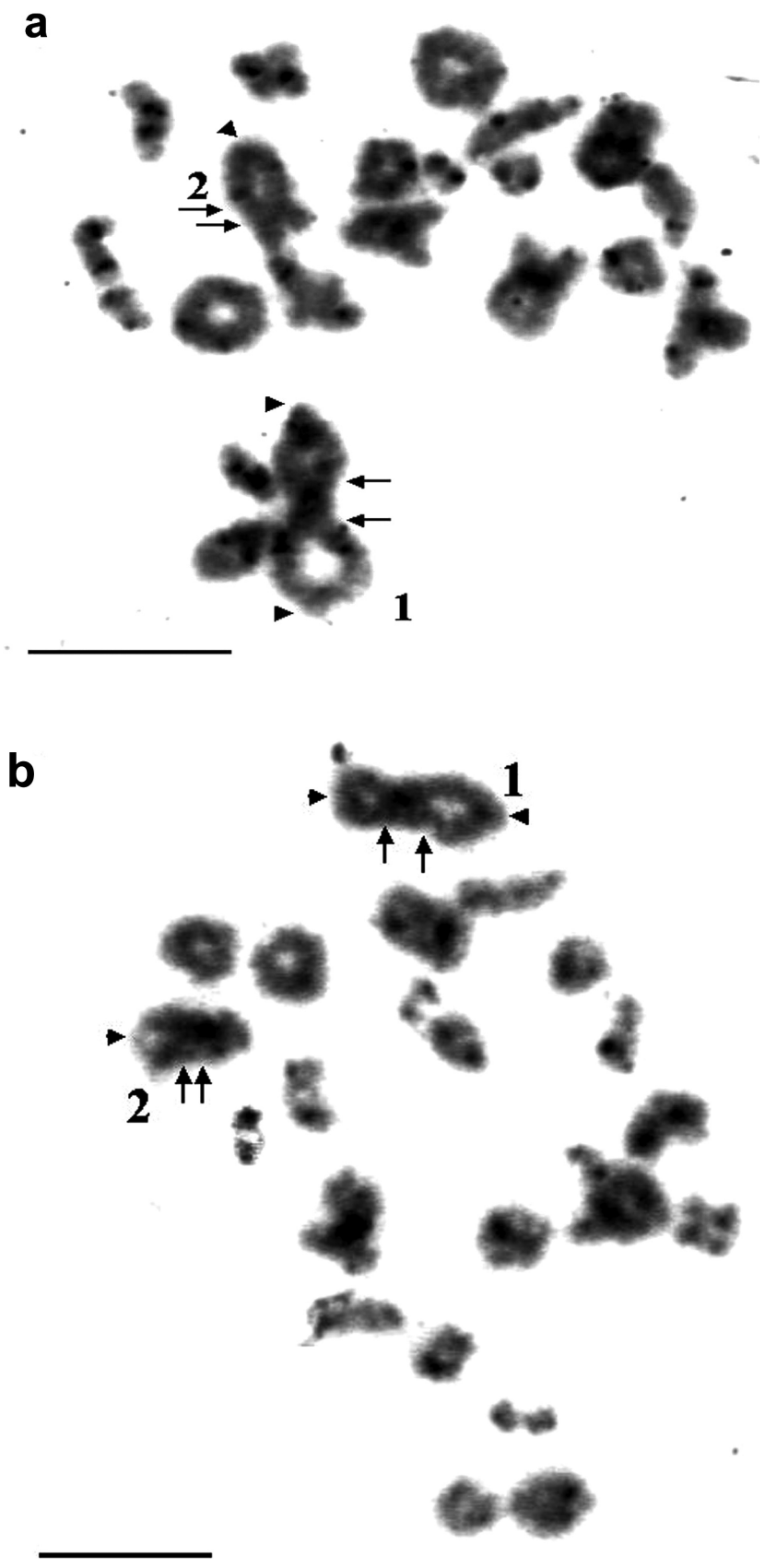

Fig. 1. – C-heterochromatin and chiasma distribution pattern of *Allactaga tetradactyla*. a. Diakinesis stage. b. Metaphase I stage. Scale bar = 10 μm. Arrows and arrowheads in this and the following figures refer to the interstitial chiasmata and terminal chiasmata, while the numbers refer to the bivalents no. 1 and 2.

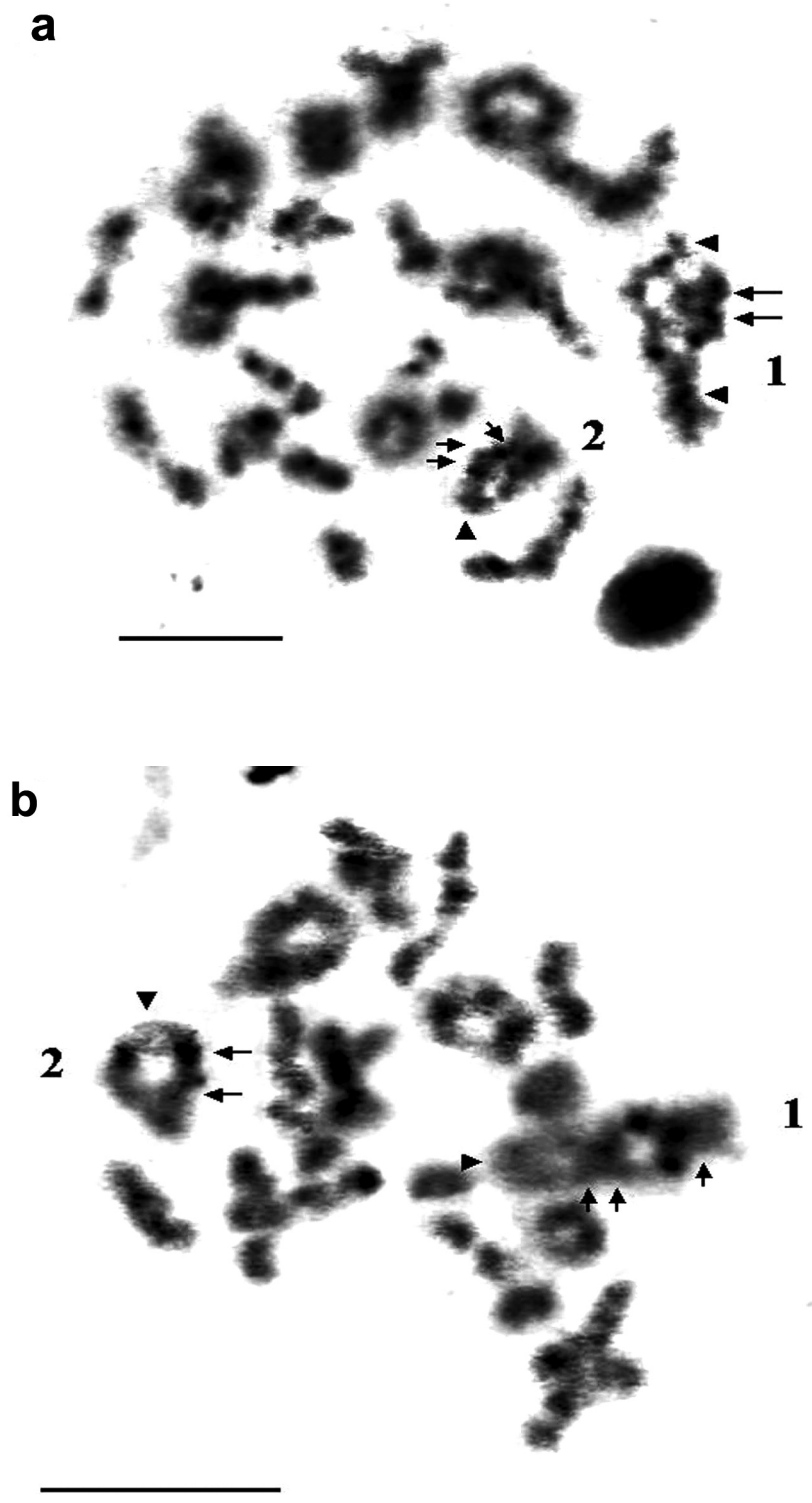

Fig. 2. – C-heterochromatin and chiasma distribution pattern of *Jaculus jaculus jaculus*. a. Diakinesis stage. b. Metaphase I stage. Scale bar = 10 μm.

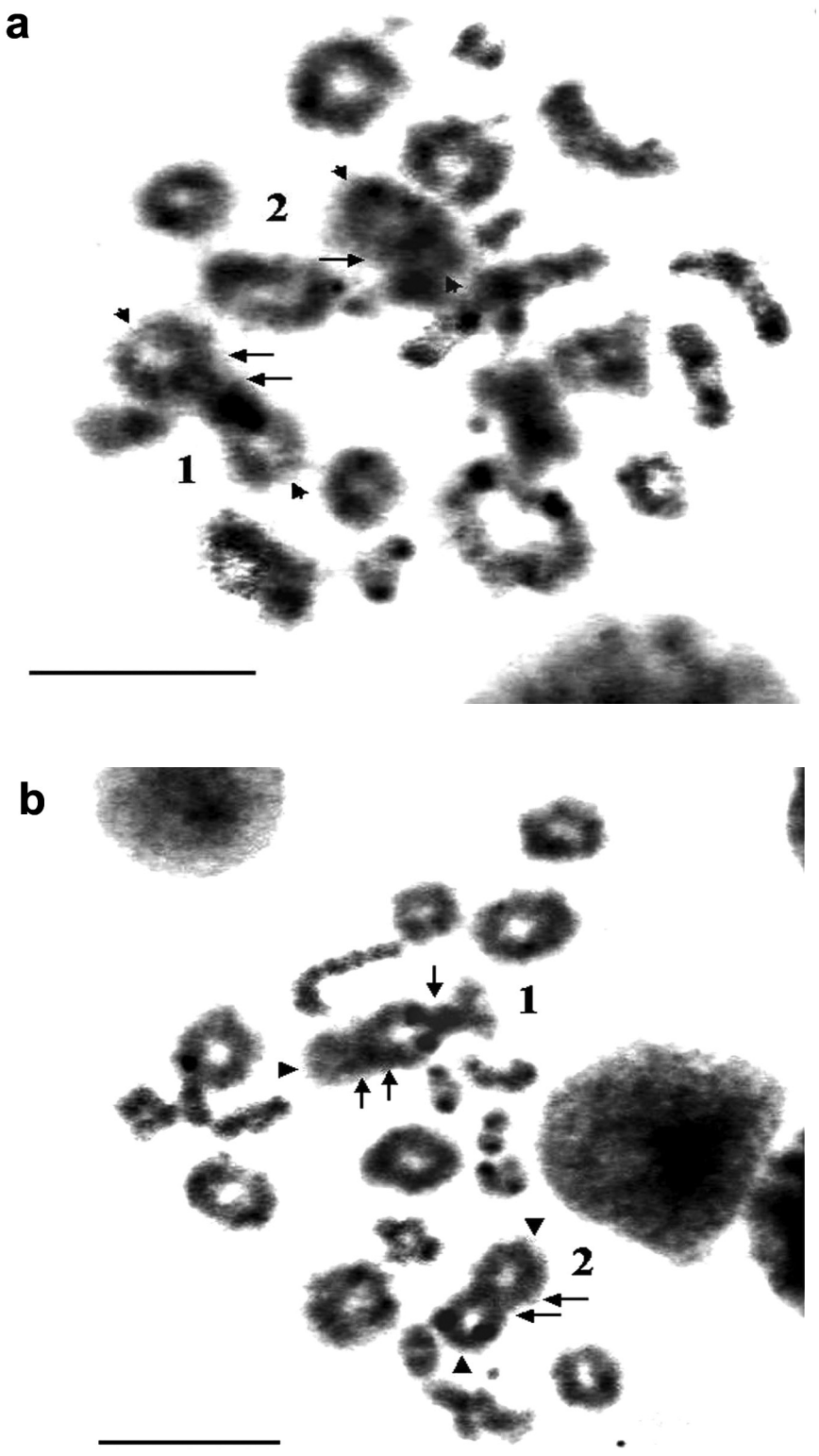

Fig. 3. – C-heterochromatin and chiasma distribution pattern of *Jaculus orientalis*. a. Diakinesis stage. b. Metaphase I stage. Scale bar = 10 μm.

TABLE 1

Mean relative distance values of the distribution of interstitial chiasmata on the long arms of the bivalents no. 1 and 2 in the three dipodid species examined. Values are in micrometers (μm)

Species			***A. tetradactyla***		***J. jaculus***		***J. orientalis***	
Stages			*Diakinesis*	*Metaphase I*	*Diakinesis*	*Metaphase I*	*Diakinesis*	*Metaphase I*
Mean relative distance of interstitial chiasmata ± SE of mean (range)	Bivalent no. 1		1.65 ± 0.08 (1.13-2.08)	1.51 ± 0.10 (1.08-2.36)	1.71 ± 0.07 (1.64-1.77)	1.84 ± 0.09 (1.75-1.92)	1.47 ± 0.15 (0.94-2.30)	1.55 ± 0.10 (1.02-2.14)
		T and (P)* values	1.01 (0.324)*		1.21 (0.438)*		-0.43 (0.670)*	
	Bivalent no. 2		1.59 ± 0.22 (0.85-2.36)	1.86 ± 0.16 (1.29-2.89)	1.01 ± 0.47 (1.53-1.84)	1.69 ± 0.08 (1.53-1.78)	1.58 ± 0.08 (1.24-2.02)	1.40 ± 0.28 (1.18-2.57)
		T and (P)* values	-1.00 (0.331)*		1.11 (0.468)*		0.59 (0.567)*	

* P values are non-significant (P>0.05).

TABLE 2

Mean relative distance values of the distribution of terminal chiasmata on the long and short arms of the bivalents no. 1 and 2 in the three dipodid species examined. Values are in micrometers (μm)

Species			***A. tetradactyla***		***J. jaculus***		***J. orientalis***	
Stages			*Diakinesis*	*Metaphase I*	*Diakinesis*	*Metaphase I*	*Diakinesis*	*Metaphase I*
Mean relative distance of terminal chiasmata ± SE of mean (range)	Bivalent no. 1	Short arm	0.06. ± 0.00 (0.05-0.06.)	0.05 ± 0.00 (0.04-0.06)	0.06 ± 0.00 (0.04-0.06)	–	0.06 ± 0.00 (0.05-0.06)	0.01 ± 0.01 (0.00-0.06)
		T and (P) values	0.36 (0.732)***		18.73 (0.000)**		6.00 (0.001)**	
		Long arm	2.13 ± 0.34 (0.06-3.18)	–	2.16 ± 0.08 (1.89-2.45)	2.31 ± 0.09 (1.98-2.64)	2.67 ± 0.15 (1.88-3.33)	0.70 ± 0.46 (0.00-3.00)
		T and (P) values	6.25 (0.000)**		-1.19 (0.278)***		(0.0204)*	
	Bivalent no. 2	Short arm	0.06 ± 0.00 (0.05-0.06)	–	–	–	–	0.02 ± 0.01 (0.05-0.06)
		T and (P) values	30.98 (0.000)**		–		1.58 (0.175)***	
		Long arm	0.94 ± 0.66 (0.06-3.97)	2.09 ± 0.77 (1.51-2.50)	2.29 ± 0.09 (1.89-2.48)	2.71 ± 0.15 (2.30-3.17)	2.55 ± 0.10 (2.17-2.91)	2.81 ± 0.19 (2.08-3.39)
		T and (P) values	1.49 (0.197)***		-2.24 (0.066)***		-1.07 (0.333)***	

No terminal chiasmata were found.

* Mann-Whitney test is significant at the values given in the Table.

** P values are significant (P≤ 0.05) and non-significant (***) at P>0.05.

DISCUSSION

As regards the occurrence of interstitial and distal chiasmata amongst karyotypes of the three species examined, ATA (2000) pointed out that the number of bivalent types in *A. tetradactyla* is ten, while it is only eight in both of *J. jaculus* and *J. orientalis* (ATA et al., 2001). Furthermore, it has been found that only the larger bivalent of these bivalent types has two patterns (2I.2D and 2I. 1D) at diakinesis and metaphase I in both of *J. jaculus* and *J. orientalis* (ATA et al., 2001), while in *A. tetradactyla* it has two patterns for each stage; 3I.2D and 2I.2D at diakinesis and 3I.1D and 2I.1D at metaphase I (ATA, 2000). The other bivalents, on the contrary, have only one pattern with different frequencies at diakinesis and metaphase I in all species (ATA, 2000; ATA et al., 2001). Therefore, the latter authors, following ZICKLER & KLECKNER (1999), attributed the differences in the number of bivalent types to the relative variation of the genome length, DNA quantity and the genetic background which may play an important role in pairing, synapsis and subsequently in chiasma formation and distribution between the two genera *Allactaga* and *Jaculus*. Nonetheless, they suggested that the variation in the number of bivalent forms of the pair no. 1 between the two genera is due to that one of the two distal chiasmata at diakinasis is more stable or not terminalized at metaphase I. Further, ATA et al. (2001) reported that there are significant differences in the mean frequency of interstitial chiasmata between the three dipodid species at both diakinesis and metaphase I, while no significant differences are found within each species. On the contrary, they added that the mean frequency of distal chiasmata is significantly different at the two stages not only among individuals of the same species, but also among the three species. The non-significant variations observed in the mean frequency of interstitial chiasmata from diakinesis to metaphase I in each of the three dipodid species has been attributed to the fact that all of the chiasmata recorded are interstitial and nothing is terminal (for review, see ATA et al., 2001).

In the present investigation, the non-significant differences observed in the distribution of interstitial chiasmata on the larger bivalents no. 1 and 2 of each species at both diakinesis and metaphase I means that these chiasmata

are fixed in their positions, *viz*. they do not move or change their positions at the two stages. If the interstitial chiasmata move or terminalize toward the ends of chromosomes, then all chiasmata should appear terminally in position, i.e. they form terminal (or distal) chiasmata, at metaphase I. Thus, the interstitial chiasmata could not terminalize to the ends of chromosomes during meiosis. These findings contradict the hypothesis of chiasma terminalization by DARLINGTON (1932) and strongly confirm the assumption of JONES (1978; 1987), KANDA & KATO (1980), IMAI & MORIWAKI (1982), JOHN (1990), WADA & IMAI (1995), IMAI et al. (1999), ZICKLER & KLECKNER (1999) and ATA et al. (2001).

On the other hand, the significant differences appeared in the distribution of terminal chiasmata on both of the short and long arms of the bivalents no. 1 and 2 at the two stages in one or more of the species examined suggest that these chiasmata scored at the diakinesis are achiasmatic terminal associations resulting from the telomere-nuclear membrane association (HRAI et al., 1996). These achiasmatic terminal associations serve for binding the bivalents during crossing-over, and then they gradually disappear or terminalize as crossing-over ceases at metaphase I.

Moreover, specific variations in the distribution of C-heterochromatin are observed among bivalents of the three species. These variations could be attributed, as described by SHAHIN & ATA (2004), either to transformation of heterochromatin into euchromatin or *vice versa*, deletion of heterochromatic segments resulting from pericentric inversions, or to variation of euchromatin content and its correlation with the chromosome size and arrangement of heterochromatin. A comparison of the occurrence of C-bands in both of the meiotic chromosomes surveyed in this study and mitotic chromosomes (SHAHIN & ATA, 2004) shows a close similarity of the amount and pattern of C-heterochromatin in the mitotic and meiotic chromosomes of the three species except the chromosome pair no. 1 in *A*. *tetradactyla*. This pair appears herein having a large dark block of C-heterochromatin, while it is completely devoid of heterochromatin in the mitotic metaphase chromosomes (SHAHIN & ATA, 2004). This heteromorphic feature could be due to the transformation of euchromatin into heterochromatin and subsequently duplication of the amount of heterochromatin needed for silencing nearby genes (for more details, see AVRAMOVA, 2002 and refs. therein).

As regards the distribution of both C-heterochromatin and interstitial chiasmata in the 24 bivalents and in particular the larger bivalents no. 1 and 2, no noteworthy relation could be found between C-heterochromatin and chiasma formation. This is firstly because C-heterochromatin in the species examined is located in the centromeric region and both kinds of chiasmata are basically distributed in the euchromatin regions. Secondly, as reported by ATA et al. (2001), the frequency of interstitial chiasmata is not significantly different in each species at diakinesis and metaphase I. This interpretation is inconsistent with the conclusions of BIDAU (1993) and TORREZAN & POGLIARINI (1995) who mentioned that there is an inverse relation between chiasma frequency and terminalization and the amount of heterochromatin and the latter seems to serve as a barrier against terminalization since terminalization beyond the C-bands is not observed in any bivalent.

A conclusion of this study is 1) the meiotic chromosomes of the three species examined have C-heterochromatin pattern and amount similar to the mitotic chromosomes (SHAHIN & ATA, 2004) except the pair no. 1 in *A*. *tetradactyla*, which has a large darkly stained C-band, 2) the interstitial chiasmata are stable in their positions in the euchromatin regions at diakinesis and metaphase I and they do not terminalize to the chromosomes termini, and 3) the centromeric C-heterochromatin in the chromosomes of the species examined does not affect the distribution and frequency of interstitial chiasmata during meiosis.

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Sublittoral megabenthos along cliffs of different profile (Aegean Sea, Eastern Mediterranean)

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ABSTRACT. The sublittoral megabenthos was studied in selected areas of the Aegean Sea. Data were collected with a visual, non-destructive, method and the technique of randomly placed frames was adopted. Eighty-nine megabenthic species were recorded. The spatial and temporal variation in population density, as well as the pattern of dispersion, was estimated for the most dominant species. Spatial analysis of the population densities suggested the separation of the studied sites in three major groups, reflecting differences in cliff profile. Temporal analysis revealed the discrimination of summer samples. The pattern of dispersion for most of the examined species was random without seasonal differentiations. *Agelas oroides* and *Leptopsammia pruvoti* showed a clumped pattern, while *Halocynthia papillosa* and *Microcosmus sabatieri* were evenly distributed.

KEY WORDS : megabenthos, sublittoral, Aegean Sea, hard substrate, population density, spatial dispersion

INTRODUCTION

In the lower sublittoral zone, where light conditions are reduced, an algal-dominated community develops on hard substratum throughout the Mediterranean (AUGIER, 1982; ANTONIADOU & CHINTIROGLOU, 2005). This community, characterized by the development of sciaphilous species, constitutes a special environment, according to the typology of the European Water Framework Directive, on which few data exist for the Eastern Mediterranean (STERGIOU et al., 1997; MORRI et al., 1999; PANSINI et al., 2000; ANTONIADOU et al., 2004a; ANTONIADOU et al., 2004c). This environment is quite sensitive to anthropogenic pressure, such as coastal technical constructions (breakwaters, seawalls, docks, harbours etc.), terrigenous water outfall, toxic wastes, fishing or collection of rare species (red coral, sponges), invasion of introduced species, sport or recreational activities etc., as most megabenthic species are slow growing and long-living (WARWICK, 1993; BELLAN-SANTINI et al., 1994; GARRABOU et al., 1998, 2002; PEREZ et al., 2000; GARRABOU & ZABALA, 2001; BOUDOURESQUE & VERLAQUE, 2002; PAPADOPOULOU & KANIAS, 2003; CHINTIROGLOU et al., 2005). Many of these species are of great economic importance, either as a food source, or as potential sources of therapeutic drugs. Examples are the tunicate *Microcosmus sabatieri*, which is intensively harvested and commercially exploited at many locations in the Aegean (ANTONIADOU et al., 2004b) and the sponges of some genera such as *Agelas*, *Ircinia*, *Axinella*, *Dysidea*, which are currently studied for their bioactivity (e.g. SCHMITZ, 1994). Therefore, there is a growing need for studies focusing on the ecology and stock availability of megabenthic species, in order to establish efficient management plans for the exploitation and conservation of their populations and habitats (SALA et al., 1996; GARRABOU et al., 1998; PEREZ et al., 2000; GARRABOU & HARMELIN, 2002).

Furthermore, substrate inclination is thought to be a major factor controlling the spatial dispersion of sublittoral megabenthic species in rocky shore communities, reducing both illumination (UV radiation) and the deposition of sediment (GLASBY, 1999; PANSINI et al., 2000; BELL & SMITH, 2004). Nevertheless, its effects have been scarcely investigated directly (PRECIADO & MALDONADO, 2005). Considering this, the aim of the present investigation is to study the composition of the megabenthic fauna associated with a sciaphilic algae community along a wide range of cliff profiles in the Aegean Sea, as well as to detect the spatial and temporal variability of the most dominant species.

MATERIALS AND METHODS

Study area

The study was carried out in the northern part of the Aegean Sea (Fig. 1) at seven coastal locations sharing some common physical characteristics. These included hard substrate down to a depth of 30-40 m and inclinations greater than 50° (for details see ANTONIADOU et al., 2004c). Sampling stations were set at a variety of cliff profiles and depth ranges, which were classified in three groups : (1) vertical cliffs (85-90°), (2) moderately inclined cliffs (60-80°) and (3) gently sloping (55-60°) bio-constructed, i.e. colonies of the scleractinian *Cladocora caespitosa* (Linnaeus, 1767), cliffs (Fig. 2). The cliffs were subjected to low current intensities (5-12 cm s^{-1}), at least at the sampling depth (15-40 m). Water clarity overpassed 18 m, with the exception of station 5, where it was reduced to about 12 m, as this site constitutes a very closed and sheltered area, in which muddy sediment covers the sea bottom (ANTONIADOU et al., 2004c).

All cliffs were sampled during summer 1998 (stations 1-6) or summer 1999 (station 7) for the needs of spatial analysis. Station 3 was selected for the temporal analysis

because it is sheltered from the N, NE and NW winds that usually blow in this area during winter. Temporal sampling was carried out on a 3-month basis, from summer 1998 to spring 1999 (i.e. July 1998, October 1998, January 1999 and April 1999).

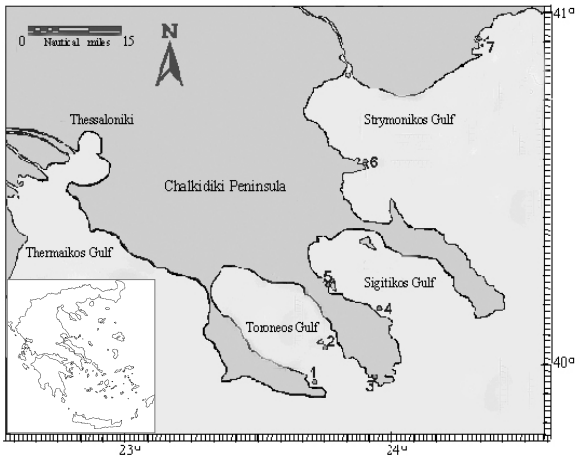

Fig. 1. – Map of the North Aegean Sea indicating sampling stations.

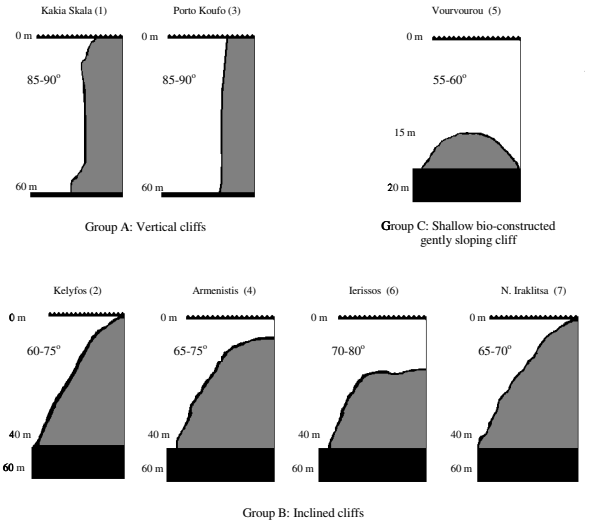

Fig. 2. – Schematic representation of the cliff profiles at the seven sampling stations.

Fauna

Preliminary sampling in all cliffs involved the collection of random qualitative samples with SCUBA diving (3 x 1 hour dives per station at a depth level of 15 to 40 m). All the collected material was identified down to the species level in the laboratory and revealed the presence of a large number of megabenthic species. The most conspicuous and abundant of these species were selected for quantitative investigation : the sponges *Agelas oroides* (Schmidt, 1864), *Axinella cannabina* (Esper, 1794), *Axinella verrucosa* (Esper, 1794), *Chondrosia reniformis* Nardo, 1833, *Diplastrella bistellata* (Schmidt, 1862), *Dysidea fragilis* (Montagu, 1818), *Ircinia variabilis* (Schmidt, 1862), *Petrosia ficiformis* (Poiret, 1789), the scleractinian *Leptopsammia pruvoti* Lacaze-Duthiers, 1897, the bryozoan *Pentapora fascialis* (Pallas, 1766) and the tunicates *Halocynthia papillosa* (Linnaeus, 1767) and *Microcosmus sabatieri* Roule, 1885. All these species are epibenthic, sessile and large enough to permit a visual, hence non-destructive quantification (WARWICK, 1993; GARRABOU et al., 2002).

Data collection

Sampling was carried out by SCUBA diving at the same depth range as the preliminary sampling and a non-destructive method was used to obtain data (HISCOCK, 1987; WARWICK, 1993; RHUMOHR, 1995; GARRABOU et al., 2002). To achieve this a combination of *in situ* counting and underwater photography was used. The method of randomly placed frames was employed for the estimation of population density and spatial dispersion of the 12 megabenthic species (ELLIOT, 1971; BAKUS, 1990). The number of individuals contained in 10 frame samples (1 x 1 m each) was recorded (BAKUS, 1990) for the estimation of numerical abundances. In order to estimate the pattern of spatial dispersion, preliminary sampling was performed to detect the optimal quadrate size and number, for each organism. The number of replicates depended on the precision required. For most benthic samples, a standard error ranging between 10 and 20 % is acceptable (BAKUS, 1990). In our case, 3 frames of different dimensions (30 x 30 cm, 50 x 50 cm, 1 x 1 m) were tested. Each frame was placed randomly 10 times and the mean (χ) and variance (σ) were calculated. On the basis of these data, the 30 x 30 cm frame was chosen for the estimation of spatial dispersion ($\sigma < \chi$) for all selected organisms. The number of replicates required was calculated according to the formula proposed by BAKUS (1990).

Data analysis

In order to check the null hypothesis, stating that population density of each species does not differ significantly spatially (among stations), an ANOVA test (one-way) was carried out. A logarithmic transformation (logx+1) was used to normalize the variance of numerical abundances data (ZAR, 1984). The Fisher PLSD test was used to detect any pair of stations with significantly different abundance.

The data obtained per sampling station were analyzed using hierarchical cluster analysis and multidimensional scaling techniques, based on the Bray-Curtis semimetric distance and log transformed numerical abundances, with PRIMER package (CLARKE & WARWICK, 1994). The significance of the multivariate results was assessed using ANOSIM testing. SIMPER analysis was applied in order to identify the percent contribution of each species to the overall similarity within a site and the dissimilarity among sites (CLARKE & WARWICK 1994). Finally, Morisita's index was calculated to estimate the spatial dispersion of the 12 megabenthic species. This index equals one for a random distribution, is greater than one for a clumped dispersion, and is less than one for a regular dispersion (ELLIOT, 1971). The advantage of Morisita's index is that it does not vary with sample size (BAKUS,

1990). A chi-square test was used to determine the significance of deviation from random (ELLIOT, 1971; BAKUS, 1990). All the above techniques were employed both in spatial and temporal analyses.

RESULTS

I. *Spatial Analysis*

Sampling at all sites revealed the presence of 89 megabenthic species : 27 Porifera, 15 Cnidaria, 1 Echiura, 7 Polychaeta, 5 Bryozoa, 15 Mollusca, 5 Crustacea, 11 Echinodermata and 3 Tunicata (Table 1). The cliffs can be classified in three groups with respect to species richness : (1) high richness cliffs (number of species > 80 % of the total number of megabenthic species recorded) as in stations 1 and 3, (2) intermediate richness cliffs (number of species between 50 % and 80 % of the total number of megabenthic species recorded) as in stations 2 and 4, and (3) low richness cliffs (number of species < 50 % of the total number of megabenthic species recorded) as in stations 5, 6 and 7.

Population density of each megabenthic species per sampling site is given in Fig. 3. One-way ANOVA test showed that the numerical abundance was not equally distributed in space, for the majority of the species (stations in which a species was absent were not taken into account) (Table 2). The only exceptions were *L. pruvoti* and *M. sabatieri*, which showed an equal dispersion of abundance, although they occurred only at two and five stations respectively. The results of the Fisher PLSD test, localizing the existing differences are depicted in Fig. 4. For example, *A. oroides* showed significantly decreased abundance at stations 2 and 7, in comparison to the rest of the stations, whereas *A. verrucosa* showed significantly increased abundance at stations 1 and 3 (absent from station 6), in comparison to the rest of the stations (see Fig. 4).

Hierarchical cluster and non-metric MDS indicated, at a similarity level of 65%, three groups of stations (Fig. 5). The performance of a one-way ANOSIM test gave global R : 0.81 at a significance level of $p<0.1\%$, indicating a satisfying discrimination of the three groups. Further examination, in order to localize differences among groups by means of a pairwise test, did not reveal any significant variation in R-values between each pair of groups, but it did show the higher similarity of groups A and C. SIMPER analysis showed that in-group similarity in station group B reached 90.4%, while 5 species (*L. pruvoti, A. oroides, D. bistellata, C. reniformis, P. fascialis*) contributed 65% to the average similarity. In-group similarity in station group C reached 80.6%, with 4 species (*A. oroides, D. bistellata, H. papillosa, A. cannabina*) contributing 62%.

Dispersion of the megabenthic species per sampling site (except for the cases where the abundance of a species was too low to permit calculation of Morisita's index) is presented in Table 3. The majority of the species were randomly or evenly dispersed, depending on the variance of abundance.

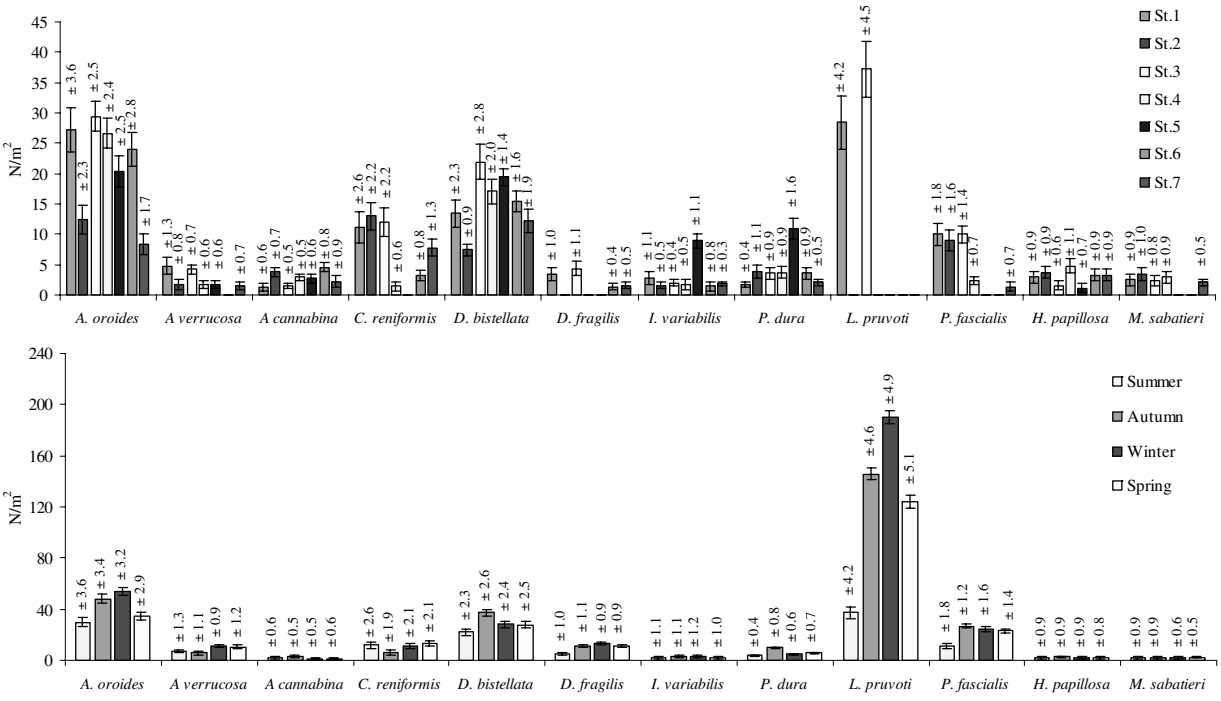

Fig. 3. – Population density (N/m^2) of megabenthic species per sampling station (up) and season (down). Values are mean of ten $1m^2$ plots and error bars represent standard deviation.

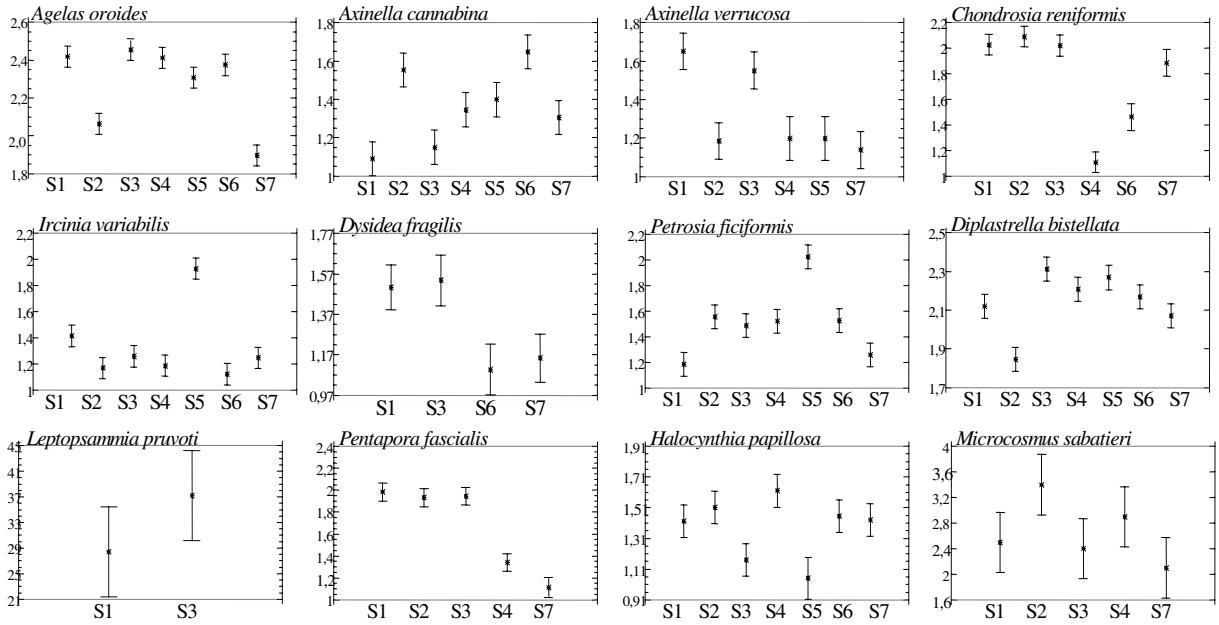

Fig. 4. – Spatial dispersion of log-transformed numerical abundances of megabenthic species showing significant differences among stations. Error bars represent standard deviation.

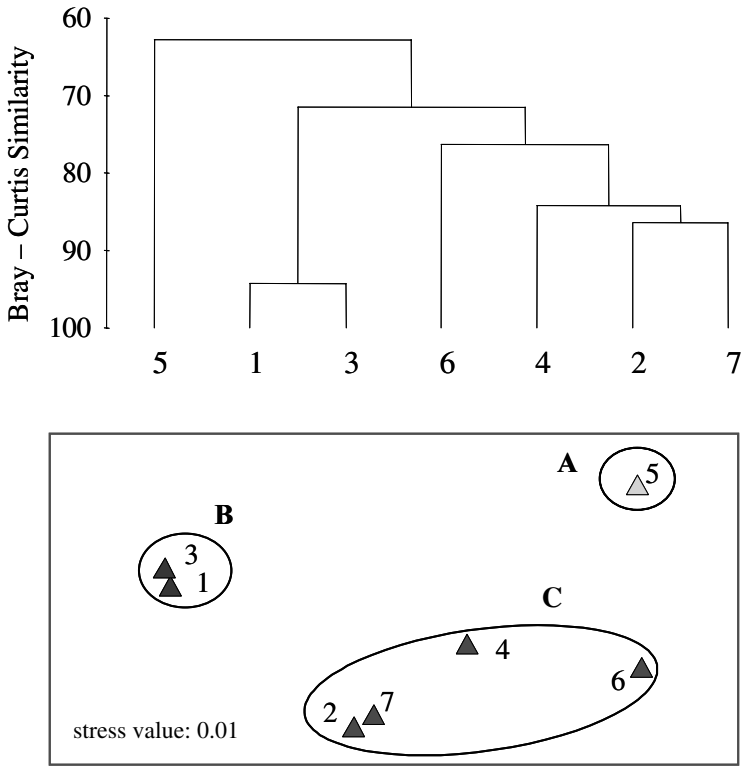

Fig. 5. – Spatial hierarchical cluster analysis and non-metric multidimensional scaling, calculated from log transformed numerical abundance data.

TABLE 1

List of megabenthic species recorded in the study area.

Species	St. 1	St. 2	St. 3	St. 4	St. 5	St. 6	St. 7
Porifera							
Agelas oroides (Schmidt, 1864)	*	*	*	*	*	*	*
Aplysina aerophoba Schmidt, 1862	*		*		*		*
Axinella cannabina (Esper, 1794)	*	*	*	*	*	*	*
Axinella damicornis (Esper, 1794)	*		*				
Axinella verrucosa (Esper, 1794)	*	*	*	*	*		*
Chondrilla nucula Schmidt, 1862	*	*	*	*	*	*	*
Chondrosia reniformis Nardo, 1833	*	*	*	*	*	*	*
Cliona sp.	*	*	*	*	*	*	*
Coscinoderma sporadense Voults,Van Soe & Kouk., 1991			*				
Crambe crambe (Schmidt, 1862)	*	*	*	*			
Diplastrella bistellata (Schmidt, 1864)	*	*	*	*	*	*	*
Dysidea avara (Schmidt, 1862)	*		*				
Dysidea fragilis (Montagu, 1818)	*		*			*	*
Erylus euastrum (Schmidt, 1870)			*			*	
Halisarca dujardini Johnston, 1842			*				
Hemimycale collumella (Bowerbank, 1874)	*	*	*	*			
Hippospongia communis (Lamarck, 1813)	*			*			
Ircinia pausifilamentosa Vacelet, 1961			*				
Ircinia variabilis (Schmidt, 1862)	*	*	*	*	*	*	*
Oscarella lobularis (Schmidt, 1826)			*				
Penares helleri (Schmidt, 1864)			*				
Petrosia ficiformis (Poiret, 1789)	*	*	*	*	*	*	*
Sarcotragus muscarum Schmidt, 1862	*		*			*	
Sarcotragus spinosulus Schmidt, 1862	*		*	*			
Spongia officinalis Linnaeus, 1759	*	*	*	*			
Spongia nitens (Schmidt, 1862)			*				
Stryphnus mucronatus (Schmidt, 1868)	*		*	*			
Cnidaria							
Aiptasia mutabilis (Gravenhorst, 1831)	*		*	*			
Alcyonium palmatum Pallas, 1766	*						
Balanophyllia europaea (Risso, 1826)	*	*	*	*			*
Calliactis parasitica (Couch, 1838)							
Caryophyllia smithii Stokes and Broderip, 1828	*		*	*			
Cerianthus membranaceus (Spallanzani, 1784)	*	*	*	*			
Cladocora caespitosa (Linnaeus, 1767)	*	*	*	*	*		
Condylactis aurantiaca (DelleChiaje, 1825)	*		*	*			
Cotylorhiza tuberculata (Macri, 1778)	*	*	*	*			
Eunicella cavolinii (Koch, 1887)	*		*				
Eunicella singularis (Esper, 1791)		*	*	*			
Eunicella verrucosa (Pallas, 1766)	*		*				
Leptopsammia pruvoti Lacaze-Duthiers, 1897	*	*	*	*			
Parazoanthus axinellae (Schmidt, 1862)	*	*	*	*	*		*
Paramuricea clavata (Risso, 1826)	*		*				
Echiura							
Bonellia viridis Rolando, 1821	*	*	*	*			
Polychaeta							
Arenicola marina (Linnaeus, 1758)	*		*				
Hermodice carunculata (Pallas, 1776)	*		*	*			
Myxicola infundibulum (Renier, 1804)			*				
Protula sp	*	*	*	*	*	*	*
Sabella pavonina Savignyi, 1820	*	*	*	*			
Serpula sp.	*	*	*	*	*	*	*
Spirographis spallanzanii (Viviani, 1805)	*	*	*	*	*	*	*
Bryozoa							
Bugula sp.	*	*	*	*	*	*	*
Myriapora truncata (Pallas, 1766)	*		*				
Pentapora fascialis (Pallas, 1766)	*	*	*	*			*
Sertella septentrionalis Harmer, 1933	*	*	*	*			
Smittina sp.	*		*	*			
Mollusca							
Aplysia sp.	*						
Charonia seguenziae Aradas et Benoit, 1876		*					
Coryphella lineata (Loven, 1846)	*	*	*	*			
Discodoris atromaculata Bergh, 1880	*	*	*	*	*	*	*
Flabellina affinis (Gmelin, 1791)	*	*	*	*	*	*	*
Halliotis tuberculata Linnaeus, 1758	*	*	*	*	*	*	*
Hypselodoris sp.	*		*				
Janolus cristatus (DelleChiaje, 1841)	*			*			
Lima sp.	*		*				
Luria lurida (Linnaeus, 1758)	*	*	*	*	*	*	*
Octopus vulgaris Cuvier, 1797	*	*	*	*	*	*	*
Ostrea edulis Linnaeus, 1758	*		*	*			
Pinna nobilis Linnaeus, 1758	*	*	*	*	*	*	*
Sepia officinalis Linnaeus, 1758	*	*	*	*			
Spondylus gaederopus Linnaeus, 1758	*	*	*	*			

TABLE 1

List of megabenthic species recorded in the study area.

Species	St. 1	St. 2	St. 3	St. 4	St. 5	St. 6	St. 7
Decapoda							
Dardanus calidus (Risso, 1827)				*			
Dromia personata (Linnaeus, 1758)	*	*	*	*		*	
Gallathea strigosa (Linnaeus, 1767)	*	*	*	*	*	*	*
Homarus gammarus (Linnaeus, 1758)	*						
Palinurus elephas (Fabricius, 1787)	*	*	*	*			
Echinodermata							
Antedon mediterraneum (DeLamarck, 1816)	*	*	*	*		*	*
Centrostephanus longispinus (Philippi, 1845)	*		*	*			
Echinaster sepositus (Retzius, 1783)	*	*	*	*	*	*	*
Hacelia attenuata Gray, 1840	*	*	*	*	*	*	*
Holothuria forskali DelleChiaje, 1823	*	*	*	*			
Marthasterias glacialis (Linnaeus, 1758)	*	*	*	*	*	*	*
Ophidiaster ophidianus (DeLamarck, 1816)	*			*			
Ophiothrix fragilis (Abildgaard, in O.F. Muller, 1789)	*	*	*	*	*	*	*
Sphaerechinus granularis (DeLamarck, 1816)	*	*	*	*		*	*
Paracentrotus lividus DeLamarck, 1816	*	*	*	*	*	*	*
Peltaster placenta (J.Muller & Troschel, 1842)	*			*			
Tunicata							
Clavelina lepadiformis Muller, 1776	*		*				
Halocynthia papillosa (Linnaeus, 1767)	*	*	*	*	*	*	*
Microcosmus sabatieri Roule, 1885	*	*	*	*			*

TABLE 2

One-way ANOVA values (F, p) testing for differences among sites.

Species	Spatial	
	F	p
Agelas oroides	28.79	0.000
Axinella verrucosa	10.41	0.000
Axinella cannabina	10.26	0.000
Chondrosia reniformis	45.22	0.000
Diplastrella bistellata	12.14	0.000
Dysidea fragilis	7.60	0.000
Ircinia variabilis	23.39	0.000
Petrosia ficiformis	23.39	0.000
Leptopsammia pruvoti	1.74	0.200
Pentapora fascialis	45.01	0.000
Halocynthia papillosa	5.69	0.000
Microcosmus sabatieri	2.32	0.070

TABLE 3

Spatial pattern of dispersion of megabenthic species; χ is the mean, σ the variance, N the number of frames required, D the acceptable error, I Morisita's index, x^2 and d are parameters from a chi-square test, at 95% confidence level.

Station	χ	σ	N	D	I	x^2	d	Pattern of Dispersion
				Agelas oroides				
St.1	3,40	2,28	72	10	1,48	108,550	2,81	clumped
St.2	2,80	2,25	64	10	1,35	113,900	3,91	clumped
St.3	2,94	2,28	78	10	1,26	136,150	4,14	clumped
St.4	2,92	2,37	66	10	1,28	125,030	4,46	clumped
St.5	2,48	1,01	20	10	1,07	7,720	-	even
St.6	3,08	2,44	66	10	1,32	125,640	4,49	clumped
St.7	2,28	0,68	10	10	1,14	1,850	-	even
				Axinella cannabina				
St.1	1,14	0,37	10	10	1,12	1,200	-	even
St.2	1,25	0,34	7	10	1,15	0,647	-	even
St.3	1,10	0,90	16	20	1,22	11,780	-	random
St.4	1,42	0,87	17	15	1,34	8,520	-	random
St.5	1,33	0,33	10	10	1,21	0,736	-	even
St.6	1,44	0,27	6	10	1,25	0,253	-	even
St.7	2,78	2,26	23	15	1,28	40,690	-	clumped
				Axinella verrucosa				
St.1	1,86	0,90	38	15	1,23	16,110	-	random
St.3	1,26	0,68	23	15	1,29	8,070	-	random
St.5	1,30	0,23	12	10	0,99	0,447	-	even
St.7	1,37	0,26	4	10	1,15	0,156	-	random

TABLE 3

Spatial pattern of dispersion of megabenthic species; χ is the mean, σ the variance, N the number of frames required, D the acceptable error, I Morisita's index, x^2 and d are parameters from a chi-square test, at 95% confidence level.

Station	χ	σ	N	D	I	x^2	d	Pattern of Dispersion
				Diplastrella bistellata				
St.1	2,92	1,43	42	10	1,24	32,780	0,54	random
St.2	1,26	0,69	23	15	1,29	8,110	-	random
St.3	2,55	1,43	81	10	1,31	89,720	0,73	random
St.4	2,10	0,87	18	10	1,02	6,127	-	even
St.5	2,23	1,12	30	10	1,25	16,370	-	random
St.6	3,28	2,98	82	10	1,25	219,300	8,26	clumped
St.7	3,00	1,75	15	15	1,22	14,290	-	random
				Dysidea fragilis				
St.1	1,83	0,93	35	15	1,26	16,910	2,60	random
St.3	1,55	0,82	30	15	1,28	8,250	-	random
				Chondrosia reniformis				
St.1	3,03	1,62	12	15	1,28	71,020	0,83	random
St.2	3,88	2,11	13	15	1,11	13,700	-	random
St.3	1,79	0,90	37	15	1,22	15,900	2,86	random
St.7	3,34	1,45	47	10	1,24	32,890	1,53	random
				Ircinia variabilis				
St.1	1,42	0,45	12	10	1,24	1,136	-	even
St.5	1,75	0,59	12	10	1,16	2,188	-	even
				Petrosia ficiformis				
St.1	1,60	0,80	25	10	1,21	10,000	-	random
St.2	1,55	0,91	15	15	1,27	8,010	-	random
St.3	1,03	0,69	20	15	1,19	8,970	-	random
St.4	1,47	0,93	40	10	1,32	22,900	-	random
St.5	4,25	4,14	43	15	1,22	169,370	9,29	clumped
St.6	1,60	0,48	9	10	1,13	1,152	-	even
St.7	1,33	0,25	4	10	1,14	0,140	-	even
				Leptopsammia pruvoti				
St.1	5,95	3,57	44	10	1,35	92,100	4,24	clumped
St.3	9,54	6,58	71	10	1,47	317,680	13,30	clumped
				Pentapora fascialis				
St.1	2,89	1,74	47	10	1,36	48,190	0,17	random
St.2	1,73	0,64	14	10	1,16	3,154	-	even
St.3	1,98	1,07	54	10	1,29	30,640	-2,51	random
				Halocynthia papillosa				
St.1	1,34	0,47	12	10	1,17	1,970	-	even
St.2	1,80	0,70	15	10	1,21		-	even
St.3	1,14	0,37	10	10	1,10	1,200	-	even
St.4	1,42	0,41	8	10	1,19	0,830	-	even
St.5	1,33	0,26	6	10	1,14	0,254	-	even
St.6	1,25	0,31	7	10	1,23	0,462	-	even
St.7	2,78	2,26	23	15	1,28	40,690	-	clumped
				Microcosmus sabatieri				
St.1	1,13	0,35	10	10	1,21	1,081	-	even
St.2	1,61	0,59	13	10	1,23	2,801	-	even
St.3	1,30	0,48	13	10	1,13	2,126	-	even
St.4	1,54	0,60	11	10	1,20	2,330	-	even
St.7	1,25	1,25	8	10	1,31	0,256	-	even

II. *Temporal Analysis*

Most species showed small-scale fluctuations in temporal variability in population density values (Fig. 3), with the exception of the anthozoan *L. pruvoti* and in a lesser degree the sponge *A. oroides*. Non-metric MDS (Fig. 6) indicated the grouping of autumn, winter and spring samples, while summer was separated at a similarity level of about 60%. One-way ANOSIM gave global R : 1 at a significance level of $p<0.1\%$, thus confirming the above discrimination. SIMPER analysis showed that the average similarity of the 3-seasons group reached 86.8%, while 3 species (*L. pruvoti, A. oroides, P. fascialis*) contributed 85% to it. Alike, the average dissimilarity between the 3-seasons group and summer reached 32.2%, while the same three species contributed 85% to the dissimilarity.

The dispersion of megabenthic species per season is given in Table 4. Most species did not show seasonal changes in the pattern of dispersion, with the exception of *A. cannabina*, which showed an even dispersion during winter. This change was related to a decrease in variance value, while population density was practically stable throughout the year.

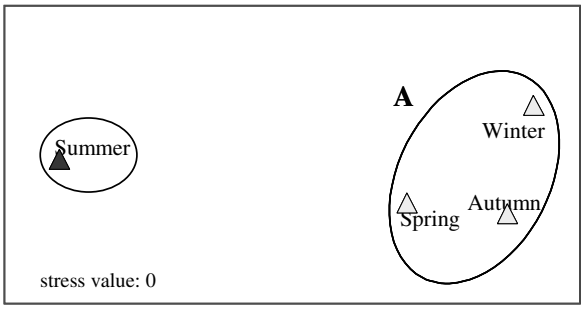

Fig. 6. – Temporal non-metric multidimensional scaling, calculated from numerical abundance data for station 3.

TABLE 4

Seasonal pattern of dispersion of megabenthic species at St.3; χ is the mean, σ the variance, N the number of frames required, D the acceptable error, I Morisita's index, x^2 and d are parameters from a chi square test, at 95% confidence level.

Station	χ	σ	N	D	I	x^2	d	Pattern of Dispersion
				Agelas oroides				
Winter 1998	4,85	4,82	70	10	1,20	330,52	14	clumped
Spring 1998	3,20	3,17	106	10	1,33	329,70	11,20	clumped
Summer 1998	2,94	2,28	78	10	1,26	136,15	4,14	clumped
Autumn 1998	3,84	5,73	120	10	1,34	1017,4	29,70	clumped
				Axinella cannabina				
Winter 1998	1,1	0,45	18	10	1,12	3,31	-	even
Spring 1998	1,1	0,85	23	15	1,21	15,10	-	random
Summer 1998	1,1	0,90	16	20	1,22	11,78	-	random
Autumn 1998	1,1	1,06	23	20	126	23,40	-	random
				Axinella verucosa				
Winter 1998	1,86	0,90	38	15	1,23	16,113	2,87	random
Spring 1998	1,78	0,95	47	15	1,28	23,32	2,70	random
Summer 1998	1,26	0,68	23	15	1,29	8,07	-	random
Autumn 1998	1,41	0,59	39	15	1,17	9,38	4,44	random
				Diplastrella bistellata				
Winter 1998	2,69	1,70	115	10	1,39	122,4	0,52	random
Spring 1998	2,94	1,45	47	10	1,25	32,89	1,53	random
Summer 1998	2,55	1,43	81	10	1,31	89,72	0,73	random
Autumn 1998	3,34	1,45	62	10	1,18	38,39	2,33	random
				Dysidea fragilis				
Winter 1998	1,83	0,94	36	15	1,26	16,89	2,61	random
Spring 1998	1,86	1,08	37	15	1,33	22,57	1,82	random
Summer 1998	1,55	0,82	30	15	1,28	8,25	-	random
Autumn 1998	1,81	0,89	32	15	1,24	13,56	2,73	random
				Chondrosia reniformis				
Winter 1998	1,55	0,82	20	15	1,28	8,25	-	random
Spring 1998	1,86	0,90	38	15	1,23	16,12	2,87	random
Summer 1998	1,79	0,90	37	15	1,22	15,9	2,86	random
Autumn 1998	1,78	0,95	47	15	1,28	23,32	2,70	random
				Petrosia ficiformis				
Winter 1998	1,26	0,68	23	15	1,29	8,07	-	random
Spring 1998	1,41	0,59	39	15	1,17	9,38	4,44	random
Summer 1998	1,03	0,69	20	15	1,19	8,97	-	random
Autumn 1998	1,55	0,81	20	15	1,28	8,25	-	random
				Leptopsammia pruvoti				
Winter 1998	16,9	12,70	119	10	1,55	1120,8	31,90	clumped
Spring 1998	11,5	6,58	32	10	1,51	120,47	3,17	clumped
Summer 1998	9,54	6,58	71	10	1,47	317,68	13,30	clumped
Autumn 1998	14,1	10,00	103	10	1,49	723,4	23,70	clumped
				Pentapora fascialis				
Winter 1998	3,03	1,63	82	10	1,28	71,026	0,84	random
Spring 1998	2,94	1,63	90	10	1,3	80,43	0,69	random
Summer 1998	1,98	1,07	54	10	1,29	30,64	-2,51	random
Autumn 1998	2,89	1,74	47	10	1,36	48,19	0,174	random

TABLE 4

Seasonal pattern of dispersion of megabenthic species at St.3; χ is the mean, σ the variance, N the number of frames required, D the acceptable error, I Morisita's index, x^2 and d are parameters from a chi square test, at 95% confidence level.

Station	χ	σ	N	D	I	x^2	d	Pattern of Dispersion
				Halocynthia papillosa				
Winter 1998	1,37	0,42	12	10	1,21	1,54	-	even
Spring 1998	1,30	0,48	13	10	1,13	2,304	-	even
Summer 1998	1,14	0,37	10	10	1,10	1,20	-	even
Autumn 1998	1,23	0,53	18	10	1,19	4,11	-	even
				Microcosmus sabatieri				
Winter 1998	1,12	0,48	18	10	1,19	3,70	-	even
Spring 1998	1,08	0,46	17	10	1,16	3,33	-	even
Summer 1998	1,30	0,48	13	10	1,13	2,12	-	even
Autumn 1998	1,22	0,62	10	15	1,22	3,15	-	even

DISCUSSION

The eight sponges studied in the present work are all common Mediterranean species, previously reported from the Aegean Sea (PÉRÈS & PICARD, 1958; KOUKOURAS et al., 1985, 1998; PANSINI et al., 2000; VOULTSIADOU, 2005). Most of them have been characterized as sciaphilic species inhabiting the sciaphilic algae, the coralligenous and the semi-dark cave communities (PÉRÈS & PICARD, 1958; BIBILONI et al., 1989; URIZ et al., 1992; GARRABOU et al., 2002). The species *C. reniformis*, *P. ficiformis* and *I. variabilis* have been found also in the community of photophilic algae (URIZ et al., 1992), while *A. cannabina* was also found in *Posidonia oceanica* meadows (KOUKOURAS et al., 1996). The scleractinian *L. pruvoti* is a solitary species, widespread throughout the Mediterranean, vertically distributed to the lower sublittoral zone and showing a preference to shaded conditions (VAFIDIS et al., 1997). The bryozoan *P. fascialis* is well distributed in the Mediterranean (SALA et al., 1996) and seems to prefer the depth range of 30 - 60 m in the Eastern basin. The friable structure of its colonies governs its ecological niche (MOISETTE, 1988), limiting its presence in relatively sheltered areas, where food for this suspension-feeding organism is provided by the moderate water flow. Finally, the two ascidians, *H. papillosa* and *M. sabatieri*, are considered as Mediterranean endemics (KOUKOURAS et al., 1995). Both are solitary and prefer the most exposed sites. Their strong rhizoids help them withstand strong water currents, which facilitate their nutrition (MONNIOT, 1965).

The diversity of the megabenthos increased in vertical cliffs, especially for sponges and anthozoans. This result is in accordance to the reported positive influence of reduced light conditions and also to the detrimental effects of intense sedimentation, which is much heavier in horizontal surfaces (GLASBY, 1999; PANSINI et al., 2000; BELL & SMITH, 2004).

The faunistic similarity among the three cliff profile groups was high, since in all sites the sponges *A. oroides* and *D. bistellata* dominated. However, the quantitative analyses of the megabenthic species at each site revealed a considerable spatial variation. Three discernible facies were documented in relation to the cliff profile, which has been considered as one of the determining factors for the range of distribution of the megabenthos on hard substrates (PANSINI et al., 2000; BELL & SMITH, 2004). The first facies was recorded on vertical cliffs, the second one on a shallow bio-constructed, gently sloping cliff (dead colonies of the scleractinian *Cladocora caespitosa*) and the third on moderately inclined cliffs. The population density for the two dominant sponge species was found to be higher at the steeper cliffs. Furthermore, increased abundances of the sponge *C. reniformis*, the scleractinian *L. pruvoti* and the bryozoan *P. fascialis* were noted in the first facies, of the sponge *P. ficiformis* in the second, and of the sponge *A. cannabina* and the tunicate *H. papillosa* in the third one.

The dispersion pattern of sessile megabenthic species appeared to be random in most cases, but with certain exceptions. For one, the specific ecological needs of some species, e.g. the sciaphilic nature of *A. oroides* and *L. pruvoti*, led to a clumped dispersion. At shaded conditions clumped dispersions seemed to prevail, as the percentage of clumped species was positively correlated to decreasing levels of shading (MARTI et al., 2004a). For the two ascidians, *H. papillosa* and *M. sabatieri*, the even distribution that was observed may be the result of a territorial behaviour, commonly observed among solitary tunicates (MONNIOT et al., 1991). Another behavioral factor that may influence the dispersion of adults in many sponge, bryozoan and ascidian species may be the selectivity towards settlement surfaces that the larval stages of these organisms show (FROMONT, 1994; WIECZOREK & TODD, 1997; BHAUD, 2000). Furthermore, spatial competition among sessile species also plays a decisive role in their final distribution, as do other biotic interactions such as trophic relations, predation and physical disturbances (GARRABOU et al., 1998, 2002; GARRABOU & ZABALA, 2001; BELL et al., 2003; BELL & SMITH, 2004; MARTI et al., 2004b).

Some authors have reported a seasonality in algal-dominated communities from the lower sublittoral zone, which is weak compared to the one of the upper sublittoral, e.g. above 15 m (GARRABOU et al., 2002; MARTI et al., 2004a, 2004b; ANTONIADOU et al., 2004c). Temporal variations observed in the present work were limited to the discrimination of summer samples, when a significant decline in population density of the scleractinian *Leptopsammia pruvoti* (species responsible for the seasonal differentiation) was observed. This decline did not affect the

annual pattern of dispersion of the species, which remained clumped, or its abundance in deeper waters, which remained increased (personal observations). Population density of the sponge *Agelas oroides* decreased slightly in summer, although the clumped pattern of dispersion was left unaffected. This algal-dominated community, in contrast to the animal dominated one (coralligenous) of deeper waters (below 50 m in the study area), is generally characterized by a seasonal growth of algae in late spring and summer, which overgrow several animal species (Garrabou et al., 2002). Therefore, the recorded decrease in population density of *L. pruvoti* and *A. oroides* could possibly be a sampling artefact due to algal overgrowth (Perez et al., 2000), a known limitation of visual sampling. However, the dominant (in terms of percent cover) algal species, i.e. the rhodomelacea *Womersleyella setacea* (Hollenberg) R.E. Norris, showed a rather stable coverage throughout the year (Antoniadou et al., 2004c), as this species is capable of a continuous asexual reproduction (Athanassiadis, 1997). Algal growth can easily obscure smaller animal species like *L. pruvoti*, a fact not necessarily detrimental for the animal, as its population upturns in autumn. As a result, *L. pruvoti* is probably not completely overgrown by algae, but instead masked enough to be visually missed (Garrabou et al., 2002). Another explanation could be the well documented increased "fall out" of several sessile species (mainly sponges in summer) occurring in temperate sublittoral cliffs (Bell et al., 2003). This holds true mostly for *A. oroides*, which due to its large massive form is more likely to be damaged by the activities of free-living motile species, such as wrasses (Bell et al., 2003), which are a major part of the fish population in the study area (unpublished data). Additionally, SCUBA diving activities significantly increase in the area in summer, resulting in many animal species being detached, especially those with a 3-D morphology (Sala et al., 1996; Garrabou et al., 1998; Bell et al., 2003).

The studied algal-dominated community hosted a large number of megabenthic species and had variability more apparent in the spatial than in the temporal scale. Cliff profile was the greater factor influencing spatial variability, with vertical cliffs being the most diverse. Taking into account the fragility of these habitats and the potential damage that diving activities can cause to them (Sala et al., 1996; Garrabou et al., 1998; Bell et al., 2003), a detailed study of these communities would provide us with much needed information in order to come up with an efficient and viable management plan.

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A species complex in the genus *Notogynaphallia* Ogren and Kawakatsu (Platyhelminthes : Tricladida : Terricola) with a taxonomic revision of homonyms of *Geoplana marginata* Schultze & Müller and a reinterpretation of *Notogynaphallia caissara* (Froehlich) anatomy

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ABSTRACT. Three species of Geoplaninae from Southern Brazil with elongated body, parallel margins and yellowish dorsum with five to seven dark longitudinal stripes are studied. The three species, besides *Notogynaphallia ceciliae*, constitute a complex presenting a long prostatic vesicle, a folded and usually very long male atrium, a female atrium ending in a dorsally or dorso-anteriorly directed diverticulum (vagina), and a long common glandular oviduct approaching dorso-anteriorly. Besides details of the external morphology, the species are mainly differentiated by anatomical characters, such as thickness of the cutaneous musculature (mc : index), position of the ovary as related to first testes, posterior limit of the serial testes, rising of the oviducts as related to the gonopore, site of the efferent duct entrance into the prostatic vesicle, morphology of the prostatic vesicle, and morphology of the male and female atria. Two of the five-striped species were identified, one as *Geoplana marginata* sensu Graff, and the other as *G. marginata* sensu Marcus, confirming that they are different species. Both are re-defined and re-named, respectively as *N. graffi* sp. nov. and *N. ernesti* sp. nov. The third, seven-striped, species was identified as *N. abundans* and its anatomy is described for the first time.

KEY WORDS : land planarians, Geoplaninae, morphology, taxonomy

INTRODUCTION

Leal-Zanchet & Froehlich (2001) proposed a complex of four species within the genus *Notogynaphallia* Ogren & Kawakatsu, 1990, all of them characterized by an elongate body with parallel margins, and dorsum with five or seven dark longitudinal stripes on yellowish background. In addition, comments were made on the present taxonomic status of *Geoplana marginata* Schultze & Müller, 1857 and of the four species misidentified and described by several subsequent authors as this same species.

One of the four species of the complex, *Notogynaphallia ceciliae*, was described by Froehlich & Leal-Zanchet (2003). The remaining three species of the complex, the three among those previously misidentified as *G. marginata*, are described now.

MATERIAL AND METHODS

Material of *N. ernesti* sp. nov. (=*G. marginata* sensu Marcus) comprised specimens from Jundiaí (23°10'60''S, 46°52'W), Valinhos (22°56'60''S, 47°1'W) and the Botanical Garden of São Paulo (23°31'60''S, 46°37'W), state of São Paulo; from Curitiba (25°25'S, 49°15'W), state of Paraná; and from the National Forest of São Francisco de Paula (29°23'-29°27'S, 50°23'-50°25W), São Francisco de Paula, state of Rio Grande do Sul. Specimens from São Paulo and Paraná belong to the Land Planarian Scientific Collection of E. M. Froehlich (EMF coll.). Studied specimens of *N. graffi* sp. nov. (=*G. marginata* sensu Graff) were from São Francisco de Paula (National Forest of São Francisco de Paula), Salvador do Sul (29°26'60''S, 51°31'W) and Três Coroas (29°31'60''S, 50°47'60''W), state of Rio Grande do Sul. Studied specimens of *Notogynaphallia abundans* (Graff, 1899) were from Campo Bom (29°40'60''S, 51°2'60''W), Glorinha (29°52'S, 50°47'60''W), Novo Hamburgo (29°40'60''S, 51°7'60''W), Salvador do Sul (29°26'60''S, 51°31'W), Poço das Antas (29°26'60''S, 51°40'W), São Leopoldo (29°46'S, 51°8'60''W) and Tupandi (29°28'S, 51°25'W), state of Rio Grande do Sul. In addition, Marcus' original slides of *N. ernesti* as well as C. G. Froehlich's slides of *N. abundans* were examined. Besides, slides of the pharynx and copulatory apparatus of three other species of the same intrageneric group, as delimited in Froehlich & Leal-Zanchet (2003), *N. caissara* (E.M. Froehlich, 1955), *N. muelleri* (Diesing, 1861) and *N. fita* (Froehlich, 1959), were studied for comparison. The material of the three species belongs to EMF coll.

For analysis of external and internal characters as well as processing of the newly collected material, methods described in FROEHLICH & LEAL-ZANCHET (2003) were used. The material was sectioned at 6-10µm. Worms from EMF coll. were treated in the same way; they had been previously fixed in Formalin/Alcohol/Acetic Acid (FAA) or 4% formalin (ROMEIS, 1989) and maintained in 70% ethanol.

The ratio of the height of the cutaneous musculature to the height of the body (mc :h index in FROEHLICH, 1955) was determined in the median region of a transversal section of the pre-pharyngeal region. Mesenchymatic muscle fibers were counted in transversal sections of the same region. Colour descriptors, based on the uptake of dyes of particular colours, were used for classifying secretions with trichrome methods : erythrophil (red-loving), xanthophil (orange-loving) and cyanophil (dark blue-loving). The term cyanophil also applies to secretions which have an affinity for the green dye of Goldner's Masson.

Specimens and type-material have been deposited in the following reference collections : Museu de Zoologia da Universidade do Vale do Rio dos Sinos (MZU), São Leopoldo, Rio Grande do Sul, Brazil, the Helminthological Collection of Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo, São Paulo State, Brazil, as well as in the Land Planarian Scientific Collection of E.M. Froehlich (EMF), Department of Zoology, Universidade de São Paulo, São Paulo, São Paulo State, Brazil.

TAXONOMIC PART

Family Geoplanidae Stimpson, 1857

Subfamily Geoplaninae Stimpson, 1857

Notogynaphallia Ogren & Kawakatsu, 1990

Notogynaphallia ernesti sp. nov.

Geoplana marginata : Marcus, 1951

Geoplana abundans : Almeida, Yamada & E.M. Froehlich, 1988 (misidentification)

Geoplana marginata (auctorum) : Almeida, Yamada & E.M. Froehlich, 1991

Notogynaphallia marginata (in part) : comb. nov. Ogren, Kawakatsu & Froehlich, 1992

Notogynaphallia sp. 5 : Leal-Zanchet & Carbayo, 2000

Notogynaphallia marginata sensu Marcus, 1951 : Leal-Zanchet & Froehlich, 2001; Carbayo, Leal-Zanchet & Vieira, 2001

Geoplana marginata sensu Marcus, 1951 : Carbayo, Leal-Zanchet & Vieira, 2002

Nec *Geoplana marginata* Schultze & Müller, 1857

Nec *Geoplana marginata* : Graff, 1899

Nec *Geoplana marginata* : Riester, 1938

Etymology

The specific name is homage to Ernst Gotthelf Marcus and the importance of his, in several aspects, pioneer work regarding Brazilian turbellarians.

Type material

Holotype : EMF Nr. 4 : Cidade Jardim, São Paulo/ SP. Collected (03.V.47) and studied for the first time by E. Marcus. Copulatory apparatus : sagittal sections on 4 slides/ Fig. 153 (Marcus, 1951 : p. 181); pharynx : sagittal sections on one slide/ Fig. 152 (Marcus, 1951 : p. 181); and pre-pharyngeal region : transversal sections on one slide (Marcus, 1951 : p. 181).

Paratypes : EMF Nr. 5 : Cidade Jardim, São Paulo/ SP. Collected (03.V.47) and studied for the first time by E. Marcus. Copulatory apparatus : sagittal sections on 4 slides/ Fig. 154 (Marcus, 1951 : p. 181); EMF Nr. 595a : O. Froehlich & M. Schweiger, leg. 22.VII.85, Valinhos/ SP– preserved in ethanol 70º; EMF Nr. 595b : O. Froehlich & M. Schweiger, leg. 22.VII.85, Valinhos/ SP, anterior region at level of ovaries : sagittal sections on 7 slides; pre-pharyngeal region : transversal sections on 4 slides; pharynx : sagittal sections on 4 slides; copulatory apparatus : sagittal sections on 10 slides; EMF Nr. 677 : O. Françoso Júnior & M. Ramos, leg. 09.I.87, Botanical Garden, São Paulo/ SP, pre-pharyngeal region : transversal sections on 4 slides; pharynx : sagittal sections on 9 slides; copulatory apparatus : sagittal sections on 10 slides; EMF Nr. 761a : O. Françoso Júnior, leg. 09.XI.87, Parque do Museu de História Natural, Curitiba/ PR – preserved in ethanol 70º; EMF Nr. 761b : O. Françoso Júnior, leg. 09.XI.87, Parque do Museu de História Natural, Curitiba/ PR, pre-pharyngeal region : transversal sections on 3 slides; pharynx : sagittal sections on 6 slides; copulatory apparatus : sagittal sections on 8 slides; EMF Nr. 927 : C. F. Rocha, leg. 22.VI.96, Serra do Japi, Jundiaí/ SP, pre-pharyngeal region : transversal sections on 5 slides; pharynx : sagittal sections on 16 slides; copulatory apparatus : sagittal sections on 7 slides; MZUSP PL.173 : F. Carbayo, leg. 25.IX.98, São Francisco de Paula/ RS, anterior region at level of ovaries : sagittal sections on 11 slides; pre-pharyngeal region : transversal sections on 5 slides; pharynx : sagittal sections on 7 slides; copulatory apparatus : sagittal sections on 11 slides; MZUSP PL.174 : R.A. Castro, leg. 05.XII.2000, São Francisco de Paula/ RS - preserved in ethanol 70º; MZU PL.00046 : F. Carbayo, leg. 25.IX.98, São Francisco de Paula/ RS – preserved in ethanol 70º; MZU PL.00047 : F. Carbayo, leg. 25.IX.98, São Francisco de Paula/ RS, pre-pharyngeal region : transversal sections on 8 slides; pharynx in two fragments : sagittal sections on 13 slides; copulatory apparatus in two fragments : sagittal sections on 18 slides; MZU PL.00048 : F. Carbayo, leg. 23.X.98, São Francisco de Paula/ RS, region anterior to ovaries : sagittal sections on 12 slides; anterior region at level of ovaries : sagittal sections on 17 slides; pre-pharyngeal region in two fragments : transversal sections on 17 slides; pharynx : sagittal sections on 13 slides; copulatory apparatus : sagittal sections on 20 slides; MZU PL.00049 : F. Carbayo, leg. 13.V.99, São Francisco de Paula/ RS, copulatory apparatus : horizontal sections on 8 slides; MZU PL.00050 : F. Carbayo, leg. 14.IX.99 – pre-pharyngeal region : transversal sections on 6 slides; pharynx : sagittal sections on 7 slides; copulatory apparatus : sagittal sections on 9 slides.

Type-locality

Cidade Jardim, São Paulo, state of São Paulo (SP), Brazil.

Distribution

São Paulo (Ribeirão Pires, São Paulo, Jundiaí, Valinhos), Paraná (Curitiba), Rio Grande do Sul (São Francisco de Paula) - Brazil.

Diagnosis

Dorsum yellowish with five brownish to black longitudinal stripes; median stripe thin and discontinued; paramedian and lateral stripes distinct, continued, of variable width; eyes dorsal, without clear halos except those in lateral stripes; glandular margin mainly with abundant xanthophil cells; mc :h, 15-20%; pharynx bell-shaped with folded margins; most anterior testes level with ovaries, most posterior ones anterior or lateral to pharynx; efferent ducts opening into anterior third of prostatic vesicle; extrabulbar prostatic vesicle, oval to oval-elongate, unforked; male atrium, long, highly folded, with many secretory cells, less frequent proximally and distally; oviducts emerging from dorsal side of median third of ovaries and rising anteriorly to gonopore; common glandular oviduct dorsal to female atrium; female atrium relatively short, with some folds, continuing dorsally by slightly anteriorly bent vagina; length of male atrium, 4.0 to 5.0 times that of female one.

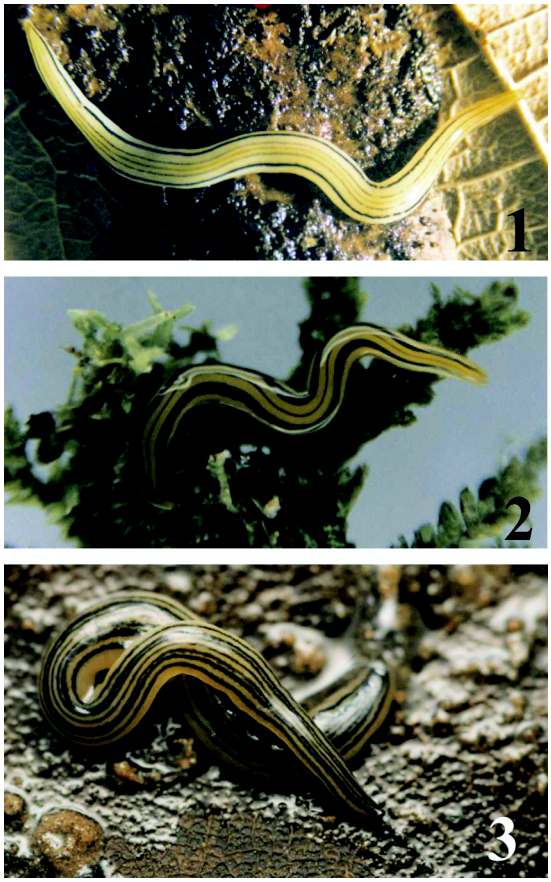
External morphology

Body elongate with parallel margins. Anterior end obtuse, posterior pointed. When creeping maximal length 70mm (Table 1). Mouth and gonopore distance from anterior tip vary a great deal in specimens of different provenances (Table 1), in part due to different maturation stages of the worms. Back pale yellow, ventral side cream. Live specimens from São Francisco de Paula, with anterior tip orange dorsally and ventrally. Dorsum with five longitudinal stripes, one median, two paramedian and two lateral ones (Figs 1, 4-6), former brownish, others black. In preserved specimens groundcolour fades, but stripes maintain colour.

TABLE 1

Measurements, in mm, of type-specimens of *N. ernesti* sp. nov. - : not measured; * : After fixation; ** Specimens with damaged anterior tip;
DG : distance of gonopore from anterior end; DM : distance of mouth from anterior end;
DMG : distance between mouth and gonopore; DPVP : distance between prostatic vesicle and pharyngeal pouch.
The numbers given in parentheses represent the position relative to body length.

	paratype EMF 595a	**paratype EMF 595b**	**paratype EMF 677**	**paratype EMF 761a****	**paratype EMF 761b****	**paratype EMF 927**	**paratype MZUSP PL.173**	**paratype MZUSP PL.174**	**paratype MZU PL.00046**	**paratype MZU PL.00047**	**paratype MZU PL.00048**	**paratype MZU PL.00049**	**paratype MZU PL.00050**
Maximum length in extension	-	-	-	-	-	70	35	60	30	42	57	49	35
Maximum width in extension	-	-	-	-	-	2.0	2.5	3.0	2.0	2.5	2.5	2.5	2.5
Length at rest	-	-	-	-	-	-	22	-	16	20	22	35	15
Maximum width at rest	-	-	-	-	-	-	4	-	3.5	3	4.5	3.0	4.0
Length*	21	35	38	28	27	59	29	42	21.5	33	37	31	30
Width*	2.0	2.5	2.5	2.5	1.5	2.5	2.5	3.0	2.0	2.0	3.5	2.5	2.5
DM*	6	20.5	22	12	10	35.5	18	29	11	21.5	23	18.5	16
	(29%)	(58%)	(58%)	(43%)	(37%)	(60%)	(62%)	(69%)	(51%)	(65%)	(77%)	(60%)	(53%)
DG*	10	27.5	28	18	17	45.5	22	32	15	24	28	23.5	22
	(47%)	(79%)	(74%)	(64%)	(63%)	(77%)	(76%)	(76%)	(70%)	(73%)	(76%)	(76%)	(73%)
DMG*	4.0	7.5	6.0	6.0	7.0	10	4.0	3.0	4.0	2.5	5.0	5.0	6.0
DPVP*	-	1.1	3.8	-	0.2	2.7	0.4	-	-	0.35	0.45	-	0.8
Creeping sole	-	81%	86%	-	82%	-	-	-	-	79%	79%	-	-
Ovaries	-	9.0	9.0	-	-	-	7.0	-	-	-	10	-	-
		(27%)	(24%)				(23%)				(27%)		
Anteriormost testes	-	9.5	9.0	-	-	-	7.0	-	-	-	10	-	-
		(27%)	(24%)				(23%)				(27%)		
Posteriormost testes	-	19.5	21	-	-	35	17	-	-	-	23	-	-
		(56%)	(55%)			(59%)	(59%)				(61.5%)		
Prostatic vesicle	-	1.5	0.3	-	0.7	0.55	0.6	-	-	0.35	0.35	-	0.7
Male atrium	-	1.9	1.3	-	3.5	2.3	1.5	-	-	2.2	2.0	-	1.7
Female atrium	-	0.5	0.6	-	0.7	0.4	0.3	-	-	0.6	0.4	-	0.4

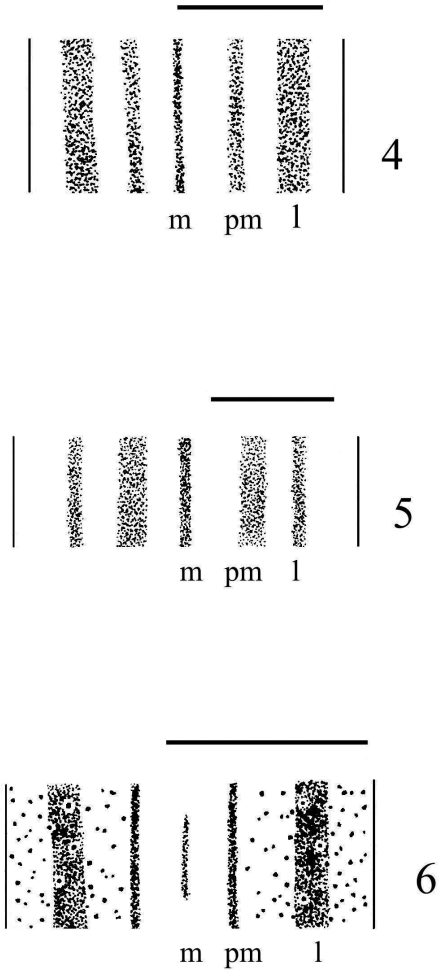


Figs 1-3. – Photographs of live specimens, dorsal view : (1) *N. ernesti* sp. nov., from São Francisco de Paula; (2) *N. graffi* sp. nov., from São Francisco de Paula; (3) *N. abundans* (Graff, 1899), from Poço das Antas.

In specimens from São Paulo state (Valinhos, Botanical Garden and Jundiaí), the median, paramedian and lateral stripes begin between ca. 0.5mm and 2mm from the anterior end (2% to 4% of body length). However, in paratype from Jundiaí, paramedian stripes begin very close to anterior tip (less than 0.5mm or 0.8% of body length). Median and paramedian stripes extend up to 0.5mm, 1.5mm or 2mm from posterior end (96% to 98% of body length); lateral stripes converge towards this tip. Lateral stripes are the widest (approx. 0.3mm or 11% of body width), then paramedian (approx. 0.1mm or 5% of body width) and median (approx. 0.05mm or 2% of body width) ones (Fig. 4).

In paratype EMF 761a, from Curitiba, with damaged anterior tip, median stripe becomes discontinued a little before ending at 2mm from posterior tip (93% of body length), paramedian stripes extend up to 1mm from posterior end (96% of body length); lateral stripes converge and extend up to this tip. Paramedian stripes the widest (approx. 0.2mm or 8% of body width), followed by lateral (approx. 0.09mm or 4% of body width) and median (approx. 0.05mm or 2% body width) ones (Fig. 5).

In paratype MZU PL.00046, from São Francisco de Paula, median stripe begins at ca. 4mm from anterior end (ca. 19% of body length) and extends up to ca. 2.5mm from posterior one (88% of body length). Paramedian and lateral stripes begin at approx. 1.7mm (ca. 8% of body length), former ends ca. 1.0mm from posterior tip (ca. 95% of body length), later converges towards it. Lateral stripes the widest (approx. 0.16mm or 8% of body width), followed by paramedian (approx. 0.05mm or 2.5% of body width) and then median (approx. 0.03mm or 1.5% of body width) stripes (Fig. 6). Latter is discontinuous.



Figs 4-6. – Detail of colour pattern of preserved specimens of *N. ernesti* sp. nov. : (4) paratype EMF 677, from Botanical Garden, São Paulo; (5) paratype EMF 761b, from Curitiba; (6) paratype MZU PL.00046, from São Francisco de Paula. (l) lateral stripe, (m) median stripe, (pm) paramedian stripe. Scale bar : 1mm.

Eyes, initially uniserial, contour anterior tip. In paratype from Jundiaí, become pluriserial approx. 1.5mm behind anterior tip (2% of body length). Between 5mm and 12mm (ca. 8% and 20% of body length) from the tip they are more abundant, and spread up to near paramedian stripes. After 15mm (25% of body length) behind the tip they become sparser. In paratype MZU PL.00046, from São Francisco de Paula, eyes become pluriserial after approx. 0.65mm from anterior end (3% of body length). More numerous between second and sixth millimeter (ca. 9% and 28% of body length) spread up to

near paramedian stripes. Sparser backwards, become exclusively marginal from ninth millimeter (ca. 42% of body length) on. Those in lateral stripes are surrounded by clear halos (Fig. 6).

Epidermis and musculature at pre-pharyngeal region

Width of creeping sole, measured in five specimens, varied from 79% to 86% of body width (Table 1).

Four types of secretory cells discharge through dorsal epidermis and body margins : (1) cells with xanthophil secretion of coarse very dense granulation; (2) rabdithogen cells; (3) cells with fine, weakly erythrophil secretion; (4) cells with amorphous cyanophil secretion. First two cell types are very numerous; xanthophil, even more abundant at body margins, creates a kind of glandular border. Creeping sole receives abundant cells with amorphous cyanophil secretion, cells with erythrophil granular secretion, and small quantity of rabdithogen cells.

Cutaneous musculature with the usual three layers, longitudinal layer with thick bundles (Table 4). Musculature higher paramedianly, especially on ventral side where may be 30µm higher than medianly. Towards body margins progressively lower. Mc :h 15% to 20% (Table 4). Well developed mesenchymatic musculature composed of four layers : dorsal subcutaneous with oblique fibers variously oriented (ca. 3-5 fibers thick); supra-intestinal transversal (approx. 5 fibers thick); sub-intestinal transversal (6-8 fibers thick); and subneural transversal (ca. 3 fibers thick). In addition, scattered ventral subcutaneous oblique fibers as well as dorsoventral ones are present. Longitudinal fibers are indiscernible, if existent, few and very scattered.

Pharynx

Pharynx bell-shaped with folded margins (Fig. 7). Mouth at end of anterior third or at median one of pharyngeal pouch, slightly anterior to or on same transversal level of dorsal insertion. Proximal part of pharyngeal lumen communicating directly with intestine, so esophagus is absent. Pharyngeal glands, with cell bodies in mesenchyme, mainly anterior and lateral to pharynx, of four types : two types of secretory cells with erythrophil secretion, one with strongly stained, irregular granules, mostly fine, other with weakly stained, fine granules; cells with amorphous cyanophil secretion; and cells with dense, granulous xanthophil secretion.

Outer musculature of pharynx constituted of thin longitudinal subepithelial layer (ca. 3µm thick), followed by circular one (ca. 25µm thick), mixed internally with few longitudinal fibers. Towards pharyngeal tip, circular layer becomes as thin as longitudinal one. Inner pharyngeal musculature composed of thick circular subepithelial layer (ca. 29µm thick), followed by some longitudinal fibers. Inner musculature gradually thins outwards, and, mainly dorsally, also inwards.

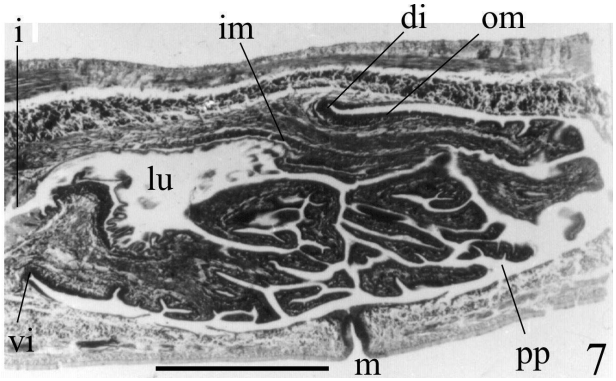


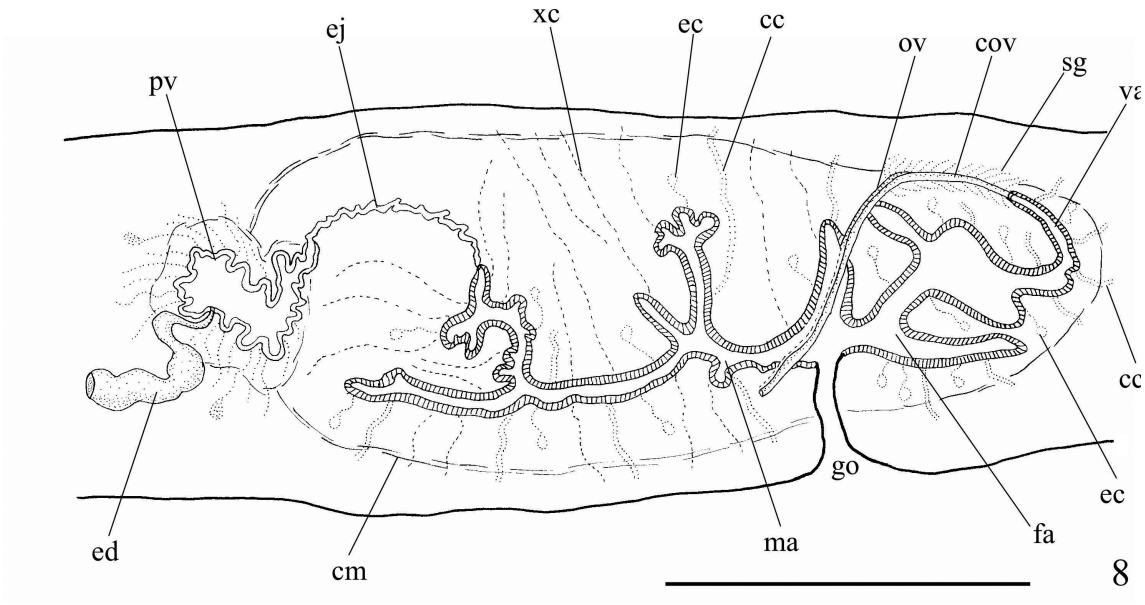
Fig. 7. – Pharynx of *N. ernesti* sp. nov. (paratype MZUSP PL.173) from São Francisco de Paula. Sagittal section. (di) dorsal insertion, (i) intestine, (im) internal musculature, (lu) pharyngeal lumen, (m) mouth, (om) outer musculature, (pp) pharyngeal pouch, (vi) ventral insertion. Scale bar : 500µm.

Reproductive organs

Most anterior testes approximately level with ovaries; most posterior ones anterior or lateral to pharynx, up to mouth level (Table 1). Efferent ducts, dorsolateral to oviducts in pre-pharyngeal region, run backwards, form false seminal vesicles behind pharynx, and, laterally to prostatic vesicle, turn anteriorly, ascend and enter into the ventral (Figs 8-10) or dorsal (Fig. 11) wall of vesicle near its anterior third. They are lined with ciliated cuboidal epithelium.

Extrabulbar prostatic vesicle spacious, oval to oval-elongate (Figs 8-13); more globose in paratype from Botanical Garden (Fig. 8), more elongate in that from Valinhos (Fig. 9). With no diverticula or branches; and although with a folded internal wall, the thick muscularis gives it a rather smooth external surface. Length of vesicle and distance between vesicle and pharyngeal pouch considerably variable (Table 1). Lining epithelium columnar, ciliated, of irregular height, traversed by abundant glands with coarse xanthophil secretion and cell bodies in surrounding mesenchyme. Muscularis (ca. 23µm thick) composed of interwoven longitudinal and circular fibers. Entering penis bulb, vesicle narrows, constituting a nearly rectilineous to mostly sinuous ejaculatory duct, which opens into bottom of male atrium. This opening dorsally dislocated in the worm from Botanical Garden (Fig. 8). Epithelial lining of ejaculatory duct with cuboidal to columnar ciliated cells, irregular in height, and with few openings of cyanophil secretory cells. Muscle coat thin (7µm thick) with intermixed longitudinal and circular fibers.

Male atrium long (Table 1), with numerous high folds that greatly restricts the whole cavity. However, in some specimens, folds near to gonopore, mainly those of ventral wall, lower, leaves atrial cavity more spacious. Folds vary considerably, regarding size, form and localization in atrial wall, in different specimens (Figs 8-12). In specimen from Valinhos, similarly to that described by MARCUS (1951) for one of his specimens (Fig. 154, p. 181), there is a circular fold, delimiting a long canal in bottom of which lies the opening of ejaculatory duct, bulging from the atrial wall. This set occupies the ental half of male cavity (Fig. 9).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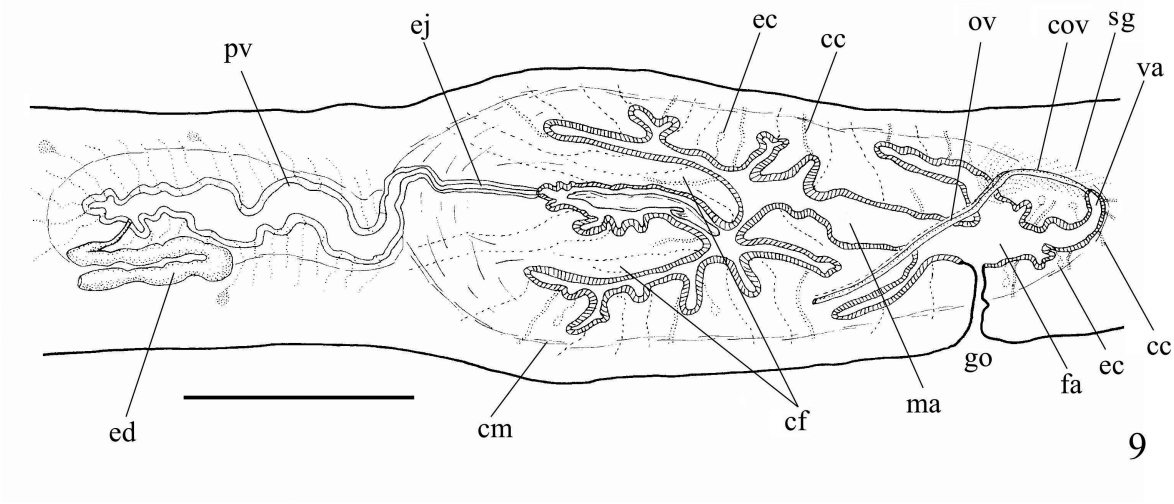
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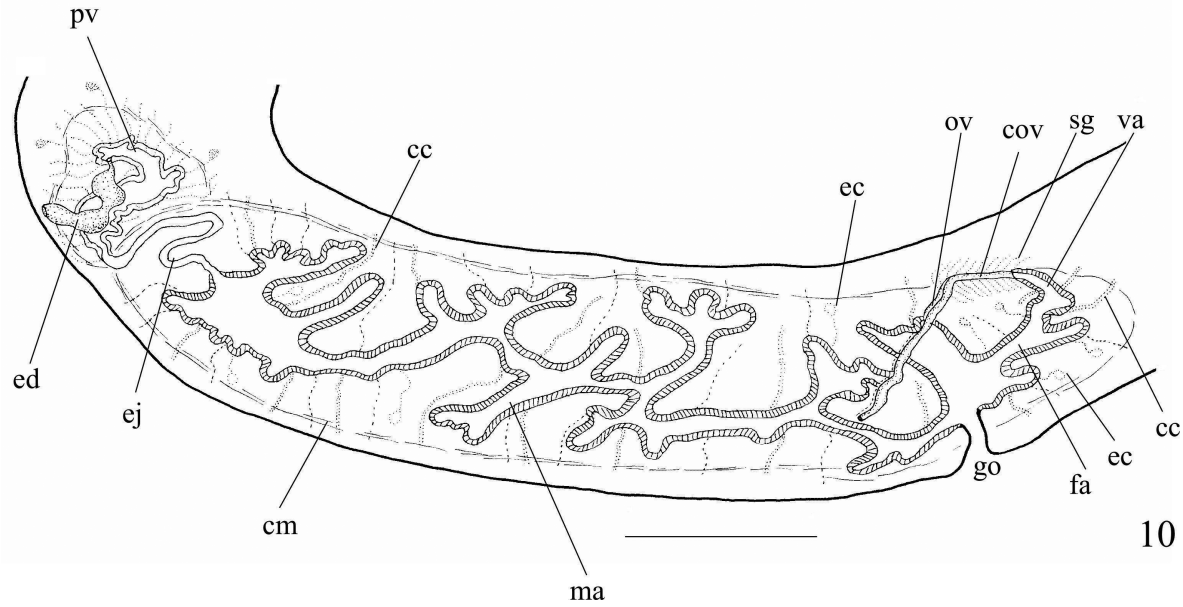
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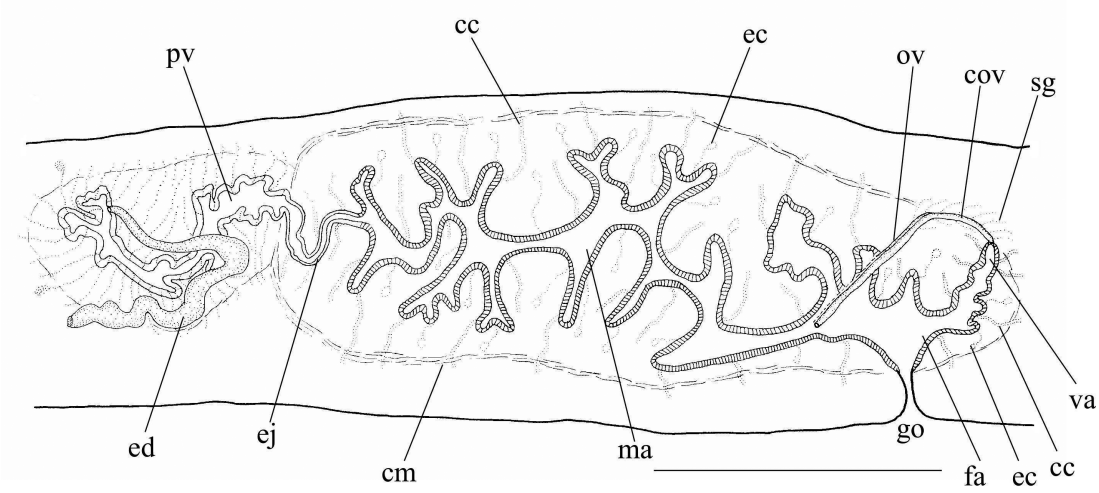
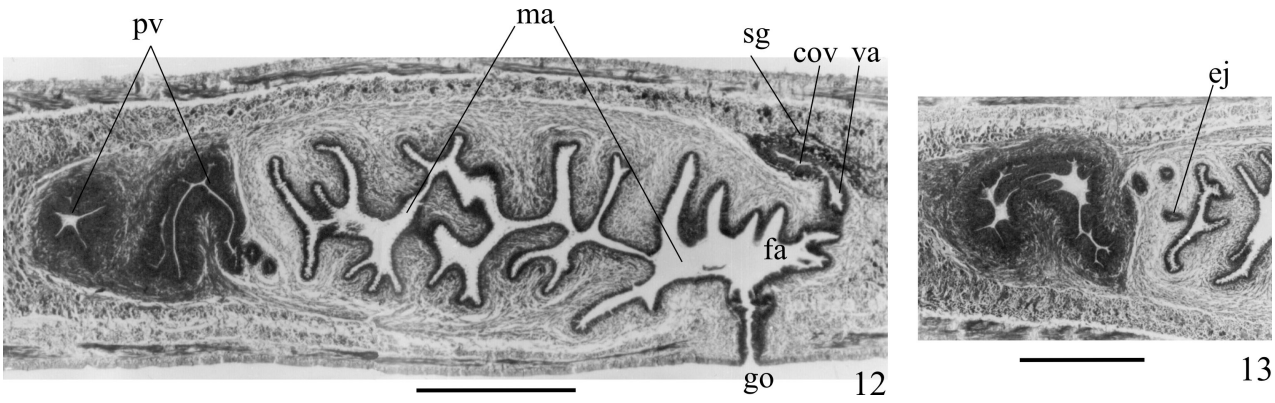


Figs 8-11. – Diagrammatic sagittal composite reconstruction of the copulatory apparatus of *N. ernesti* sp. nov. : (8) from Botanical Garden, São Paulo (paratype EMF 677); (9) from Valinhos/ SP (paratype EMF 595b); (10) from Curitiba (paratype EMF 761b); (11) from São Francisco de Paula (paratype MZU PL.00048). (cc) cyanophil secretory cells, (cf) circular fold, (cm) common muscle coat, (cov) common glandular oviduct, (ec) erythrophil secretory cells, (ed) efferent duct, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ma) male atrium, (ov) oviducts, (pv) prostatic vesicle, (sg) shell glands, (va) vagina, (xc) xanthophil secretory cells. Scale bar : 1mm.



Figs 12-13. – Copulatory apparatus of *N. ernesti* sp. nov. from São Francisco de Paula (paratype MZUSP PL.173) in sagittal section : (12) overall view; (13) detail of proximal portion of male atrium. (cov) common glandular oviduct, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ma) male atrium, (pv) prostatic vesicle, (sg) shell glands, (va) vagina. Scale bar : 500µm.

Male atrium epithelium with columnar non-ciliated cells of irregular height, and xanthophil irregular apical surface, partially broken and discharged into male atrium cavity. Three types of abundant secretory cells pearce the epithelium : cells with xanthophil granulous secretion and cells with fine granulous erythrophil secretion, both with bodies internal to common muscle coat; third type cells with fine granulous cyanophil secretion and bodies external to common muscle coat in surrounding mesenchyme. Secretory cells less numerous in the proximal atrial portion, where ejaculatory duct opens, and in the most distal one. Muscularis thick (61-68µm) constituted of circular subepithelial and subjacent longitudinal fibers, partially interwoven. In proximal atrial region, towards ejaculatory duct opening, muscularis becomes thinner, and crossed by some fibers of stroma between muscularis and common muscle coat.

Oviducts emerging dorsally from median third of ovaries. Anterior to gonopore, oviducts ascend posterior and medially inclined, dorsally to the female atrium unite and form common glandular oviduct (Figs 8-12). The latter, a long canal slightly inclined to dorsum that leads backward to comunicate with vagina. Paired oviducts as well as common oviduct lined with columnar ciliated epithelium, and coated with thin layer mainly of circular muscles. Abundant shell glands empty into common oviduct besides distally in paired oviducts.

Vagina, curved dorsoanteriorly, emerges from posterior extremity of female atrium (Figs 8-12). Female atrium short (Table 1) with some folds (Figs 8-12), and length

equal to one-quarter or one-fifth part of male atrium length in specimens from São Francisco de Paula, Valinhos and Curitiba, to half male atrium length in specimen from Botanical Garden.

Vagina and atrium lined with columnar epithelium, distally ciliated in the vagina, and with irregular height and xanthophil surface. Glands with cyanophil amorphous secretion with bodies external to common muscle coat and erythrophil glands with granulous secretion, short necks, and subepithelial bodies, discharge into the whole epithelial surface. Muscularis, weakly developed when compared with male atrium muscularis (15-25µm thick in female atrium), composed of circular fibers mixed with some longitudinal ones.

Gonopore canal vertical or slightly inclined backwards (Figs 8-12).

Common muscle coat with longitudinal, oblique and circular fibers, thicker around male (ca. 32µm thick) than around female (ca. 17µm thick) atrium. Between atrial muscularis and common muscle coat, a stroma with many, variously orientated muscle fibers (Eigenmusculatur of GRAFF, 1899), is well developed.

Notogynaphallia graffi sp. nov.

Geoplana marginata : Graff, 1899 (Tafel V, Fig. 27-29 : external aspect; Textfig. 36 : copulatory apparatus). Localization of original material unkown

Notogynaphallia marginata (in part) : comb. nov. Ogren, Kawakatsu & E.M. Froehlich, 1992

Notogynaphallia sp. 3 : Leal-Zanchet & Carbayo, 2000

Notogynaphallia marginata sensu Graff, 1899 : Leal-Zanchet & Froehlich, 2001; Carbayo, Leal-Zanchet & Vieira, 2001

Geoplana marginata sensu Graff, 1899 : Carbayo, Leal-Zanchet & Vieira, 2002

Nec *Geoplana marginata* Schultze & Müller, 1857

Nec *Geoplana marginata* : Riester, 1938

Nec *Geoplana marginata* : Marcus, 1951

Etymology

The specific epithet is homage to Ludwig von Graff who first described the species, and his Herculean work regarding turbellarians, and land planarians in particular.

Type material

Holotype : MZUSP PL. 176 : F. Carbayo, leg. 25.IX.98, São Francisco de Paula/ RS, region anterior to ovaries : sagittal sections on 4 slides; anterior region at the level of the ovaries : sagittal sections on 12 slides; pre-pharyngeal region : transversal sections on 5 slides; pharynx : sagittal sections on 10 slides; copulatory apparatus : sagittal sections on 11 slides.

Paratypes : MZUSP PL.175 : F. Carbayo, leg. 10.VII.98, São Francisco de Paula/ RS – preserved in ethanol 70º; MZUSP PL.177 : F. Carbayo, leg. 14.X.99, São Francisco de Paula/ RS, copulatory apparatus : horizontal sections on 12 slides; MZU PL.00051 : F. Carbayo, leg. 26.X.97, Três Coroas/ RS, copulatory apparatus in two fragments : sagittal sections on 15 slides; MZU PL.00052 : M. Cardoso, leg. 11.XII.97, São Francisco de Paula/ RS, anterior region at the level of the ovaries : sagittal sections on 10 slides; pre-pharyngeal region in two fragments : transversal sections on 16 slides; pharynx : sagittal sections on 5 slides; copulatory apparatus : sagittal sections on 9 slides; MZU PL.00053 : F. Carbayo, leg. 03.III.98, São Francisco de Paula/ RS – preserved in ethanol 70º; MZU PL.00054 : M. Cardoso, leg. 04.V.98, São Francisco de Paula/ RS, preserved in ethanol 70º; MZU PL.00055 : I.A. Fick, leg. 11.II.99, São Francisco de Paula/ RS, pre-pharyngeal region : transversal sections on 3 slides; pharynx : sagittal sections on 6 slides; copulatory apparatus : sagittal sections on 8 slides; MZU PL.00056 : I.A. Fick, leg. 13.V.99, São Francisco de Paula/ RS, copulatory apparatus : horizontal sections on 4 slides; MZU PL.00057 : F. Carbayo, leg. 13.V.99, São Francisco de Paula/ RS, copulatory apparatus : sagittal sections on 10 slides; MZU PL.00058 : A.M. Leal-Zanchet, coll. 26.VII.2000, Salvador do Sul/ RS – preserved in ethanol 70º.

Type-locality

São Francisco de Paula, state of Rio Grande do Sul (RS), Brazil.

Distribution

Rio Grande do Sul (São Francisco de Paula, Salvador do Sul, Três Coroas, Taquara, São Leopoldo).

Diagnosis

Dorsum gold-yellow with five black well-delimited longitudinal stripes; median and lateral ones thin, paramedian wide; eyes dorsal, with clear halos when in paramedian stripes; without glandular margin; mc :h, 13-15%; pharynx cylindrical with dorsal insertion posteriorly displaced, folded margins; foremost testes anterior to ovaries, most posterior ones near root of pharynx; efferent ducts open into median third of prostatic vesicle; prostatic vesicle extrabulbar, long, spacious; male atrium, relatively short, almost filled by large annular ental fold separating from general male cavity a restrict, intra-antral, cavity with irregular contour; ejaculatory duct with two histologically distinct portions, opening through a small projection into bottommost part of intra-antral cavity; oviducts emerging dorsally from anterior or median third of ovaries, and ascending anteriorly to gonopore; common glandular oviduct dorsal to female atrium, long, with few openings of shell glands on proximal third; vagina directed dorsally and forwards; female atrium long, highly folded, approx. as long as male atrium.

External morphology

Body elongate with parallel margins, anterior end obtuse and posterior pointed. When crawling, maximal length reaches 50mm (Table 2). Mouth distance from anterior tip varying from 52% to 67% relative to body length, gonopore from 75% to 86% (Table 2). Alive, dorsum gold-yellow, becoming cream in some preserved worms. Dorsally, five black longitudinal stripes, one median, two paramedian and two lateral (Figs 2, 14). In paratype MZUSP PL.175, median stripe begins at 1.5mm

from anterior tip (ca. 7.5% of body length), paramedian at 0.3mm (ca. 1.5% of body length), and lateral at 3.0mm (ca. 15% of body length). Median stripe extends up to 0.9mm (ca. 95% of body length), paramedian and lateral stripes up to 0.3mm (ca. 98% of body length) from posterior tip. All stripes well delimited, median and lateral ones thin (approx. 0.05mm or 3% of body width), and paramedian stripes comparatively very wide (approx. 0.3mm or 19% of body width) (Fig. 14). Venter whitish or yellowish.

TABLE 2

Measurements, in mm, of type-specimens of *N. graffi* sp. nov. - : not measured; * : After fixation; ** Exemplar with empty pharyngeal pouch (without the pharynx); DG : distance of gonopore from anterior end; DM : distance of mouth from anterior end; DMG : distance between mouth and gonopore; DPVP : distance between prostatic vesicle and pharyngeal pouch. The numbers given in parentheses represent the position as related to body length.

	paratype MZUSP PL.175	**holotype MZUSP PL.176**	**paratype MZUSP PL.177**	**paratype MZU PL.00051**	**paratype MZU PL.00052****	**paratype MZU PL.00053**	**paratype MZU PL.00054**	**paratype MZU PL.00055**	**paratype MZU PL.00056**	**paratype MZU PL.00057**	**paratype MZU PL.00058**
Maximum length in extension	28	45	45	-	34	26	22	47	43	35	50
Maximum width in extension	1.0	2.0	2.0	-	1.5	1.5	1.5	1.0	1.0	2.0	2.0
Length at rest	12	35	20	-	-	14	12	32	18	20	-
Maximum width at rest	2.0	3.5	3.0	-	-	2.0	2.0	2.0	2.0	3.0	4.0
Length*	20	41	37	27	30	21	16	32	25	28	36
Width*	1.5	2.0	2.0	2.0	1.5	1.5	1.5	2.0	2.0	<2.0	2.5
DM*	12 (60%)	24 (58%)	23 (62%)	14 (52%)	18 (60%)	14 (67%)	8 (50%)	19 (59%)	16 (64%)	16 (57%)	23 (64%)
DG*	16 (80%)	33 (80%)	29 (78%)	21 (78%)	23 (77%)	18 (86%)	10 (62%)	25 (78%)	20 (80%)	21 (75%)	29 (80%)
DMG*	4.0	9.0	6.0	7.0	5.0	4.0	2.0	6.0	4.0	5.0	6.0
DPVP*	-	1.7	-	0.7	0.4	-	-	-	-	-	-
Creeping sole	-	83%	-	-	-	-	-	85%	-	-	-
Ovaries	-	7.0 (22%)	-	-	7.0 (17%)	-	-	-	-	-	-
Anteriormost testes	-	6.0 (19%)	-	-	5.4 (14%)	-	-	-	-	-	-
Posteriormost testes	-	23 (55%)	-	-	17.4 (58%)	-	-	-	-	-	-
Prostatic vesicle	-	2.5	2.8	2.0	2.0	-	-	-	-	-	-
Male atrium	-	0.9	0.6	0.6	0.9	-	-	-	-	-	-
Female atrium	-	1.0	0.6	0.7	0.8	-	-	-	-	-	-

In paratype MZUSP PL.175, eyes uniserially surround anterior tip, and become pluriserial immediately after. Between 2.5mm and 9.0mm (approx. 12% and 45% of body length) behind anterior end, they extend from body margins up to paramedian stripes, and may invade the latter acquiring clear halos (Fig. 14). Subsequently, up to posterior end, they become scarce and limited to body margins.

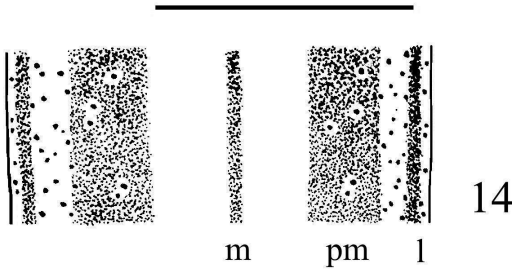


Fig. 14. – Detail of colour pattern of preserved specimen of *N. graffi* sp. nov. (paratype MZUSP PL.175). (l) lateral stripe, (m) median stripe, (pm) paramedian stripe. Scale bar : 1mm.

Epidermis and musculature at pre-pharyngeal region

Creeping sole, 83% to 85% of body width (Table 2).

Three types of secretory cells open through dorsal epidermis and body margins : (1) cells numerous with coarse erythrophil secretion; (2) cells less frequent with cyanophil amorphous secretion; (3) rhabditogen cells with xanthophil secretion. A fourth secretory cell type with fine chromophob granular secretion opens near body margins. Creeping sole receives less numerous secretory cells of three types : scarce cells with fine weakly erythrophil granular secretion; cells with cyanophil amorphous secretion; and rhabditogen cells. Glandular margin is absent.

Cutaneous musculature with constitution similar to that described for *N. ernesti*, being, however, laterally in prepharyngeal sections, as high as medially. Mc :h 13% to 15% (Table 4). Mesenchymatic musculature as in *N. ernesti*.

Pharynx

Pharynx (Fig. 15) of cylindrical type with dorsal insertion posteriorly displaced, but still in anterior third of pha-

ryngeal pouch, and with folded margins. Mouth in median third of pharyngeal pouch : posterior to dorsal insertion. No esophagus. Pharyngeal glands with cell bodies located in mesenchyme, mainly anterior and posterior to pharyngeal pouch. Three secretory cell types : (1) cells with densely arranged, xanthophil granulous secretion; (2) cells with strongly erythrophil granulous secretion; and (3) cells with cyanophil amorphous secretion. Outer and inner pharyngeal musculatures as in *N. ernesti*, but inner one less developed.

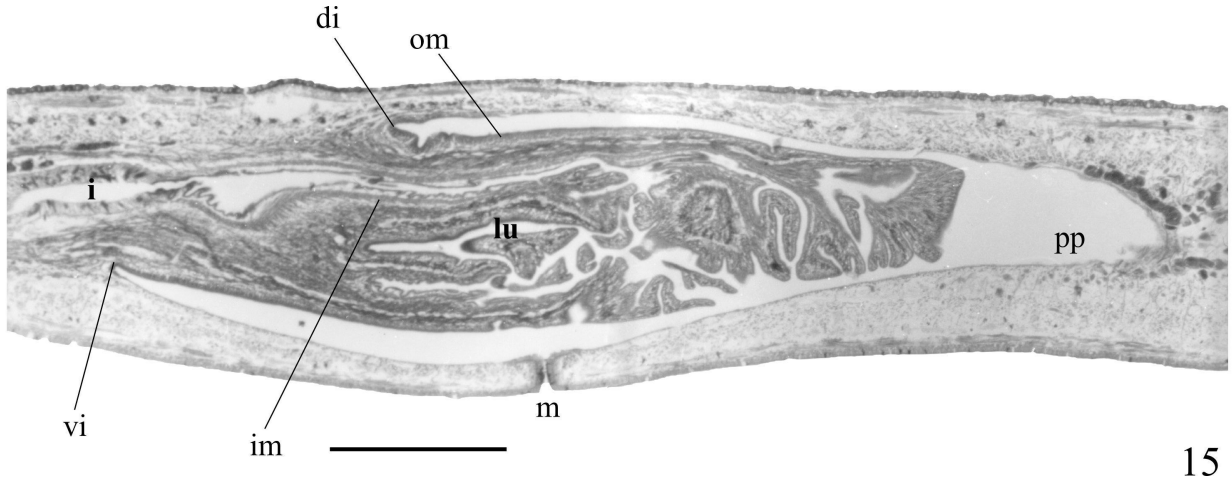
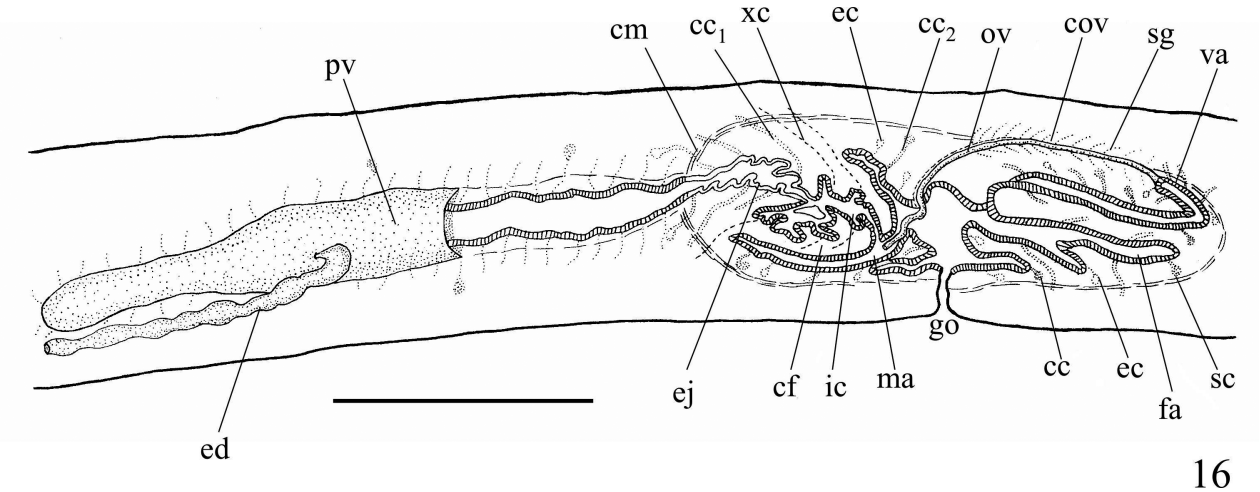


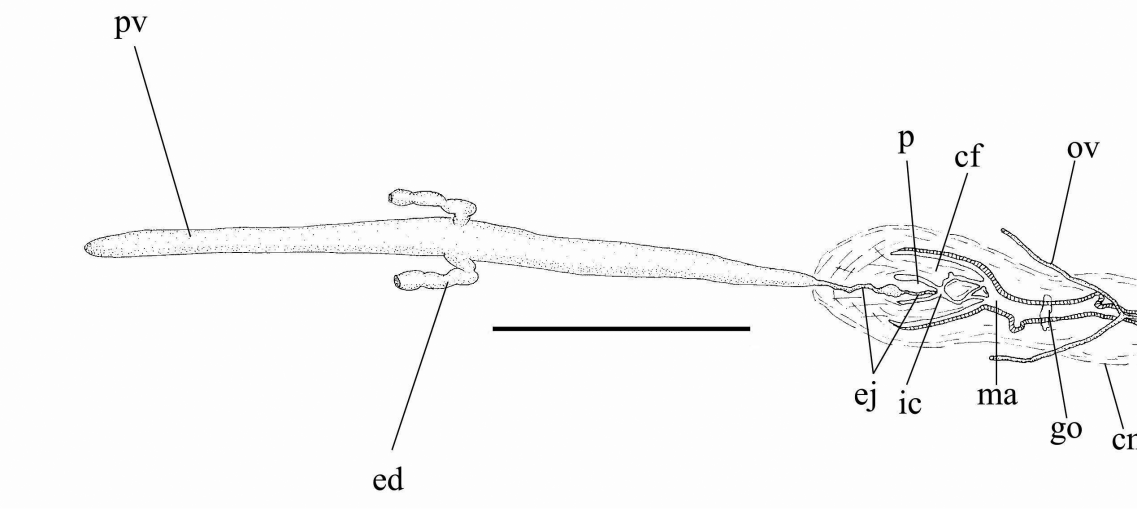
Fig. 15. – Pharynx of *N. graffi* sp. nov. (holotype). Sagittal section. (di) dorsal insertion, (i) intestine, (im) internal musculature, (lu) pharyngeal lumen, (m) mouth, (om) outer musculature, (pp) pharyngeal pouch, (vi) ventral insertion. Scale bar : 500µm.

Reproductive apparatus

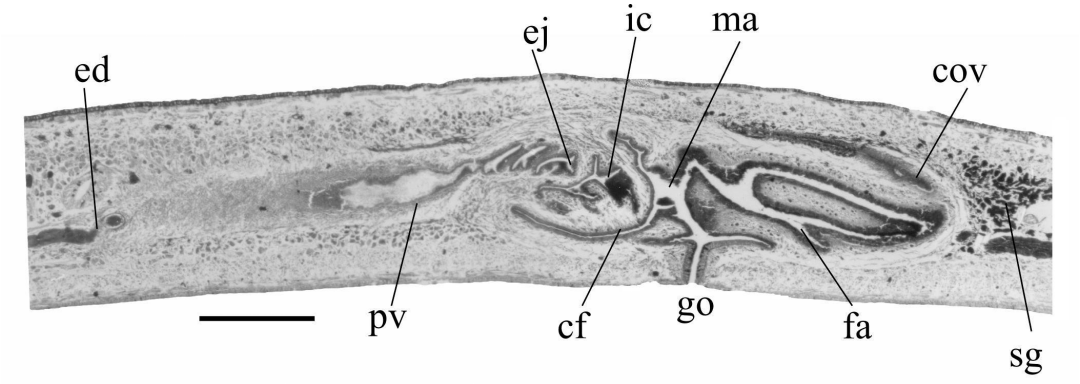
Testes beginning anteriorly to ovaries and extending up to near root of the pharynx (Table 2). Pre-pharyngeally, efferent ducts dorsal to oviducts, sometimes laterally displaced. Behind pharynx forming false seminal vesicles and opening laterally into median third of prostatic vesicle (Figs 16-19).

Extrabulbar prostatic vesicle elongate (Table 2), with spacious cavity (Figs 16-19). Proximal portion not reaching pharyngeal pouch; unforked in holotype and most analysed specimens, except for paratype 172 which a short ental furcation (0.4mm or approx. 1/5 of total length of vesicle). Lining epithelium columnar ciliated, irregular in height, receiving numerous fine-grained, erythrophil glands with poligonal cell bodies in surrounding mesenchyme. Muscularis well developed, mainly at the proximal half (35µm thick), at the distal half diminishing up to 24µm; composed of interwoven circular, oblique and longitudinal fibers. Approaching penis bulb, prostatic vesicle narrows and gives rise to sinuous ejaculatory duct, lined with columnar ciliated epithelium, higher in proximal portion, weakly cyanophil glands, with cell bodies extrabulbar around prostatic vesicle, opening into both. Coating muscularis, consisting of mixed circular and longitudinal fibers, weakly developed but thicker proximally (9µm) than distally (2µm).





Figs 16-17. – Diagrammatic composite reconstructions of copulatory apparatus of *N. graffi* sp. nov. : (16) from sagittal sections (holotype); (17) from horizontal sections (paratype MZUSP PL.177). (cc) cyanophil secretory cells, (cc_1) cyanophil secretory cells opening into the ejaculatory duct, (cc_2) cyanophil secretory cells opening into the male atrium, (cf) circular fold, (cm) common muscle coat, (cov) common glandular oviduct, (ec) erythrophil secretory cells, (ed) efferent duct, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ic) intra-antral cavity, (ma) male atrium, (ov) oviducts, (p) projection into the intra-antral cavity, (pv) prostatic vesicle, (sc) secretory cells with ill-defined stained secretion, (sg) shell glands, (va) vagina, (xc) xanthophil secretory cells. Scale bar : 1mm.



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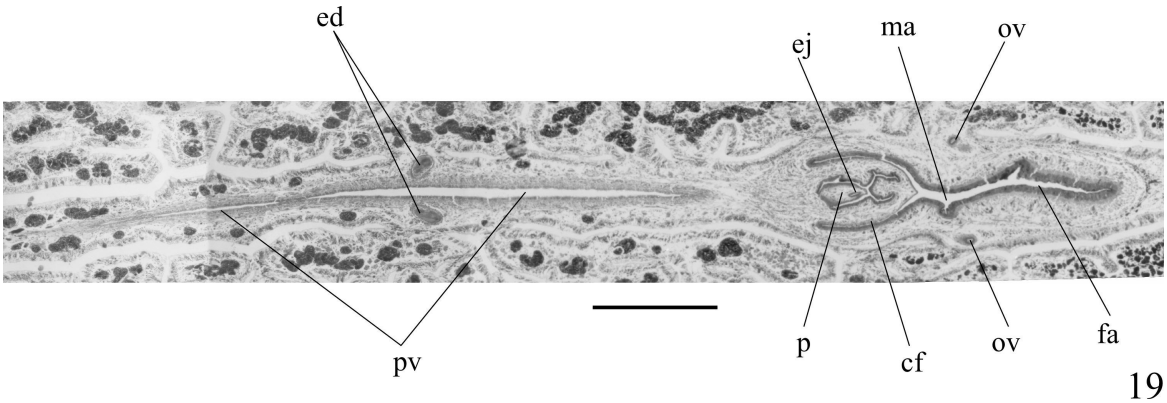

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Figs 18-19. – Copulatory apparatus of *N. graffi* sp. nov. : (18) holotype in sagittal section; (19) paratype MZUSP PL.177 in horizontal section. (cov) common glandular oviduct, (cf) circular fold, (ed) efferent duct, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ic) intra-antral cavity, (ma) male atrium, (ov) oviducts, (p) projection into the intra-antral cavity, (pv) prostatic vesicle, (sg) shell glands. Scale bar : 500µm. In fig. (18), section shows only the posterior third of the prostatic vesicle.

Relatively short male atrium (Table 2) mainly filled by a large annular fold that, arising from its bottom, encloses an intra-antral restricted cavity, with irregular contour and folded walls (Figs 16-19). Ejaculatory duct opens into a small projection of the bottommost part of intra-antral cavity. In paratypes MZU PL.00051 and MZUSP PL.177, both fixed after copulation (24h and 2h, respectively), this projection is more in evidence (Fig. 19). In two incompletely mature worms (paratypes MZU PL.00056 and MZU PL.00057), the annular fold is elongate, cylindrical, encircling a canalicular intra-antral cavity extending as a direct continuation of the ejaculatory duct. Transition from ejaculatory duct to canalicular intra-antral cavity very clear due to their different linings.

Epithelial lining of male atrium, circular fold and enclosed cavity included, with low columnar non-ciliated with erythrophil cytoplasm and xanthophil apical portion. Two types of secretory cells empty through the epithelium : (1) cells containing strongly erythrophil granulous secretion with short necks and subepithelial bodies; (2) cyanophil cells with bodies internal to muscle coat. A third type of gland, with weakly xanthophil granulous secretion and bodies in mesenchyme external to bulb, enter the circular fold and open through its epithelial lining. Muscularis of male atrium (10-12µm) less developed in the circular fold (6-7µm); composed of circular subepithelial and longitudinal subjacent layer, partially intermixed in some places.

Oviducts emerging dorsally from anterior or median third of ovaries, lead backwards immediately dorsal to nerve plate. Before gonopore, oviducts rise through an S-shaped path, postero-mediad inclined, to unite and continue dorsally to female atrium as common glandular oviduct (Figs 16-18). Common glandular oviduct long, with first two thirds horizontal and distal one slightly descendent (Figs 16-17). Lining epithelium of paired oviducts cubical to columnar ciliated, of common oviduct columnar ciliated; muscle coat of paired and common oviducts mainly with circular fibers. Shell glands open into distal ascending portion of paired and in common oviducts, becoming fewer towards distal portion of the latter.

Vagina continues dorsally and forward-directed from posterior end of female atrium (Figs 16-17). Female atrium nearly as long as male one (Table 2, Figs 17-18), with long folds that, traversing diagonally the whole cavity, ligate ventral and dorsal walls, or unite with folds of ventral distal wall of male atrium.

Epithelium lining vagina and female atrium tall columnar non-ciliated with xanthophil apical portion and pierced by erythrophil cells with short necks and subepithelial bodies, and very numerous cells with a cyanophil amorphous secretion and bodies in mesenchyme external to common muscle coat. Female atrium additionally receives a third type of gland cells with fine-graned and ill-defined staining secretion, bodies internal to common muscle coat, and distally more numerous. Muscularis constituted of intermixed circular and longitudinal muscle fibers, less developed in female atrium (8µm thick) than in male one.

Gonopore canal vertical (Figs 16, 18), slightly inclined forwards.

Common muscle coat (14µm and 19-28µm thick, respectively around female and male atria) with circular, longitudinal and oblique fibers. Between atrial muscularis and common muscle coat, a stroma with muscle fibers variously oriented.

Notogynaphallia abundans (Graff, 1899)

Geoplana marginata var. *abundans* Graff, 1899 (Tafel V, Fig. 30 : external aspect). Localization of original material unkown

Geoplana abundans : Froehlich, 1959

Notogynaphallia abundans : comb. nov. Ogren, Kawakatsu & E.M. Froehlich, 1992

Nec *Geoplana abundans* : Almeida, Yamada & E.M. Froehlich, 1991

Material examined

EMF Nr. 331a : J. Hauser, leg. 26.IX.55, São Leopoldo/ RS, copulatory apparatus sectioned in two fragments : sagittal sections on 4 slides; EMF Nr. 331b : J. Hauser, leg. 10.X.55, São Leopoldo/ RS, copulatory apparatus : sagittal sections on 2 slides; MZUSP PL.178 : F. Carbayo, leg. 14.XII.97, Boa Vista, Poço das Antas/ RS, copulatory apparatus sectioned in two fragments, 1 : sagittal sections on 19 slides, and 2 : sagittal sections on 7 slides; MZU PL.00059 : F. Carbayo, leg. 18.X.96, São Sebastião do Caí/ RS, copulatory apparatus : sagittal sections on 46 slides; MZU PL.00060 : D.C. Vara, leg. 06.III.97, Campo Bom/ RS, copulatory apparatus : sagittal sections on 10 slides; MZU PL.00061 : W.H. Santos & L. Bonneau, leg. 19.VIII.97, São Leopoldo/ RS, anterior region level with ovaries : sagittal sections on 16 slides; pre-pharyngeal region : transversal sections on 8 slides; pharynx : sagittal sections on 13 slides; copulatory apparatus : horizontal sections on 37 slides; MZU PL.00062 : W.H. Santos & L. Bonneau, leg. 19.VIII.97, São Leopoldo/ RS, copulatory apparatus : sagittal sections on 18 slides; MZU PL.00063 : M. Cardoso, leg. 13.X.97, Novo Hamburgo/ RS, preserved in ethanol 70º; MZU PL.00064 : M. Cardoso, leg. 21.X.97, Novo Hamburgo/ RS, anterior region level with ovaries : sagittal sections on 12 slides; pre-pharyngeal region : transversal sections on 4 slides; pharynx : sagittal sections on 14 slides; copulatory apparatus : sagittal sections on 14 slides; MZU PL.00065 : M. Cardoso, leg. 21.X.97, Novo Hamburgo/ RS, pharynx : sagittal sections on 12 slides; copulatory apparatus : sagittal sections on 8 slides; MZU PL.00066 : M. Cardoso, leg. 16.X.97, Novo Hamburgo/ RS, preserved in ethanol 70º; MZU PL.00067 : I.A. Fick, leg. 02.VIII.98, Glorinha/ RS, copulatory apparatus : horizontal sections on 35 slides; MZU PL.00068 : F. Carbayo, leg. 25.VIII.98, São Leopoldo/ RS, anterior region level with ovaries in two fragments – sagittal sections on 24 slides; pre-pharyngeal region : transversal sections on 10 slides; pharynx : sagittal sections on 26 slides; copulatory apparatus : sagittal sections on 49 slides; MZU PL.00069 : M. Cardoso, leg. 22.XI.99, Salvador do Sul/ RS, pharynx and copulatory apparatus : horizontal sections on 29 slides.

Type-locality

Taquara, state of Rio Grande do Sul (RS), Brazil.

Distribution

Rio Grande do Sul (Poço das Antas, Salvador do Sul, Tupandi, Taquara, Novo Hamburgo, Campo Bom, São Leopoldo, Glorinha) – Brazil

Diagnosis

Dorsum cream, yellowish or pale brownish with seven longitudinal dark brown stripes; median, the thinnest; lateral and submarginal, the darkest; paramedian and lateral often poorly delimited; eyes dorsal, without clear halos except those in submarginal stripes; glandular margin absent; mc :h, 12-13%; pharynx bell-shaped with folded margins; foremost testes anterior to ovaries, most posterior anterior to root of pharynx; efferent ducts opening into median third of prostatic vesicle; prostatic vesicle extrabulbar, long and laterally sinuous, proximal portion, most often unforked, exceeding a little the posterior end of pharyngeal pouch; male atrium, elongate, highly folded with proximal wall histologically diferentiated; oviducts arising from dorsal side of median third of ovaries, and rising behind gonopore; common glandular oviduct dorsal to female atrium; vagina short, dorso-anteriorly directed; female atrium, long and folded; male atrium length, 2.5 to 4.0 times that of female atrium.

External morphology

Body elongate with parallel margins, anterior end obtuse and posterior pointed. When creeping, maximal length may reach 60mm (Table 3). Mouth distance from anterior tip varies from 54% to 62% relatively to body length, gonopore from 70% to 81% (Table 3). Dorsum cream, yellowish or pale brownish with seven dark brown longitudinal stripes, one median, two paramedian, two lateral, two submarginal; lateral and submarginal being the darkest (Figs 3, 20). Venter cream. In specimen MZU PL.00063, median stripe begins at 1.5mm from anterior tip, a little behind the others which begin between 0.7 and 1.0mm (between 2 and 3% of body length). Stripes are not evenly distributed throughout dorsum width : interval between paramedian and lateral stripes being the narrowest, sometimes near virtual. Near posterior end (approx. 1mm or ca. 97% of body length), on each side of body, paramedian and lateral stripes converge and finish; median stripe ends at approximately the same level, whereas submarginal stripes of both sides converge and lead to posterior tip. Median stripe is the thinnest (approx. 0.05mm or 2% of body width), followed by paramedian and submarginal (approx. 0.07mm or 4% of body width), and lateral (approx. 0.15mm or 8% of body width) stripes (Fig. 20).

TABLE 3

Measurements, in mm, of type-specimens of *N. abundans* (Graff, 1899). - : not measured; * : After fixation; DG : distance of gonopore from anterior end; DM : distance of mouth from anterior end; DMG : distance between mouth and gonopore.
** This exemplar had the posterior end damaged. The numbers given in parentheses represent the position as related to body length.

	MZUSP PL.178	**MZU PL.00059****	**MZU PL.00060**	**MZU PL.00061**	**MZU PL.00062**	**MZU PL.00063**	**MZU PL.00064**	**MZU PL.00065**	**MZU PL.00066**	**MZU PL.00067**	**MZU PL.00068**	**MZU PL.00069**
Maximum length in extension	45	45	>30	35	50	38	58	52	42	60	-	58
Maximum width in extension	2.5	3.0	1.5	2.0	2.0	2.0	2.5	2.5	2.0	2.5	-	2.0
Length at rest	-	-	-	-	-	-	-	-	-	35	-	32
Maximum width at rest	-	-	-	-	-	-	-	-	-	4	-	4.0
Length*	33	38**	20	44	34	29	49	33	34	54	53	42
Width*	3	3.0	2.0	3.0	4.0	2.5	3.0	3.0	2.5	3.5	3	3
DM*	18.5 (56%)	23 (-)	11 (55%)	26 (59%)	20.5 (60%)	17 (59%)	29 (59%)	18 (54%)	21 (62%)	33 (61%)	30 (57%)	25 (59%)
DG*	26 (79%)	34.5 (-)	14 (70%)	34 (77%)	26.5 (78%)	22 (76%)	39 (80%)	25.5 (77%)	27 (79%)	42 (78%)	40 (76%)	34 (81%)
DMG*	7.5	11.5	3.0	8.0	6.0	5.0	10	7.5	6.0	9.0	10	9.0
Creeping sole	-	-	-	73%	-	-	68%	-	-	-	70%	-
Ovaries	-	-	-	-	-	-	10.5	-	-	-	13.5	-
Anteriormost testes	-	-	-	-	-	-	12 (21%)	-	-	-	14 (25%)	-
Posteriormost testes	-	-	-	-	-	-	28 (24%)	-	-	-	28 (26%)	-
Prostatic vesicle	2.7	>3.3	1.4	-	2.3	-	4.6 (57%)	3.3	-	-	4.3 (53%)	-
Male atrium	2.5	4.4	1.7	-	2.7	-	2.7	2.2	-	-	3.4	-
Female atrium	0.7	1.0	0.6	-	0.7	-	0.8	0.6	-	-	1.0	-

Eyes, initially marginal and uniserial, surrounding anterior end. In specimen MZU PL.00063, become pluriserial between 1.5 and 3.5mm from tip (approx. 5% and 12% of body length). Following, up to approx. 10mm from anterior tip (ca. 34% of body length), they spread as far as between submarginal and lateral stripes (Fig. 20),

being here the most numerous. Backwards become limited to body margins, occurring up to posterior tip. Those in submarginal stripes are surrounded by clear halos (Fig. 20).

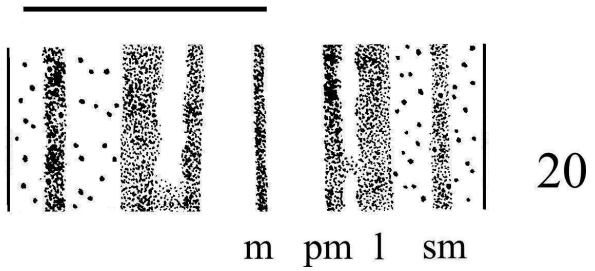


Fig. 20. – Detail of the colour pattern of a preserved specimen of *N. abundans* (specimen MZU PL.00063). (l) lateral stripe, (m) median stripe, (pm) paramedian stripe, (sm) submarginal stripe. Scale bar : 1mm.

Epidermis and musculature at pre-pharyngeal region

Creeping sole, 68% to 73% of body width (Table 3).

Three types of secretory cells discharge through dorsal epidermis and body margins : (1) abundant cells with coarse erythrophil secretion; (2) few cells with cyanophil amorphous secretion; (3) rhabditogen cells with xanthophil secretion, less frequent dorsally when compared with those of first two species, however, very numerous through body margins. Creeping sole receives cells with a coarse erythrophil secretion, very numerous cells with amorphous cyanophil secretion and few rhabditogen cells. Glandular margin absent.

Cutaneous musculature with similar constitution as in precedent species; mc :h 12% to 13% (Table 4). Mesenchymatic musculature as described for *N. ernesti*, subintestinal transversal layer being, however, thicker (12 fibers thick).

TABLE 4

Table 4. Cutaneous musculature in the median region of a transversal section of the pre-pharyngeal region and ratio of the height of the cutaneous musculature to the height of the body (mc :h index) of specimens of N. ernesti, N. graffi and N. abundans.

	N. ernesti						***N. graffi***		***N. abundans***		
	paratype EMF 595b	**paratype EMF 677**	**paratype EMF 761b**	**paratype EMF 927**	**paratype MZU PL.00047**	**paratype MZU PL.00048**	**paratype MZU PL.00052**	**paratype MZU PL.00055**	**specimen MZU PL.00061**	**specimen MZU PL.00064**	**specimen MZU PL.00068**
Circular ventral	2	3	2	2	2	2	2	2	2	2	2
Oblique ventral	10	18	10	9	10	10	7	10	11	10	18
Longitudinal ventral	49	67	60	65	56	60	30	42	50	70	62
Ventral total	61	88	72	76	68	72	39	54	63	82	82
Circular dorsal	2	2	2	2	2	2	2	2	3	3	2
Oblique dorsal	9	13	11	10	8	11	6	9	8	7	10
Longitudinal dorsal	54	72	71	60	75	95	31	40	42	31	36
Dorsal total	65	85	84	72	85	108	39	51	53	41	48
mc :h	15%	18%	18%	17%	20%	17%	13%	15%	12%	13%	13%

Pharynx

Pharynx bell-shaped with folded margins. Mouth in median third of pharyngeal pouch, on the same transversal level of dorsal insertion or slightly posterior (Fig. 21). No esophagus. Pharyngeal glands as those of *N. ernesti*. Outer and inner pharyngeal muscle layers similar to those of *N. ernesti* except for outer longitudinal layer, more developed in *N. abundans* (8µm thick).

Reproductive apparatus

Foremost testes anterior to ovaries; most posterior ones, anterior to pharynx (Table 3). Efferent ducts run dorsally to oviducts, laterally displaced in some points, and enter prostatic vesicle in median third (Figs 22-23). Form false seminal vesicles from pharynx level or slightly posterior. Lining epithelium cuboidal ciliated.

Prostatic vesicle, long (Table 3) and laterally sinuous, extends anteriorly a little beyond and ventrally to posterior end of pharyngeal pouch, without forking in most analysed specimens (Figs 22-23). In specimen 376, however, proximal portion, corresponding to 1/6 of the whole length of the organ, is forked. Lining epithelium, columnar to pseudostratified ciliated, receives abundant erythrophil granulous secretion from secretory cells with bodies lying in mesenchyme around or anterior to vesicle. Muscularis (31µm thick) constituted of longitudinal subepithelial layer, followed by circular fibers. Entering bulbar muscular coat, prostatic vesicle gradually narrows and becomes more sinuous (Fig. 22), forming ejaculatory duct which, after short course, ascends, describing an arc before opening dorsally into proximal portion of male atrium. Ejaculatory duct lined with columnar ciliated epithelium and coated with weakly developed muscularis (ca. 7µm thick). There are no secretory cells opening into ejaculatory duct.

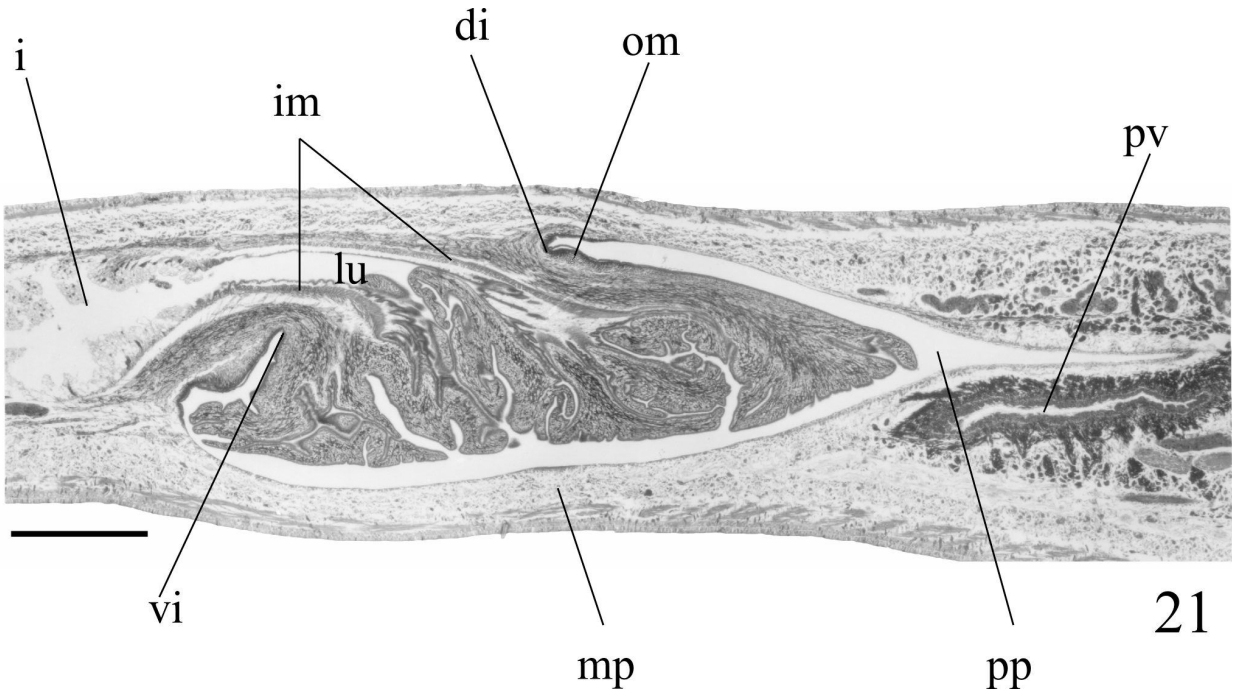


Fig. 21. – Pharynx of *N. abundans* (specimen MZU PL.00068). Sagittal section. (di) dorsal insertion, (i) intestine, (im) internal musculature, (lu) pharyngeal lumen, (mp) mouth's position, (om) outer musculature, (pp) pharyngeal pouch, (pv) prostatic vesicle, (vi) ventral insertion. Scale bar : 500µm.

Male atrial cavity elongate (Table 3) with very irregular contour as a consequence of its highly folded walls (Figs 22-24). In most specimens it is entally very dorsoventrally narrowed due to a wide, complex, asymmetrical fold, variously shaped in different specimens and occupying variable extension (from one fifth to one half) of male atrium (Figs 22-25). Lining of male atrium columnar nonciliated epithelium of irregular height, and free surface differentiated, being more strongly or more weakely stained. Those of ental fold with cuboidal epithelium penetrated by two secretory cell types which are restricted to it : (1) cells with densely arranged, granulous cyanophil secretion; (2) cells with densely arranged, granulous erythrophil secretion. However, mostly of male atrial wall receives three other secretory cell types : (1) cells with weakly cyanophil amorphous secretion, distally more abundant; (2) cells with fine xanthophil secretion; (3) less frequent cells with coarse xanthophil secretion. Bodies of all secretory cells in mesenchyme, anteriorly or laterally to copulatory apparatus. Muscularis well developed (ca. 46-48µm) with subepithelial layer of circular fibers, followed by, and partially intermixing with, longitudinal fibers. On ental fold muscularis mainly composed of longitudinal fibers intermixed with some circular ones, and crossed by radial fibers from the stroma between muscularis and common muscle coat.

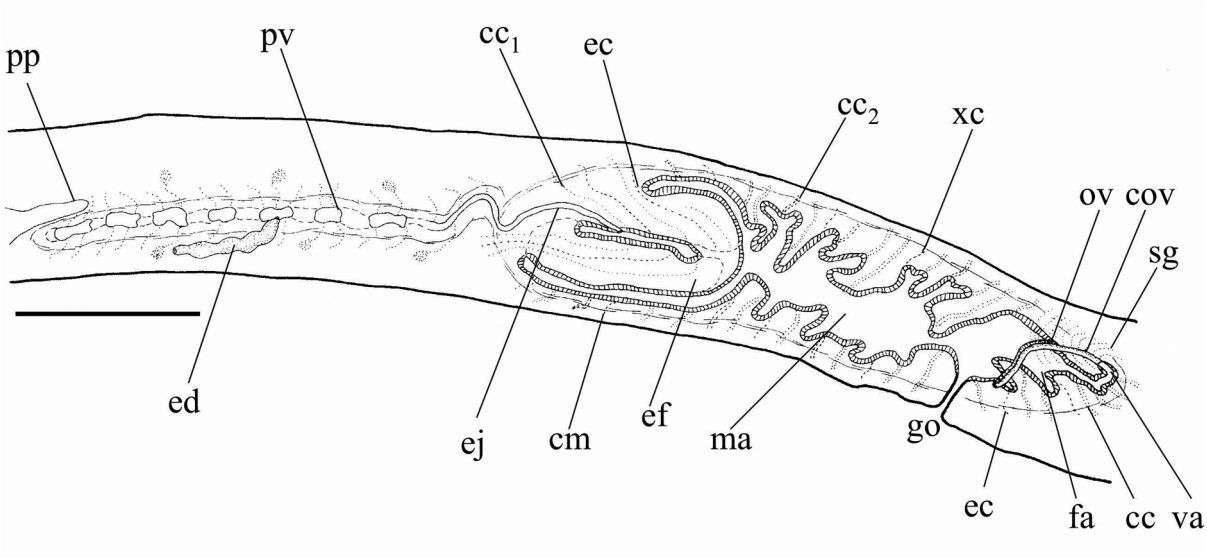
Oviducts arise from dorsal side of median third of ovaries and run posteriorly, immediately above nerve plate. Behind gonopore, oviducts proceed dorsomediad, unite above female atrium forming common glandular oviduct which continues backwards, slightly inclined ventrally, to enter into vagina (Figs 22-23). Cells of epithelium lining oviducts and common glandular oviduct columnar ciliated; that on paired oviducts, with basal nuclei and, mainly in apical half, cyanophil cytoplasm. Muscle coat of oviducts constituted of circular fibers, that of common glandular oviduct of mixed circular and longitudinal fibers. Shell glands opening into distal ascending portion of paired oviducts, besides into common glandular oviduct.

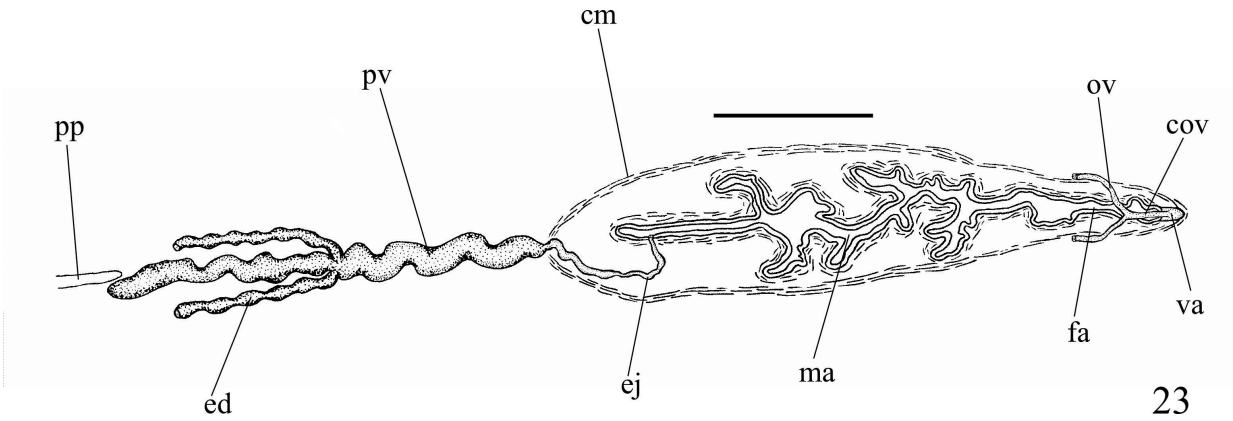
Vagina short arising from dorsal ental wall of female atrium, sligthly curved forward (Figs 22, 24). Female cavity with intensily folded walls; some folds arising from ental wall traverse the whole cavity to unite with folds from wall of male atrial near to gonopore (Fig. 24). Length of female atrium (Table 3) equal to one third or one quarter of male atrial length.

Vagina and atrium lined with tall columnar epithelium, distally ciliated in the vagina and with apical surface weakly erythrophil in the atrium. Glands with cyanophil amorphous secretion discharge into whole epithelium, numerous in atrium and fewer towards distal end of vagina. A second type of gland; less numerous, with erythrophil granulous secretion, empties into the atrium. Cell bodies of both gland types lateral or posterior to female atrium. Muscularis consisting of circular layer with some longitudinal subjacent fibers in vagina, and of layer (approx. 20µm thick) with loosely arranged circular and longitudinal fibers in atrium.

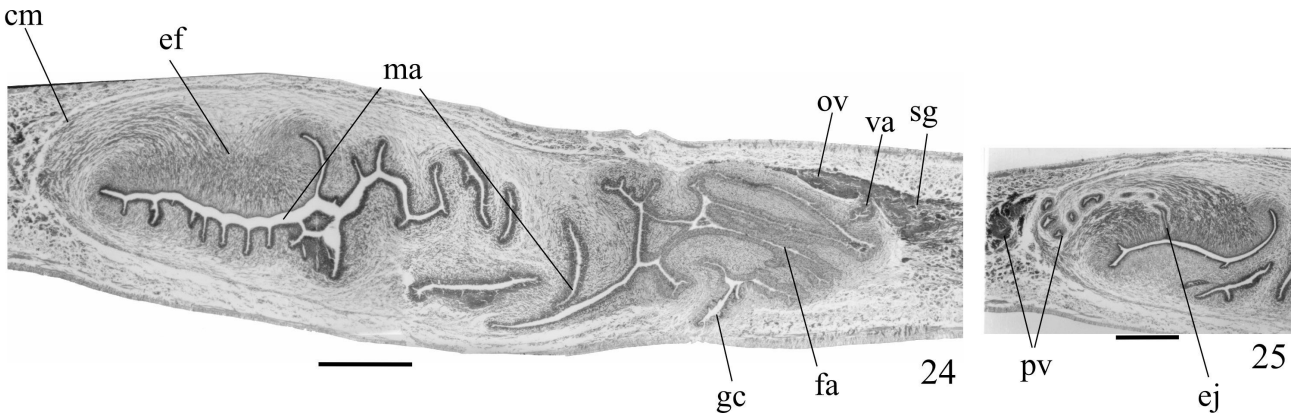
Gonopore canal slightly inclined backwards (Figs 22, 24).

Common muscle coat well developed around male atrium (19-22µm thick), and very thin (2µm thick) at level of female one. Between muscularis and common muscle coat, a stroma with muscle fibers variously oriented.





Figs 22-23. – Diagrammatic composite reconstructions of copulatory apparatus of *N. abundans* : (22) from sagittal sections (specimen MZUSP PL.178); (23) from horizontal sections (specimen MZU PL.00069). (cc) cyanophil secretory cells, (cc_1) cyanophil secretory cells opening into the ejaculatory duct, (cc_2) cyanophil secretory cells opening into the male atrium, (cm) common muscle coat, (cov) common glandular oviduct, (ec) erythrophil secretory cells, (ed) efferent duct, (ef) ental fold, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ma) male atrium, (ov) oviducts, (pp) pharyngeal pouch, (pv) prostatic vesicle, (sg) shell glands, (va) vagina, (xc) xanthophil secretory cells. Scale bar : 1mm.



Figs 24-25. – Copulatory apparatus of *N. abundans* (specimen MZU PL.00068) in sagittal section : (24) overall view; (25) detail of proximal portion of male atrium. (cm) common muscle coat, (ef) ental fold, (ej) ejaculatory duct, (fa) female atrium, (gc) gonopore canal, (ma) male atrium, (ov) oviduct, (sg) shell glands, (pv) prostatic vesicle, (va) vagina. Scale bar : 500µm.

DISCUSSION

When describing *N. ceciliae* FROEHLICH & LEAL-ZANCHET (2003) commented on the heterogeneity of the species in the genus *Notogynaphallia* and distinguished, in a first general approach, two groups. One of them included, besides *N. guaiana* Leal-Zanchet & Carbayo, 2001, *N. muelleri* (Diesing, 1861), *N. fita* (Froehlich, 1959), and *N. caissara* (E.M. Froehlich, 1955), also *N. ceciliae* and the three species herein described.

Regarding external morphology, and as already shown for *N. ceciliae*, the first three species are readily separable from *N. ernesti, N. graffi, N. abundans* and *N. caissara. N. guaiana* shows a plainly grey dorsum and exclusively marginal eyes. *N. fita* has a much larger size, exclusively marginal eyes, and dorsum with four stripes along the major part of body. *N. muelleri* has a greater length and leanness and a dorsum with only one or three stripes; when with three stripes the lateral ones are always ferrugineous, never brownish or black as the median.

Of the remaining five species of the group, *N. abundans* is the only one with seven dark stripes, whereas the other four have five. The latter species, despite their striking external similarities, can be separated from each other by details such as the relative width of the different stripes, arrangement of stripes relative to body width and procedure of stripes near the extremities of body, specially the caudal one.

In *N. ceciliae* stripes, all of similar width run closer to each other, and nearer the median line of body, than in *N. caissara, N. ernesti* and *N. graffi*, so as to leave the widest band of ground-colour on body margins, as compared to the three latter species. Besides, all stripes end at the same distance from the posterior extremity without converging. In *N. caissara* from Rio de Janeiro all stripes are of similar width, the lateral being darker and slightly wider; in specimens from Ubatuba, however, the median stripe is some five times wider than the others. Median and paramedian stripes end, at the same level, a little before both extremities; lateral ones, as a continuous line, contour both extremities. As already commented by MARCUS (1951) when comparing specimens from V. Atlântica and São Paulo, in *N. ernesti* width of paramedians and lateral stripes are characteristically variable according to worm provenance, the former being the widest in worms from V. Atlântica, and the latter the widest in most specimens from São Paulo. Specimens from Curitiba and S. Francisco de Paula, studied herein, are similar, respectively, to those from V. Atlântica and S. Paulo. Procedure of stripes regarding body ends also varies in different specimens, except for the lateral, which are always convergent towards caudal end. However, in no specimen lateral stripes are continuous around both extremities, as in *N. caissara*. This detail constitutes the only constant external difference between *N. ernesti* from S. Paulo and *N. caissara* from Rio de Janeiro. This might explain why Marcus, despite important incongruities he verified and commented on, between Riester's description and drawing of copulatory apparatus and copulatory apparatus of his own worms, surprisingly decided on the conspecificity of his and Riester´s material. Finally, in *N. graffi* the stripes are very densely pigmented and sharply delimited as in none of the five-striped species dealt with above. Paramedian stripes are consistently some four to six times as wide as median and lateral ones. GRAFF (1899) described and drew the intense darkness and the greater width of the paramedian stripes, which was registered and commented on by Marcus as this did not occur in his own worms. He might have attributed it to geographical variation since at that time *N. ernesti* was not known from Rio Grande do Sul. In *N. graffi*, all stripes finish at slightly different levels from both extremities, but it is the only among the five-striped species in which paramedian and lateral stripes converge on each side of body before posterior end.

Eye distribution throughout body length is similar in the four species of the complex. In *N. abundans*, *N. ernesti* and *N. graffi* they become pluriserial at approx. 1mm (2 to 5% of body length) from the anterior end, and in *N. ceciliae* at approx. 4mm (ca. 14% of body length) from the tip; thereafter they become dorsal and are abundant up to 9 to 12mm (20 to 45% of body length) from the anterior extremity. In *N. caissara* eye distribution is also similar.

GRAFF (1899, p. 333) described a slender longitudinally striped species, *G. bohlsi*, from Asuncion, Paraguay, based on two fragments without either of the extremities. The stage of development was not commented upon, but its colour pattern was described as a median dark line and two grey bands, one at each side, on a yellowish background; both margins of grey bands, the external and the internal, the latter twice as wide as the first, were darker. Judging from Fig. 17 (Taf. VII), they were also rather clearly delimited. Continuing, GRAFF (1899) commented that if the grey between them were fainter one would be able to consider the worm as being five striped, although to him the fragments "sind so charakteristisch gezeichnet, das es nicht schwer sein wird, danach die Art wiederzuerkennen" (show a very peculiar pattern, so that it will be not difficult to recognize the species). MARCUS (1951), however, suggested that *G. bohlsi* was probably a synonym of *G. marginata* sensu Graff. Nevertheless, additionally to the peculiarities of its colour pattern, the provenance of *G. bohlsi* does not seem to be consistent with the distribution of *N. graffi* or that of any of the five striped species discussed herein, taking into account the high degree of endemism generally presented by land planarians. So the assignment of the species by OGREN & KAWAKATSU (1990) to their Geoplaninae genus *Pseudogeoplana*, "collective group to temporarily assign species inquirendae and nomina dubia" (l.c., p. 90), seems a more adequate procedure.

Internal anatomy

Due to its female atrium being almost completely filled by a multilayered lining epithelium, *N. guaiana* once more stands apart from the striped species of group 2 of *Notogynaphallia* (FROEHLICH & LEAL-ZANCHET, 2003).

The seven species with a striped dorsum, regarding anatomy, can be assembled as follows : *N. graffi*, *N. caissara* and *N. fita* on the one side, *N. ernesti* and *N. muelleri* on the other, while *N. abundans* and *N. ceciliae* combine, each in their own way, characters similar to those of both groups.

N. graffi, *N. caissara* and *N. fita* have in common a very long prostatic vesicle with a comparatively narrow cavity; efferent ducts entering laterally into the prostatic vesicle. The male atrium is not very long, approximately of the same size as the female one, with folded walls, and a large penial papilla-like fold around the opening of the ejaculatory duct.

Regarding *N. graffi,* there are some important differences between Graff´s description and drawing of the copulatory apparatus, and that of the worms herein studied. GRAFF (1899) described and drew the vesicle as an elongate structure with an ample cavity and thin, richly folded walls. He considered it originated as a fusion of the efferent ducts, and so, as an unpaired false seminal vesicle (1899, p. 165). In seven specimens, studied herein, the wall – epithelial lining plus muscularis – of the vesicle, although not very thick, is much thicker than those of the efferent ducts, something never observed in false vesicles of any other species. The vesicle in the present specimens is undoubtedly a prostatic vesicle receiving plenty of erythrophil glands. Besides in none of our specimens is it a through continuation of efferent ducts as in Graff's drawing, but, instead, as above-stated, receives each of them, on either side. Still regarding the vesicle, in present specimens it is not "faltenreiche" (very folded) as in Graff's one, but with rather smooth walls. It is important to remark here that the poor histological conditions of Graff's material, as commented by him, could explain, at least in part, the important differences discussed above.

With regard to other characteristics of the copulatory apparatus, they are in agreement with those described by GRAFF (1899). It seems that there are only, in some points, divergences in the interpretation of certain structures. Thus the penial papilla described by Graff is here considered an ental ring-like fold of the male atrial wall, around the opening of the ejaculatory duct. The latter is ciliated while, like the rest of the atrial wall, the narrow cavity delimited by the fold is not. So it is not an extension of the duct as Graff considered albeit having observed the histological differences.

N. caissara, one of the species mistakenly identified as *G. marginata* Shultze & Müller (LEAL-ZANCHET & FROELICH, 2001), was described as possessing a penial papilla, thus placing the species in an ambiguous position inside *Notogynaphallia*. After the original description, some other specimens from one of the original localities were sectioned, the entire material of this species being restudied for this paper. This leads to the conclusion that the "papila penial" is actually a projection involving a variable extension of the proximal atrial wall, with irregular invaginations on its surface, in one of which the ejaculatory duct empties. As a consequence, any possible doubt about the assignment of the species to *Notogynaphallia* is removed. Further, *N. caissara* belongs to the species complex herein discussed, and is the sole species of the complex not yet found southwards from S. Paulo state. The main differences between *N. caissara* and *N. graffi* concern the form and extension of the prostatic vesicle, which in the former is very long, extending anteriorly up to the posterior extremity of the pharyngeal pocket, where it forks and continues, even arriving in some of the worms, probably depending on the grade of maturity, at the level of the ventral insertion of the pharynx. Its walls are so intricately folded, mainly in more mature specimens, that, in most of the longitudinal sections, it appears as a large agglomerate of cross-sectioned canals. The vesicle of *N. graffi* is long, and surpasses slightly the end of pharyngeal pocket, but it is not forked, and its walls are smooth.

N. fita, although the most divergent of the three species with regard to the external morphology, presents a very similar copulatory apparatus. The principal difference is the localization of the ejaculatory entrance into the distal third of the prostatic vesicle, near the penis bulb, and not into the median third, rather far from the bulb, as in *N. graffi* and *N. caissara*. The prostatic vesicle is also tubular and very long, but, does not reach the pharyngeal pouch; besides it is not forked and its walls are smooth. Another outstanding difference between the three species is that the pharynx is cylindrical in *N. graffi* and *N. caissara* but bell-shaped in *N. fita.*

In the second assemblage of species, the prostatic vesicle is a voluminous and compact organ, without branches or diverticula, and much shorter than in the species of first group. Its muscle coat is rather thick and dense, being heavily traversed by the neck of gland cells. The efferent ducts, after turning dorsoanteriorly, penetrate it not laterally into the median or ectal portion but into the ental portion, terminal or subterminally. The male atrium is very long; at least twice as long as the very short female atrium, and frequently more.

In most specimens of *N. ernesti* the opening of efferent ducts into the vesicle is subterminal, located ventrally (specimens from states of São Paulo and Paraná) or dorsally (specimens from São Francisco de Paula). In *N. muelleri* the entrance is terminal in the characteristically upturned proximal portion of the prostatic vesicle. In mature worms of both species the male atrium is comparatively more intensively and deeply pleated in *N. ernesti* than in *N. muelleri*. The walls of the gonopore canal are smooth in all specimens of *N. ernesti,* but deeply pleated in the three sectioned specimens of *N. muelleri*. The pharynx of *N. muelleri* is bell-shaped with pleated margins, and the dorsal insertion is situated on the same transversal level as the mouth. That of *N. ernesti*, here described as bell-shaped, was described by Marcus as of the cylindrical type, approaching the bell type. He depicted its dorsal insertion slightly anterior to the mouth, as in most specimens herein studied.

With such a similar internal anatomy, a fact already remarked by FROEHLICH (1959), who described the anatomy of *N. muelleri* for the first time, the separation of these two species relies heavely on the externally observable differences, mainly colour pattern, and behaviour. FROEHLICH (1959) described *N. muelleri* as "a lively species, with quick reactions when stimulated", and the second author remarked in her field notes, together with drawings of living worms, that when touched these worms quickly shorten. A similar behaviour was never observed in *N. ernesti* by both authors in worms from Rio Grande do Sul, and neither from worms from São Paulo.

MARCUS (1951) sectioned and studied two specimens of what he considered conspecific with Graff´s *G. marginata*, one of which with a ring-like fold of the ental wall of

the male atrium around the opening of the ejaculatory duct, similar to that described herein for the specimen from Valinhos. He described the whole copulatory apparatus as in a stage of evaginate penial papilla. Thus, he considered the fold a transitory structure formed by the eversion of the ental atrial wall for transfering spermatozoa during copulation. A similar fold was not found in any of the three sectioned worms of *N. muelleri*, which does not mean, of course, that its copulatory apparatus does not work in the same way on the proper occasion. However, in one of the worms, there is a cluster of spermatozoa plus eosinophil secretion fixed to the ventral wall of the male atrium, near the gonopore. The structure was well described by FROEHLICH (1959), who considered it a spermatophore. A similar structure has been also described for *Notogynaphallia sexstriata* (FROEHLICH, 1956), for *Amaga righii* (FROEHLICH & FROEHLICH, 1972) and for *Choeradoplana iheringi* Graff, 1899, in which a similar cluster of spermatozoa was also seen free in the atrial cavity (LEAL-ZANCHET & SOUZA, 2003; SOUZA & LEAL-ZANCHET, 2004). The two last mentioned species are also devoid of a penial papilla and have a long pleated male atrium. Another interesting point worthy of note is that in all these species the cluster of spermatozoa is fixed on the ventral wall of the atrium, near the gonopore. These facts appear to indicate that in *N. muelleri*, as well as in these other three species, the transference of spermatozoa would be accomplished through the eversion of the distal wall instead of the proximal wall of the male atrium.

In the discussion of his *G. marginata*, MARCUS (1951) commented that the drawings of the genital apparatus presented by him were concordant with those of RIESTER (1938) and GRAFF (1899) only with respect to general topography, as an analysis of the details unveiled inexplicable incongruities. In spite of this, he concluded the conspecificity of the three species. E.M. FROEHLICH (1955) separated Riester's and Marcus' species; the separation of Graff's and Marcus' species is accomplished herein.

The principal differences between *N. ernesti* and *N. graffi* appear immediately by the herein assignment of them to distinct well-characterised groups. Some of the main similarities between both species, as pointed out by MARCUS (1951), do not prevail after the present study of several new specimens of Graff's species. In this manner, the penial papilla in GRAFF's figure (1899, Text-fig. 36) was interpreted by MARCUS (1951) as also being a transitory structure as that in his own material, and so, contrary to what was concluded in the present work. Although not being a true penial papilla, the fold around the opening of the ejaculatory duct of *N. graffi* is present in all studied specimens. Another similarity according to MARCUS (1951), the entrance of the efferent ducts, localized by GRAFF (1899) on the proximal end of the prostatic vesicle, is also in disagreement with what was shown herein. RIESTER (1938), who studied Graff's slides and commented on its poor histological conditions, had already emended Graff's description regarding this point.

In *N. abundans* the prostatic vesicle is similar to that of *N. graffi* : a long branchless tube in most specimens, with a well-developed muscularis, and receiving the efferent ducts approximately in the same position. However, differently from that of *N. graffi,* it is laterally sinuous and extends anteriorly beyond the posterior end of the pharyngeal pouch in all studied specimens. The male atrium, in turn similar to that of *N. ernesti* and *N. muelleri*, is very long, at least twice as long as the female one. Again, resembling species of the first assemblage, there is a large fold in the proximal wall of the male atrium, delimiting a small cavity, where the ejaculatory duct opens, from the rest of the atrium. In *N. abundans,* this fold is variously shaped in different specimens and occupies a comparatively minor portion of the atrial cavity. The pharynx of *N. abundans* is bell-shaped as in *N. fita* and in both species of the second assemblage.

In *N. ceciliae* the prostatic vesicle is a long tube as that of species of the first assemblage and that of *N. abundans*. It approaches that of *N. caissara,* being anteriorly forked and extended beyond the end of the pharyngeal pouch, although considerably less so than in *N. caissara*. That of *N. abundans* also shortly surpasses the end of the pharyngeal pouch but is not forked in most specimens. In *N. graffi* and *N. fita* the vesicle stands at a distance from the pharyngeal pouch and is not forked. Concerning the atrial cavities, *N. ceciliae* very much resembles species of the second assemblage; the atria have a similar general structure, besides similar size and relative proportions. The pharynx is bell-shaped as that of *N. fita*, *N. abundans, N. ernesti* and *N. muelleri*, the dorsal insertion lying in the median third of the pouch, on the same transversal level as the mouth. Nevertheless, *N. ceciliae* is distinct from the other five striped species, as well as from the remaining species of *Notogynaphallia* gathered in group 2 of FROEHLICH & LEAL-ZANCHET (2003), by its distally branched efferent ducts, each branch opening separately into the vesicle, a very rare trait in the Terricola.

To conclude, what GRAFF (1899), RIESTER (1938) and MARCUS (1951) considered as *G. marginata* SCHULTZE & MÜLLER, 1857, currently, after E.M. FROEHLICH (1955), and the present study, corresponds, to three species : respectively *N. graffi,* herein described; *N. caissara* (FROEHLICH, 1955); and *N. ernesti*, herein described. Further, these three species plus *N. abundans*, the anatomy of which was herein described for the first time, and *N. ceciliae* FROEHLICH & LEAL-ZANCHET, 2003, constitutes a complex of species with the characteristics presented in the introduction.

Geoplana marginata Schultze & Müller was transferred by OGREN et al. (1992) to *Pseudogeoplana,* as its anatomy remains unknown.

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Annotated checklist of the umagillid turbellarians infesting echinoids (Echinodermata)

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ABSTRACT. A literature survey of the taxonomically complex taxon *Syndesmis* Silliman, 1881 is presented, resulting in the recognition of 22 valid species and three species mentioned in several works but not yet described. For each of these species the host(s) are mentioned and the distribution is given. These results are compared with earlier checklists and the differences are discussed.

KEY WORDS : Biogeography, *Marcusella*, symbiosis, *Syndesmis*, *Syndisyrinx*, Turbellaria.

INTRODUCTION

The family Umagillidae Wahl, 1910 (Platyhelminthes, Rhabdocoela) is the largest taxon of symbiotic turbellarians. It includes 68 valid species (WESTERVELT, 1981; CANNON, 1982, 1987; SHINN, 1987; CANNON, 1990; HERTEL et al., 1990; JANGOUX, 1990; KOZLOFF & WESTERVELT, 1990; WESTERVELT & KOZLOFF, 1990, 1992; MOENS et al., 1994; JONDELIUS, 1996; KOZLOFF, 1997; VASS & NAPPI, 1998). Most umagillids are common endosymbionts of echinoderms, particularly echinoids and holothuroids, and a few occur in sipunculids (see CANNON, 1982; JANGOUX, 1990). Our knowledge of the biology of umagillids is, however, very scant. For instance, although they are frequently found in the digestive tract or coelomic cavity of their hosts, almost nothing is known about the nature of their symbiotic relationships. The life cycle itself is completely elucidated for only four species : *Anoplodium hymanae* Shinn, 1983 and *Wahlia pulchella* Kozloff & Shinn, 1987 (both infesting holothuroids) (SHINN, 1983a, 1985a, b, 1986a), *Fallacohospes inchoatus* Kozloff, 1965 (infesting crinoids) (SHINN, 1986b) and *Syndesmis franciscana* (Lehman, 1946) (infesting echinoids) (SHINN, 1980, 1981, 1983b). Most umagillid species are host-specific, though a few apparently infest several, often phylogenetically unrelated, echinoderm species (see CANNON, 1982; SHINN, 1984; JANGOUX, 1990).

Research on the biology of these worms is greatly hampered by the complex taxonomy of the group, which often leads to wrong assumptions. For instance, for almost sixty years all umagillids found in echinoids were attributed to *Syndesmis echinorum* François, 1886 (see VON GRAFF, 1903). Although already very early on, it was suggested that more species existed (see BRAUN, 1889), it was not until recently that *S. echinorum* was split into six valid species (see KOZLOFF & WESTERVELT, 1987, 1990; WESTERVELT & KOZLOFF, 1990, 1992; KOZLOFF, 1997). After the description of the second recognized species of *Syndesmis*, *S. franciscana* (Lehman, 1946), many more species were described and some redescribed. Yet, authors often did not agree on the taxonomic importance of many characters. For instance, the validity of the genera *Syndisyrinx* Lehman, 1946 and *Marcusella* Westblad, 1953 has been the subject of many impassioned debates (LEHMAN, 1946; MARCUS, 1949; STUNKARD & CORLISS, 1951; WESTBLAD, 1953; HYMAN, 1960; CANNON, 1982, 1987; KOZLOFF & WESTERVELT, 1987, 1990; HERTEL et al., 1990; WESTERVELT & KOZLOFF, 1990, 1992). In the most recent literature, all umagillid species infesting echinoids were brought together into the genus *Syndesmis* Silliman, 1881 (see CANNON, 1982; MOENS et al., 1994; GEVAERTS et al., 1995; JONDELIUS, 1996).

Taxonomic literature regarding the species of *Syndesmis* has become extremely entangling, and is almost inaccessible for novice and even experienced researchers who are not particularly interested in taxonomy. As a result, several attempts were made to review the extensive literature on these animals : STUNKARD & CORLISS (1951), HICKMAN (1956), JENNINGS (1971), BAREL & KRAMERS (1977), CANNON (1982), HERTEL et al. (1990), JANGOUX (1990), VASS & NAPPI (1998). Yet, in recent years, several works (KOZLOFF & WESTERVELT, 1987, 1990; HERTEL et al., 1990; WESTERVELT & KOZLOFF, 1990, 1992; KOZLOFF, 1997) showed that many specimens previously attributed to a given species, in fact belong to several distinct species and, consequently, some species are less opportunistic than previously thought, thereby making most earlier checklists outdated. Moreover, because of confusing synonymies, discrepancies in the spelling of species names, and because of an incomplete literature survey or bad translations of non-English literature, these reviews contain mistakes and contradictory information.

The new checklist we present here is intended to give an overview of all umagillid species infesting echinoids, based on a complete literature survey. As the names of

many species changed through time, because these were synonymized, misidentified or even misspelled, it is quite difficult to understand the literature in a way other than chronologically. Only a detailed reappraisal of all descriptions allows to attribute the most recently accepted name to species misidentified in older works, for example the "*S. echinorum*" of BAREL & KRAMERS (1970) that was later described as *S. albida* Kozloff & Westervelt, 1990 and *S. rubida* Kozloff & Westervelt, 1990. In this work, we follow the most modern views, e.g. the one that considers *Syndisyrinx* a synonym of *Syndesmis* (MOENS et al., 1994; GEVAERTS et al., 1995; JONDELIUS, 1996). Similarly, we use the most recently published taxonomy of the echinoids (SMITH, 2003).

RESULTS AND DISCUSSION

To date, the 22 valid and three undescribed species of *Syndesmis* are known to infest a total of 31 species of echinoids belonging to the orders Clypeasteroida and Spatangoida (irregular sea urchins), Diadematoida, Echinoida and Temnopleuroida (regular sea urchins) (Table 1). The taxon *Syndesmis* occurs in European waters (from the Mediterranean Sea to the Barents Sea), the Caribbean, the entire Pacific Ocean and the Indian Ocean. Some echinoid species (e.g., *Echinometra oblonga* (de Blainville, 1825)) may host up to three umagillid species and some species of *Syndesmis* are found in various (up to five) echinoid hosts. Interestingly, some species are found in hosts belonging to distinct families or even orders. For instance, *S. echinorum* is found in *Paracentrotus lividus* (Lamarck, 1816) (Echinidae) as well as *Sphaerechinus granularis* (Lamarck, 1816) (Toxopneustidae).

The superscript numbers following the species names in our checklist (Table 1) refer to major discrepancies with previous checklists, the details of which are presented here :

(1) : Based on a literature survey, JANGOUX (1990) mentioned the occurrence of *S. echinorum* in *Psammechinus microtuberculatus* (de Blainville, 1825). However, only VON GRAFF (1903) has studied this species of sea urchin (from Trieste, Italy) and he reported that all 30 specimens he inspected were uninfested. Apart from the records presented in Table 1, MEIXNER (1926) mentioned the occurrence of *S. echinorum* in sea urchins from Lessin Island (Croatia), but without specifying the host.

(2) : VON GRAFF (1903) mentioned the occurrence of *Syndesmis echinorum* in *Strongylocentrotus droebachiensis* (Müller, 1776). This is impossible to verify owing to the lack of material. However, the fact that this species of echinoids is known to host two other morphologically similar and well documented species of *Syndesmis*, *S. franciscana* and *S. inconspicua* Westervelt & Kozloff, 1992 (see SHINN, 1984; WESTERVELT & KOZLOFF, 1992), suggests that the species VON GRAFF (1903) observed was probably not *S. echinorum*. VON GRAFF (1903) also reported *S. echinorum* in *Sphaerechinus granularis* from Bergen (Norway), but as this species of sea urchins is limited from the Mediterranean to the south of the English Channel, this observation is clearly erroneous. He probably misidentified the echinoid species, which could be *Echinus esculentus* Linnaeus, 1758, which harbours two distinct umagillid species (*S. rubida* and *S. albida*, see remarks 7 and 8).

(3) : Originally, *Syndesmis franciscana* was described as *Syndisyrinx franciscanus* (LEHMAN, 1946; SHINN, 1980), but was also mentioned as *Syndesmis franciscanus* (GIESE, 1958) and *Syndesmus franciscus* (BERGER & PROFANT, 1961). We regard *Syndesmis franciscana* as the correct name of this species, the genus name being feminine.

(4) : *Syndesmis antillarum* Stunkard & Corliss, 1951 was sometimes confused with *S. franciscana* (WESTBLAD, 1953; BARNES, 1969). Before its description, the species was also mentioned as *Syndesmus* sp. (POWERS, 1935). See also remark 9 about *S. collongistyla* Hertel et al., 1990.

(5) : *Syndesmis dendrastrorum* Stunkard & Corliss, 1951, *S. atriovillosa* Westblad, 1953 and *S. pallida* Hickman, 1956 were previously attributed to *Marcusella* (WESTBLAD, 1953; HICKMAN, 1956), a genus synonymized with *Syndesmis* by CANNON (1982). The first species is reported as *S. dendrastomum* in JANGOUX (1990).

(6) : *Syndesmis compacta* Komschlies & Vande Vusse, 1980, *S. mammilata* Komschlies & Vande Vusse, 1980, *S. philippinensis* Komschlies & Vande Vusse, 1980 and *S. alcalai* Komschlies & Vande Vusse, 1980 are treated here as valid despite of their incomplete species descriptions. The second species is reported as *S. mammillata* in JANGOUX (1990).

(7) : For a long time, *Syndesmis albida* and *S. rubida* were confused with *Syndesmis echinorum* (BAREL & KRAMERS, 1970, 1977), also reported as *Syndesmus echinorum* (SHIPLEY, 1901). It is impossible to know if WESTBLAD (1926), BRUCE et al. (1963) and LYONS (1973) observed *S. rubida* or *S. albida*. See also remark 8.

[a] This list updates previous reviews and reattributes outdated taxonomic names used in original works to the valid species. Superscript numbers following species names refer to notes in Results and Discussion.

Umagillid species : Some species have been described as *Syndisyrinx* or *Marcusella* by various authors but, without complete reappraisal of the group, validity of these genera is still matter of discussion. All species are thus attributed to the genus *Syndesmis*. Species are presented in the chronological order of their description.

Sea urchin host : Species in bold are irregular sea urchins. Synonyms : *Echinus esculentus* is also known in the literature on umagillids as *Echinus sphaera*; *Paracentrotus lividus* as *Toxopneustes lividus* or *Strongylocentrotus lividus*; *Strongylocentrotus pallidus* as *S. echinoides*. Locality : BS Barents Sea; C Caribbeans; EA East Atlantic; EP East Pacific; IO Indian Ocean; M : Mediterranean Sea; WP West Pacific.

References : "/" separates the authors having studied the species in the different localities mentioned. "?" is used when the species mentioned by the author is not clearly identified but probably belongs to the one named in this list. Authors in bold are the authority to which to refer for the original description of the species.

TABLE 1

List of the umagillids infesting sea urchins, with details about host species infested, localities and references.[a]

Umagillid species	Sea urchin host	Locality	References
Syndesmis echinorum[1]	*Paracentrotus lividus*	M : Banyuls (France) / Marseille (France) / EA : Roscoff (France) / Galicia (Spain)	**François, 1886**; Kozloff & Westervelt, 1987; Pers. obs. / Briot, 1906 / Cherbonnier, 1951; Barel & Kramers, 1970?; Pers. obs. / Lamas Seco & Rodriguez Babio, 1978; Cifrian et al., 1992; Pers. obs.
	Sphaerechinus granularis	M : Naples (Italy) / Trieste (Italy), Umag (Croatia) / Marseille (France) / Banyuls (France)	Russo, 1895; Wahl, 1909 / von Graff, 1903 / Briot, 1906 / Kozloff & Westervelt, 1987; Pers. obs.
	Strongylocentrotus droebachiensis?[2]	BS : Alexandrowsk (Russia)	von Graff, 1903
	Psammechinus miliaris	EA : Galicia (Spain)	Lamas Seco & Rodriguez Babio, 1978
Syndesmis franciscana[3]	*Strongylocentrotus franciscanus*	EP : California	**Lehman, 1946**; Giese, 1958; Jennings & Mettrick, 1968; Barnes, 1969; Mettrick & Jennings, 1969; Hertel et al., 1990
	Strongylocentrotus pallidus	EP : Washington State	Orihel, 1952; Shinn, 1980?, 1981, 1983b, 1984; Shinn & Cloney, 1986
	Strongylocentrotus droebachiensis	EP : Washington State	Shinn, 1980?, 1981, 1983b, 1984; Shinn & Cloney, 1986; Westervelt & Kozloff, 1992; Shinn, 1993
	Strongylocentrotus purpuratus	EP : California	Lehman, 1946?; Giese, 1958; Barnes, 1969; Mettrick & Jennings, 1969; Mettrick & Boddington, 1972; Holt & Mettrick, 1975; Hertel **et al.**, 1990
	Lytechinus anamesus	EP : California	Barnes, 1969; Hertel **et al.**, 1990
Syndesmis antillarum[4]	*Diadema antillarum*	C : Dry Tortugas, Florida / Bermuda / Unknown	Powers, 1935; **Stunkard & Corliss, 1951** / Snyder, 1980 / Westblad, 1953
Syndesmis dendrastrorum[5]	***Dendraster excentricus***	EP : California / Washington State	**Stunkard & Corliss**, 1950, **1951**; Smith, 1973 / Orihel, 1952; Shinn, 1981, 1984, 1988
Syndesmis atriovillosa[5]	***Spatangus purpureus***	EA : Plymouth (UK)	**Westblad, 1953**
Syndesmis punicea	*Heliocidaris erythrogramma*	WP : Tasmania / Sydney	**Hickman, 1956** / McRae, 1959?; Rohde & Watson, 1988
	Amblypneustes ovum	WP : Tasmania	Hickman, 1956
Syndesmis pallida[5]	***Echinocardium cordatum***	WP : Tasmania	**Hickman, 1956**
Syndesmis glandulosa	*Diadema setosum*	IO : Madagascar / WP : Philippines	**Hyman, 1960** / Komschlies & Vande Vusse, 1980a
	Echinothrix calamaris	WP : Philippines / IO : Seychelles	Komschlies & Vande Vusse, 1980a / Martens & De Clerck, 1994
Syndesmis evelinae	Unknown, *Echinometra lucunter*	C : Saint-Barthélémy	**Marcus, 1968**; Hertel **et al.**, 1990; Hertel & Duszynski, 1991
Syndesmis compacta[6]	*Echinometra oblonga*	WP : Philippines	**Komschlies & Vande Vusse, 1980a**
Syndesmis mammilata[6]	*Echinometra oblonga*	WP : Philippines	**Komschlies & Vande Vusse, 1980b**
Syndesmis philippinensis[6]	*Echinometra oblonga*	WP : Philippines	**Komschlies & Vande Vusse, 1980b**
Syndesmis alcalai[6]	*Heterocentrotus mammillatus*	WP : Philippines	**Komschlies & Vande Vusse, 1980b**
Syndesmis aethopharynx	*Paracentrotus lividus*	M : Banyuls (France)	**Westervelt & Kozloff, 1990**; Pers. obs.
Syndesmis albida[7]	*Echinus esculentus*	EA : Plymouth (UK), Roscoff (France)	Barel & Kramers, 1970; **Kozloff & Westervelt, 1990**
Syndesmis rubida[7, 8]	*Echinus esculentus*	EA : Plymouth (UK), Roscoff (France)	Geddes, 1880?; Silliman, 1881?; Cuénot, 1892?, 1900? (Roscoff); Shipley, 1901? (Plymouth); Barel & Kramers, 1970; **Kozloff & Westervelt, 1990**
Syndesmis collongistyla[9]	*Echinometra lucunter*	C : Saint-Barthélémy, Jamaica	**Hertel et al., 1990**
	Echinometra viridis	C : Jamaica	Nappi & Crawford, 1984; Hertel et al., 1990
	Lytechinus variegatus	C : Jamaica / Puerto Rico / Haiti	Jennings & Mettrick, 1968; Mettrick & Jennings, 1969; Nappi & Crawford, 1984; Allison et al., 1987; Ubelaker et al., 1988; Hertel et al., 1990; Vass & Nappi, 1998 / Hyman, 1960; Jones & Canton, 1970 / Jones & Canton, 1970
	Lytechinus williamsi	C : Jamaica	Hertel **et al.**, 1990
	Tripneustes ventricosus	C : Puerto Rico	Jones & Canton, 1970
Syndesmis inconspicua[10]	*Strongylocentrotus droebachiensis*	EP : Washington State	Orihel, 1952?; Shinn, 1981?, 1983b?, 1984?; **Westervelt & Kozloff, 1992**
	Strongylocentrotus pallidus?	EP : Washington State	Shinn, 1981?, 1983b?, 1984?
Syndesmis neglecta[11]	*Allocentrotus fragilis*	EP : Washington State / California	Berger & Profant, 1961?; **Westervelt & Kozloff, 1992** / Giese, 1958; Boolootian et al., 1959?; Hyman, 1960
Syndesmis longicanalis	*Tripneustes gratilla*	IO : Kenya	Moens & Martens, 1992; Gevaerts et al., 1993; **Moens et al., 1994**; Gevaerts et al., 1995
	Toxopneustes pileolus	IO : Kenya	Moens & Martens, 1992; Gevaerts et al., 1993; Moens et al., 1994; Gevaerts et al., 1995
Syndesmis cannoni	***Ammotrophus arachnoides***	IO : Western Australia	**Jondelius, 1996**
Syndesmis echiniacuti[12]	*Echinus acutus*	M : Banyuls, Port-Vendres (France)	François, 1886; **Kozloff, 1997**
Syndesmis sp 1	*Evechinus chloroticus*	WP : New Zealand	McRae, 1959
Syndesmis sp 2[13]	*Strongylocentrotus franciscanus*	EP : California	Lehman, 1946; Barnes, 1969; Mettrick & Jennings, 1969
	Strongylocentrotus purpuratus	EP : California	Barnes, 1969; Mettrick & Jennings, 1969
	Lytechinus anamesus	EP : California	Barnes, 1969; Mettrick & Jennings, 1969
Syndesmis sp 3	*Diadema savigny*	IO : Kenya	Martens & De Clerck, 1994

(8) : *Syndesmis rubida* can be distinguished from *S. albida* by the colour : the former is brown red while the latter is pale pink to white (KOZLOFF & WESTERVELT, 1990). According to their descriptions, GEDDES (1880), SILLIMAN (1881), CUÉNOT (1892, 1900) and SHIPLEY (1901) likely observed *S. rubida*.

(9) : Prior to its description, *Syndesmis collongistyla* was confused with *S. franciscana* and *S. antillarum* (JENNINGS & METTRICK, 1968 ; JONES & CANTON, 1970 ; NAPPI & CRAWFORD, 1984 ; ALLISON et al., 1987).

(10) : *Syndesmis inconspicua* was mentioned as *S. "echinorum"* in older literature (ORIHEL, 1952 ; SHINN, 1981, 1983b, 1984). This species was reported in *Strongylocentrotus droebachiensis* and *S. pallidus* (Sars, 1871) but not described until WESTERVELT & KOZLOFF (1992). These authors investigated specimens from *S. droebachiensis* only.

(11) : *Syndesmis neglecta* Westervelt & Kozloff, 1992 was previously listed as *Syndesmis franciscanus* (see GIESE, 1958), *Syndesmus franciscanus* (BOOLOOTIAN et al., 1959), *Syndesmis franciscana* (HYMAN, 1960) and *Syndesmus franciscus* (BERGER & PROFANT, 1961).

(12) : Specimens of *Syndesmis echiniacuti* Kozloff, 1997 were perhaps also used by FRANÇOIS (1886) in the original description of *Syndesmis echinorum* (see KOZLOFF & WESTERVELT, 1987 and KOZLOFF, 1997).

(13) : *Syndesmis* sp. 2 could be *S. inconspicua*. BARNES (1969) stated its close similarity with *S. echinorum*.

We hope this survey will be a useful tool for further research on this ecologically important group. Recent investigations have demonstrated that literature accounts of intraspecific variation in *Syndesmis* species found in distinct hosts or from distant locations should be treated sceptically, as some of these cases may in fact be instances of interspecific variation.

Re-evaluation of the current species, and the description of new species should be based on a thorough morphological study of the taxon as a whole (including the re-examination of all material deposited in collections), combined with molecular data. Unfortunately, confusions and misidentifications have persisted for years because some works were based on material of poor quality and sometimes lacked the study of histological sections. In some cases, researchers described a species, but failed to deposit type material in a collection. As a consequence, comparison between species can be very difficult, if not impossible, unless new material is collected. Yet, with the updated checklist presented here, future researchers will have immediate access to literature and a complete overview of *Syndesmis* species and their corresponding host(s) and geographical distribution.

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Relative efficiency of three types of small mammal traps in an African rainforest

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ABSTRACT. Numerous studies have compared the efficiency of different types of traps for the survey of small mammals in temperate countries, but such comparative studies are strikingly fewer in tropical habitats. We compared the efficiency of three types of traps for sampling small mammals in an African rainforest : one type of interception trap (pitfall traps with drift fences) and two types of baited traps (Sherman live trap and metal snap trap). We captured 1884 individuals belonging to 9 shrew and 11 murid rodent species. Pitfall traps were more efficient (higher number of species trapped and higher trap success) than baited traps for capturing shrews. In contrast, they were less efficient for capturing rodents, even if some rare species were captured in pitfall traps only. Sherman and snap traps have complementary qualities for the capture of rodents. Sherman traps are more effective for trapping smaller rodent species, while snap traps tend to be more effective for trapping larger ones. Moreover, light individuals of the largest species are better captured than heavy ones in Sherman, while no significant difference was observed for small species. No sex ratio difference was detected between populations sampled by each of the three types of traps. The three types of traps used in this study have complementary effects onto the capture of small mammals in African rainforest. An assortment of traps should always be employed in studies of small mammal communities in African rainforest in order to obtain a wider range of taxa, and thus a better representation of the community.

KEY WORDS : Sherman; snap trap; pitfall; rodent; shrew

INTRODUCTION

Most studies of small mammal communities rely on sampling methods involving trapping. The quality of the data collected is thus dependent on the efficiency of the trapping techniques employed. Traps can be categorized into those catching small mammals randomly (interception or passive traps such as pitfall traps) and those attracting and eliciting orientation behaviour (active traps such as baited traps; SOUTHWOOD, 1978). Additionally, two main types of baited traps can be used to capture small mammals : live traps (such as Sherman traps) and snap traps.

In temperate countries, numerous studies compared the success of different trapping techniques for the survey of small mammals, and adequate techniques for sampling each group have been established (e.g. COKRUM, 1947; SEALANDER & JAMES, 1958; BEER, 1964; PATRIC, 1970; WIENER & SMITH, 1972; DALBY & STRANEY, 1976; ROSE et al., 1977). In contrast, such comparisons are fewer in tropical habitats (LAURANCE, 1992; WOODMAN et al., 1996; LYRA-JORGE & PIVELLO, 2001), particularly for the African rainforest ecosystem. Recent awareness of the rapid disappearance of tropical rainforests has increased interest in understanding and conserving biodiversity (WILSON, 1988, 1992). To this aim, defining appropriate trapping techniques is essential.

The aim of this study was to compare the efficiency of three types of traps for sampling small mammals (murid rodents and shrews) in an African rainforest : one type of interception trap (pitfall traps with drift fences) and two types of baited traps (Sherman live trap and metal snap trap).

Study site

Our study was conducted in the eastern part of the "Aire d'Exploitation Rationnelle de Faune (AERF) des Monts Doudou", south-western Gabon (02°09'S, 10°30'E), in undisturbed lowland forest (110 m A.S.L.). The forest canopy, which is approximately 35-45 m high, is dominated by Caesalpiniaceae and Mimosaceae. The understorey is open and *Dichostemma glaucescens, Meiocarpidium lepidotum* and a large variety of *Diospyros* species are frequently encountered (SOSSEF et al., 2004). The average annual rainfall recorded near this area is about 2300 mm (SAINT-AUBIN, 1963), with a short rainy season from March to May and a long one from October to December. The long dry season occurs from June to September and the short one in January-February. Temperature is more or less uniform along the year, with mean monthly minima between 19° and 23°, and maxima between 24° and 29°C.

MATERIAL AND METHODS

Trapping methods

Small mammals were trapped monthly, from April 2000 to March 2001, in two 875 x 1000 m study sites, standing 2500 m apart, which were localised at the same altitude and subject to similar climatic conditions. Each month, the trapping schedule was as follows :

(i) three traplines (each 1 km long) of 200 traps baited with manioc. Each trapline contained 100 Sherman live traps (7.5 X 9 X 23 cm) and 100 metal snap traps (10 X 15 cm) spaced at 5 m interval (one Sherman and one snap trap alternatively). Traplines were set 25 m apart;

(ii) one pitfall line with drift fences (150 m long) comprising 30 10-litre plastic buckets (26 cm deep, 26 cm top internal diameter, 20 cm bottom internal diameter) placed at 5 m interval (NICOLAS et al., 2003). Small holes (3-5 mm) were burned through the bottom of the buckets to allow water drainage in case of rain and thus prevent the drowning of trapped animals until the bucket was checked. Each month, the distance between the pitfall line and traplines was at least of 300 m. All traps (Sherman, snap traps and pitfall traps) were set for seven days and checked daily.

Due to the existence of sibling species, all animals captured had to be euthanized by cervical dislocation for species identification. As all animals were therefore removed from the study area, traplines were moved in space from one month to another, at a distance of 25 m from neighbouring traplines and 300 m for neighbouring pitfall lines.

Sex and weight of all the animals captured were checked and measured and muscle-tissue samples taken from killed animals were placed in surgical alcohol for molecular analysis. Skulls were extracted and cleaned for use in species determination, and bodies were preserved in 10% formalin.

Species identification

Species identification was based on external morphology and cranio-dental characteristics, and confirmed for several specimens by molecular analysis (16S rRNA sequencing). Identification was based on the most recent advances in the knowledge of shrew and rodent taxonomy, but several complexes of species are still in need of revision (NICOLAS, 2003; QUÉROUIL et al., 2006). Thus, in the text and tables, the designation "cf." (as in *Praomys* cf. *petteri*) suggests that specimens best fit within the morphological variation described for the named species, but could, under further examination, represent a different species.

Methods of analysis

At each study site, 87% of the land surface was covered by mainland forest and 13% was covered by flooded forest. Because the community structure and proportion of traps set in each habitat varied between these habitats (NICOLAS, 2003), our results take into account mainland forest only.

A trap in use for a 24-hour period is referred to as a trap-night (TN, dawn to dawn). Trap success (T) is defined as the number of individuals caught per 100 TN, i.e. $T = (N_m/N_{tn})x100$, where N_m is the number of individuals trapped and N_{tn} the number of trap-nights.

For each type of trap (pitfall, Sherman and snap trap) we compared the number and identity of species caught, and their trap success. Chi-square tests were used for trap success comparisons and a probability of $P \leq 0.05$ was considered significant. We also tested if body weight or sex of individuals had an effect on their capture by different types of traps. Chi-square tests were used for sex ratio comparisons between trap types and Mann-Whitney tests were used for body weight comparisons.

RESULTS

We captured a total amount of 1884 individuals belonging to nine shrew and 11 murid rodent species (Table 1).

Number of species and species composition

More shrew species were captured in Pitfall traps (nine) than in Sherman (six) or snap traps (two), and nearly the same number of rodent species was captured with the three types of traps (seven, nine and eight species respectively).

Species composition varied according to the type of trap used (Table 1) :

- several species of shrews (*Crocidura dolichura*, *Suncus remyi*) and rodents (*Hylomyscus parvus*) were captured in pitfall traps only;
- several rodent species were never captured in pitfall traps (*Deomys ferrugineus*, *Hybomys univittatus*, *Praomys* cf *misonnei*, *Praomys* cf *petteri)*;
- most species of shrews (*Crocidura crenata*, *Crocidura goliath*, *Crocidura grassei*, *Crocidura batesi* and *Sylvisorex ollula*) and the rodent species *Hylomyscus* cf *aeta* and *Lophuromys nudicaudus* were never captured in snap traps. The shrew species *Sylvisorex johnstoni* and the rodent species *Thamnomys rutilans* were never captured in Sherman traps.

Several shrew (*Paracrocidura schoutedeni)* and rodent (*Heimyscus fumosus*, *Hylomyscus stella*, *Malacomys longipes*) species were captured in the three types of traps.

Trap success

Trap success of shrews was significantly greater in pitfall traps than in Sherman or snap traps ($X^2 = 2462.007$ and 2815.180 respectively, $P < 0.001$), while the opposite was true for rodents ($X^2 = 43.279$ and 17.398 respectively, $P < 0.001$; Tab. 1). Trap success of shrew species was significantly greater in pitfall traps than in Sherman or snap traps ($63.358 < X^2 < 1492.965$, $P < 0{,}001$), except *C. goliath* evenly captured in pitfall and Sherman traps ($X^2 = 3.176$, $P < 0{,}075$). Trap success of most rodent species was greater in Sherman or snap traps than in pitfall traps, but several rare species (*H. parvus*, *L. nudicaudus*, *T. rutilans*, *H.* cf *aeta*) were mainly captured in pitfall traps.

Trap success of shrews vas greater in Sherman traps than in snap traps ($X^2 = 35.268$, $P < 0.001$); the same result was obtained for rodents ($X^2 = 53.880$, $P < 0{,}001$; Tab. 1). Trap success of *D. ferrugineus*, *H. fumosus*, *H.*

TABLE 1

Mean weight of species (in g); number of captures (N) and trap success (T) for each type of trap. E is the trapping effort.

	Mean weight	Pitfall traps (E=4865)		Sherman traps (E=44065)		Snap traps (E=43967)	
		N	T	N	T	N	T
Shrews							
Crocidura batesi Dollman, 1915	12	31	0,64	13	0,03	0	0,00
Crocidura crenata Brosset et al., 1965	8	30	0,62	1	0,00	0	0,00
Crocidura dolichura Peters, 1876	7	13	0,27	0	0,00	0	0,00
Crocidura goliath Thomas, 1906	44	5	0,10	19	0,04	0	0,00
Crocidura grassei Brosset et al., 1965	12	9	0,18	2	0,00	0	0,00
Paracrocidura schoutedeni Heim de Balsac, 1956	8	38	0,78	2	0,00	1	0,00
Suncus remyi Brosset et al., 1965	2	3	0,06	0	0,00	0	0,00
Sylvisorex johnstoni Dobson, 1888	3	170	3,49	0	0,00	1	0,00
Sylvisorex ollula Thomas, 1913	15	34	0,70	5	0,01	0	0,00
Total		333	6,84	42	0,10	2	0,00
Rodents							
Deomys ferrugineus Thomas, 1888	47	0	0,00	30	0,07	8	0,02
Heimyscus fumosus Brosset et al., 1965	18	6	0,12	119	0,27	30	0,07
Hybomys univittatus Peters, 1876	49	0	0,00	131	0,30	237	0,54
Hylomyscus cf *aeta* Thomas, 1911	24	3	0,06	4	0,01	0	0,00
Hylomyscus parvus Brosset et al., 1965	10	3	0,06	0	0,00	0	0,00
Hylomyscus stella Thomas, 1911	16	10	0,21	329	0,75	161	0,37
Lophuromys nudicaudus Heller, 1911	22	6	0,12	3	0,04	0	0,00
Malacomys longipes Milne-Edwards, 1877	65	2	0,04	20	0,05	24	0,05
Praomys cf *misonnei* Van der Straeten & Dieterlen, 1987	29	0	0,00	162	0,37	78	0,18
Praomys cf *petteri* Van der Straeten et al., 2003	29	0	0,00	46	0,10	28	0,06
Praomys sp		0	0,00	37	0,08	28	0,06
Thamnomys rutilans Peters, 1876	34	1	0,02	0	0,00	1	0,00
Total		31	0,64	881	2,00	595	1,35

stella, *P.* cf *misonnei* and *P.* cf *petteri* was greater in Sherman traps than in snap traps ($4.335 < X^2 < 53.051$, $P < 0{,}001$). This was not true for the two heaviest rodent species : *M. longipes* was evenly captured with the two types of traps ($X^2 = 0.372$, $P = 0.542$) and more *H. univittatus* were captured in snap traps than in Sherman traps ($X^2 = 30.641$, $P < 0.001$).

Effect of body weight

Mean weight of individuals of *H. stella* and *H. fumosus* captured in pitfall traps (6 and 13 g respectively) was smaller ($P < 0.05$) than those captured in Sherman (16 and 19 g) or snap traps (17 and 10 g). In contrast, no significant difference ($P > 0.05$) was observed between mean weight of *C. batesi*, *C. goliath* and *S. ollula* individuals captured in pitfall and Sherman traps (13 and 12 g, 49 and 43 g and 15 and 16 g respectively). Number of captures of other species was too low to conclude.

Mean weight of individuals of *D. ferrugineus*, *H. univittatus* and *P.* cf *misonnei* captured in Sherman traps (45, 43 and 30 g respectively) was smaller ($P < 0.05$) than those captured in snap traps (58, 53 and 31 g). In contrast, the difference was not significant ($P > 0.05$) for *H. fumosus* (19 and 19 g), *H. stella* (16 and 17 g) and *P.* cf *petteri* (25 and 33 g).

Sex

When possible, we tested for possible significant difference in sex ratio between populations sampled by different types of traps. There was no significant difference in sex of *H. fumosus* or *H. stella* sampled by the three types of traps ($P > 0.05$). Similarly, we did not observed any significant difference in sex of *C. goliath* or *C. batesi* sampled by Sherman and Pitfall traps, or in sex of *H. univittatus*, *M. longipes*, *P.* cf *petteri* or *P.* cf *misonnei* sampled by snap traps and Sherman traps. No significant difference in body weight between sexes was recorded in these eight species ($P > 0.05$).

DISCUSSION

Comparison between pitfall traps and baited traps

In agreement with numerous studies (Brosset, 1966, 1988; Maddock, 1992; Kirkland & Sheppard, 1994; Dickman, 1995; Stanley et al., 1996; Goodman et al., 2001) we found that pitfall traps were more efficient (higher number of species and higher trap success) than Sherman or snap traps for capturing shrews. In contrast, pitfall traps were generally less efficient than Sherman or snap traps for capturing rodents, even if some rare species (e.g. *H. parvus*) were captured in pitfall traps only. In other parts of tropical Africa, Colyn et al., (unpublished data) also found several rare rodent species to be only captured in pitfall traps (e.g. *Prionomys batesi*, *Dendromus sp*).

At least four traits could influence capture rates for different species :

(1) body size and ability to jump : mean weight of rodents individuals captured in pitfall traps was smaller than those of the same species captured in Sherman or snap traps. Few individuals of large rodent species (Table 1) were captured in pitfall traps and all of them were captured during rainy nights. This is in agreement with the observation of Handley & Varn (1994) and Silva et al., (2000) according to which wet pitfall traps are more effi-

cient than dry ones because animals cannot escape by jumping. Contrary to rodents, large shrew species (eg *C. goliath*) would not be able to jump out from dry pitfalls;

(2) trigger sensitivity and weight of animals : animals of low weight (< 10 g) rarely trigger the mechanism of the trap;

(3) shrews, in contrast to rodents, are not attracted by the bait used (BROSSET, 1966);

(4) some crocidurine shrews would be reluctant to explore new objects and therefore would not enter in Sherman or snap traps (DICKMAN, 1995).

Comparison between Sherman traps and snap traps

Sherman and snap traps are only efficient to capture rodents and have complementary effects onto their capture : Sherman traps (7.5 X 9 X 23 cm) are more effective for trapping smaller species, while snap traps (10 X 15 cm) tend to be more effective for trapping larger ones. Moreover, light individuals of the largest species (e.g. *D. ferrugineus*, *H. univittatus* and *P.* cf *misonnei*) are better captured than heavy ones in Sherman, while no significant difference was observed for small species (e.g. *H. fumosus* and *H. stella*). The tendency of the two types of traps to catch rodents of different sizes may reflect trigger sensitivity in traps, or be due to the fact that larger rodents are reluctant to, or cannot, enter the confined space of the Sherman trap, as suggested by PIZZIMENTI (1979).

CONCLUSION

The three types of traps used in this study have complementary effects onto the capture of small mammals in African rainforest. Thus, as concluded from studies in other tropical rainforests (WOODMAN et al., 1996; LYRA-JORGE & PIVELLO, 2001), we suggest that an assortment of traps should always be employed in studies of small mammal communities in African rainforest in order to obtain a wider range of taxa, and thus a better representation of the community.

Additional studies are needed to compare the efficiency of others types of traps, such as Tomahawk traps or different sizes of Sherman and snap traps. Moreover, a comparison of the efficiency of different types of bait would be interesting. Also, to define an appropriate sampling methodology for the study of small mammal communities in African rainforest, we must also pay attention to the cost of the traps and their convenience for carriage and installation in the field. For example, Sherman traps are more costly than snap traps or pitfall traps. A limitation for using pitfall traps is that their carrying and placement in the field demands a great physical effort, and their installation may become unfeasible in rocky or swampy soils.

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SHORT NOTES

A new host (*Nordmannia acaciae* (Fabricius) (Lep. : Lycaenidae)) record for *Aplomya confinis* (Fallén) (Dip. : Tachinidae) from Turkey

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KEY WORDS. *Aplomya confinis*, New host, *Nordmannia acaciae*, Almond, Turkey.

The Tachinidae is the largest family of Diptera (among the suborder Brachycera). The larvae live as endoparasites in insects (Lepidoptera, Hymenoptera, Coleoptera, Heteroptera, Orthoptera and a few others) (1). The detailed general information on host data were obtained by (2; 3), and the most recent data about hosts of Turkish Tachinidae were given by (4). This family includes approximately 10.000 species worldwide, of which about 1650 are found in the Palearctic Region (2). Because of their role as natural enemies of pests in agricultural and forest ecosystems, some species of tachinids have been the focus of applied biological control studies (5). *Nordmannia acaciae* (Fabricius) (Lep. : Lycaenidae) were recorded on Almond trees in Southeast Anatolian Region for the first time (6). It is distributed in Russia, Middle and South Europe, Iran and Turkey and found on stone fruit trees (7). There is no information about natural enemies of *N. acaciae* so far. *Aplomya confinis* (Fallén), the first recorded tachinid parasitoid of *N. acaciae.* It was previously found in Turkey by sweep net collections thus the host was unknown (8).

This study was carried out in 2004. The larvae of *Nordmannia acaciae* were collected from almond tree plantations in the provinces of Diyarbakir, Elazig and Mardin Turkey during April, June and were brought to the laboratory for rearing. During the course of the study, a total 52 larvae of the host were collected.

The larvae were reared in boxes containing almond leafs from the same field. Distilled water was provided. The larvae were reared at a temperature of 26±1°C, relative humidity of 65±5 %, and illumination of 3500 lux for 16 hours per day. The boxes were checked daily. The last instar tachinid larvae left the host larvae and transformed to the puparia next to the remains of their hosts. Host pupae and tachinid puparia were placed in separate petri dishes containing moistened cotton until the adult moths and flies emerged.

Subfamily : Exoristinae

Tribe : Eryciini

Aplomya confinis (Fallén)

Reared material :

Elazig, emergence of fly, 12.05.2004, 1♀

Nordmannia acaciae is a new host record for *Aplomya confinis*.

Biology :

It was found scrubby dry slopes, warm, dry forest edges, and dry meadows. Flight period is early may to early October. It has several generations (3).

Recorded hosts exclusive of Turkey : Specific parasitoid of Lycaenidae (3). *Callopyrus rubi* L., *Thecla w-album* Knoch (9, 10), *Chrysophanus phleas* L., *Lycaena icarus* Rott., *L. bellargus* Rott. (11), *L. coridon* Poda (9), *Cyaniris argilus* L. (9). *Zephyrus quercus* L. (12; 13).

Distribution :

It was frequently found in warmer central Europe (and in Southern Europe), rarely in the North (3) : Switzerland, France, Great Britain, Sweden, Transcaucasus, Soviet Middle Asia, East Siberia, Far East, Israel, Mongolia, Japan, Canari Is. (14).

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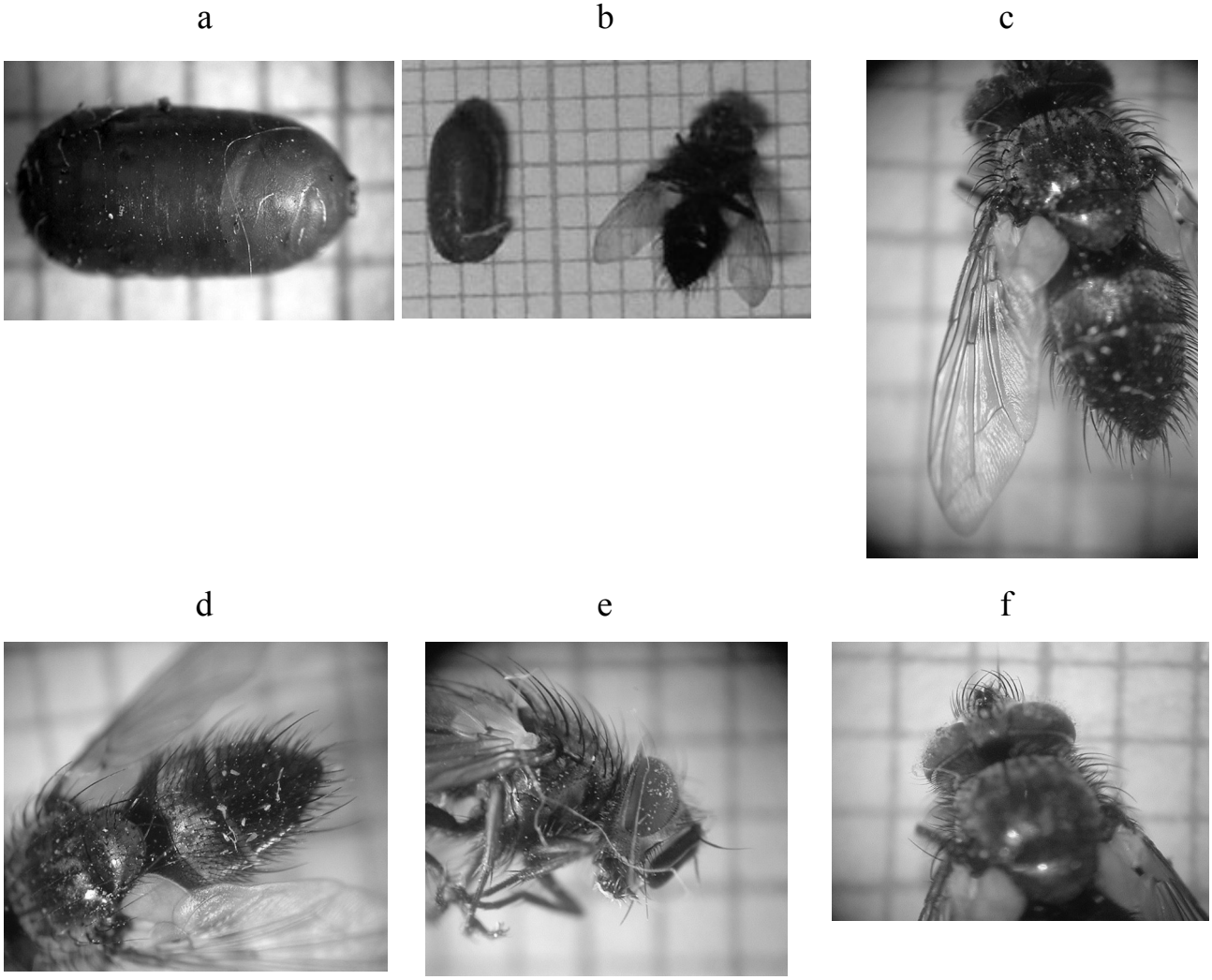

Fig. 1. – Some *Aplomya confinis* images a : Pupa; b : Pupa and adult (); c : Dorsal view; d : Dorsal view of abdomen; e : Lateral view of head; f : Dorsal view of head and thorax.

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