Belgian Journal of Zoology

(formerly: «ANNALES DE LA SOCIÉTÉ ROYALE ZOOLOGIQUE DE BELGIQUE — ANNALEN VAN DE KONINKLIJKE BELGISCHE VERENIGING VOOR DIERKUNDE»)

Published by the «KONINKLIJKE BELGISCHE VERENIGING VOOR DIERKUNDE — SOCIÉTÉ ROYALE ZOOLOGIQUE DE BELGIQUE»

Volume 128 (1)

(June, 1998)

Editor:

Prof. Dr. E. Schockaert
Department SBG
Limburgs Universitair Centrum
B-3590 Diepenbeek (Belgium)

The *Belgian Journal of Zoology* publishes original manuscripts in the field of zoology. The manuscripts are evaluated by the editorial board.

The opinions of the authors published in this journal remain, nevertheless, their own responsibility.

Manuscripts are to be sent to: Prof. Dr. E. SCHOCKAERT, Department SBG, Limburgs Universitaire Centrum, B-3590 Diepenbeek (Belgium).

EDITORIAL BOARD :

Foreign Members

R. BARBAULT (France), G. BOXSHALL (Great Britain), A. DE RICQLÈS (France), D. DINDAL (U.S.A.), A. DIXON (Great Britain), K. KLEMMER (W. Germany), P. LEGENDRE (Canada), J. OSSE (The Netherlands), H. SCHMINKE (Germany), A. VAN BRUGGEN (The Netherlands), N. WATSON (Australia)

Belgian Members

A. COOMANS, J. HULSELMANS, Ch. JEUNIAUX, PH. LEBRUN, J. M. PASTEELS, D. THYS VAN DEN AUDENAERDE, J. VAN GOETHEM

Editor: E. SCHOCKAERT
Associate editors: M. CHARDON, W. VERRAES

Belg. J. Zool. - Volume 128 (1998) - issue 1 - pages 3-11 - Brussels 1998

Received: 4 December 1997

IMPROVED SURFACE VISUALIZATION OF LIVING AVIAN BLASTODERM STRUCTURES AND NEIGHBOURING OOPLASMS BY OOCYTAL TRYPAN-BLUE STAINING

MARC CALLEBAUT, LUC VAN NASSAUW, FERNAND HARRISSON AND HILDE BORTIER

RUCA, UA, Laboratory of Human Anatomy and Embryology
Groenenborgerlaan 171, B-2020 Antwerpen (Belgium)
e-mail: macal@nets.ruca.ua.ac.be

Abstract. By injection(s) of trypan-blue solution during late oogenesis (rapid growth period of the oocytes) into the mother quail we could improve the visibility of the structural details seen from the surface in living quail blastoderms when still *in situ* on their egg yolk balls. This technique of intraoocytal yolk staining by trypan blue allows us to observe, to focus on and to photograph much better the surface morphology of unincubated or shortly incubated blastoderms and their relationship with the neighbouring ooplasms. Indeed the difference in distribution and volume of the trypan-blue-stained yolk granules in the blastoderms and neighbouring ooplasms seems to prevent excessive reflexion by the deep part of the germ disc of the light penetrating through the superficial parts. This diminished scattering of light, greatly increases the contrast between the different regions. By this method we could visualize a higher number of RAUBER's sickles from the surface in living unincubated quail blastoderms.

Key words: trypan-blue-stained yolk, avian blastoderm, RAUBER's sickle, ooplasm, Japanese quail (*Coturnix coturnix japonica*).

INTRODUCTION

It is well known by avian embryologists that it is often difficult or impossible to see from the surface in the living state all the components or the orientation of the unincubated (as represented on Fig. 1) or shortly incubated blastoderm *in situ* on its egg yolk. Thus FARGEIX (1964) and LUTZ (1964) found that in only 30% of the unincubated quail eggs could a KOLLER's (1882) (RAUBER's, 1876) sickle be observed. In the unincubated blastoderms of our laboratory Japanese quails (*Coturnix coturnix japonica*) we found a similar percentage of RAUBER's sickles (CALLEBAUT, 1987). After removal of the unincubated blastoderm from its egg yolk ball f.i. for *in vitro* culture this becomes still more difficult, as well in the chicken as in the quail because often the whole or part of RAUBER's sickle remains fixed to the yolk by the natural tendency of its cells to incorporate underlying ooplasm (CALLEBAUT, 1993a; CALLEBAUT & VAN NUETEN, 1994). In the upper layer in the concavity of RAUBER's sickle (area centralis), the first gastrulation phenomena will take place (CALLEBAUT *et al.*, 1996a) during early incubation. Just like the NIEUWKOOP centre (equatorial vegetal dorsali-

zing cells) described by NIEUWKOOP (1973) in amphibian blastulas, RAUBER's sickle belongs to the equatorial vegetal part of the germ and induces neighbouring upper layer cells of the animal hemisphere (area centralis) to differentiate in endomesoderm. A primitive streak in the living blastoderm, *in situ* on its egg yolk ball, is often also difficult to see during early incubation. The reason for these difficulties (both in unincubated and primitive streak stages) is the lack of colour contrast in the different blastodermal and ooplasmic structures. Moreover, if components of an unstained blastoderm can be made visible under the stereomicroscope, this is usually only possible by a laborious combination of incident and transmitted light. The absence of contrast and the unequal spreading of the light then causes supplementary difficulties for stereomicrography. The obvious difficulties in observation and easy photography of living blastoderms, *in situ* on their egg yolk ball, explains why most studies or experiments are performed *in vitro*. The constituent yolk layers of quail blastoderms are formed when the precursor oocyte is growing from 3 to approximately 19 mm (rapid growth period: CALLEBAUT, 1974; 1983). By injection of solutions of the acid bisazo dye, trypan blue in a laying Japanese quail, it is possible to stain the yolk protein α-livetin in her largest oocytes (D'HERDE & ROELS, 1993). By fluorescence microscopy we have shown that besides the vitelline membrane, only the yolk granules are stained in the blastoderms and egg yolks derived from these oocytes (CALLEBAUT *et al.*, 1981). The method of trypan blue labelling was previously only used for fluorescent studies on sections and not *in situ* for observation of living germ discs from the surface. Seen from the surface these egg yolks present a brown or blue to black staining, depending on the quantity of injected trypan blue. This gives a kind of contrast which permits a much better visualization of the blastodermal structures and of the subgerminal, perigerminal and paragerminal ooplasms in the neighbourhood. In the present study we compare stereomicrographs from surface views after intravital trypan blue staining of the yolk with stereomicrographs taken without staining. The different components of an unincubated quail blastoderm and neigbouring ooplasms and yolk (with current terminology) are represented on a drawing of a mediosagittal section (Fig. 1).

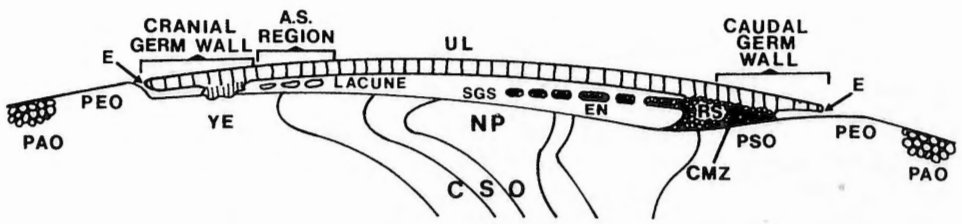


Fig. 1. – Schematic representation of a mediosagittal section through an unincubated quail blastoderm with surrounding ooplasms after fixation *in situ* on their egg yolk ball. UL: upper layer; EN: incomplete endophyll; RS: RAUBER's sickle; CMZ: caudal marginal zone; the caudal marginal zone being a more or less transparent part of the caudal germ wall adherent to the caudal peripheral subgerminal ooplasm (PSO); SGS: subgerminal space; E: edge of the blastoderm; YE: early development of yolk endoblast, growing into the peripheral subgerminal ooplasm; PEO: perigerminal ooplasm; PAO: paragerminal ooplasm; CSO: central subgerminal ooplasm in which the central nucleus of PANDER (NP) is seen.

MATERIAL AND METHODS

Thirty female adult laying Japanese quails were used for injection with unfiltered trypan blue O (SERVA, HEIDELBERG) solution in Ringer. The trypan blue solution was not filtered because it is a colloidal solution, which remains fixed to the filter paper during filtering (personal observation). Fifteen female quails were injected i.m. with 1 ml of 1% trypan blue solution every 2 days, during 1 month. This corresponds to the dose used in a previous study (CALLEBAUT & SYENS, 1985). Fifteen other quails were injected once with 1 ml of 0.25% trypan blue. This is a dose which is lower than the lowest dose (0.3 ml of 1% solution trypan blue) used by D'HERDE & ROELS (1993). The latter dose still gave a temporary slight modification of the yolk morphology exterior to the germinal disc region but had no influence on the yolk in the germinal disc region. Some of the freshly laid eggs were opened for observation and photography of their blastoderm. Special attention was given to the visibility of the morphological details present in unincubated blastoderms (as represented on Fig. 1) f.i. RAUBER's sickle (1876), the localization and eventual eccentricity of the nucleus of PANDER (1817), the caudal marginal zone, the germ wall and its borders. Other eggs were incubated for 10h at 39°C in an incubator, to observe the first signs of gastrulation from the surface, on the living egg yolk ball. The photographs were taken with a Leica camera (R3, electronic) adapted to a Wild (M5) stereomicroscope ocular. For comparison, unstained control blastoderms were photographed through the opening in a black screen placed over the egg yolk ball. This screen functions as a non-reflecting background, preventing intense light reflexion by the surface of the egg yolk. Some of the blastoderms were fixed in Susa without sublimate (ROMEIS, 1948) and after dehydration, embedded in paraffin. Mediosagittal and parasagittal sectioning was performed at 8-10 μm thickness. To demonstrate the relationship of the different components of the avian blastoderm with the subgerminal perigerminal or paragerminal ooplasm after that fixation, some sections were stained with iron hematoxylin and eosin. To compare the stereomicroscopic aspect of the living structures seen from the surface after trypan-blue labelling with the localization of the trypan blue labelled yolk in the germ disc, fluorescence microscopy was used on the sections. To do this, sections were examined under a Leitz Orthoplan microscope equipped with a HBO 100 high-pressure mercury lamp and an incident-light Ploem Opak illuminator. A 4-nm BG 38 red suppression filter and a 2-mm KG1 heat absorption filter were placed in the lamp housing. Blue excitation filters 2 x SP 490 in combination with a chromatic beam splitter (CBS) with a cut-off at 510 nm (filter setting No 3) were used (HARRISSON *et al.*, 1981). The chemical interaction between trypan blue and the yolk proteins results in a red fluorescence and permits the detection of trypan blue-marked yolk with high sensitivity. Fluorescence microscopy was only used on sections and not for surface observations. Other eggs (containing trypan-blue-labelled yolk) remained unopened and were incubated until hatching occurred.

RESULTS

Fig. 2 represents a surface stereomicrograph (without the use of fluorescent methods) from a living unincubated quail blastoderm after oocytal yolk staining with trypan blue.

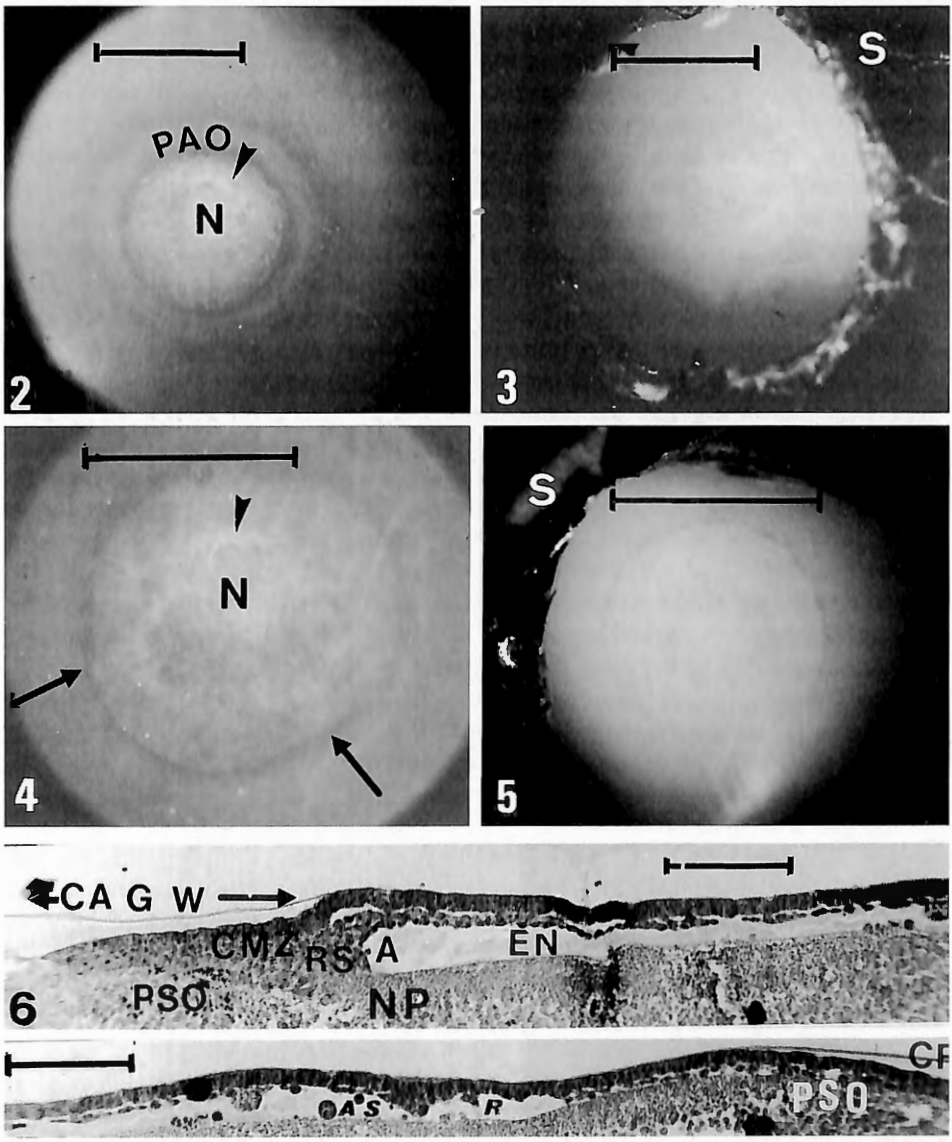


Fig. 2. – Stereomicrograph of a living unincubated quail blastoderm *in situ* on its egg yolk ball after intraoocytal staining with trypan blue (4 injections of 1 ml); N: eccentric nucleus of PANDER; arrow head indicates RAUBER's sickle, very close to the narrow caudal germ wall; PAO: white paragerminal ooplasm; bar: 3 mm.

Fig. 3. – Similar unstained living unincubated quail blastoderm *in situ* on its egg yolk ball; the stereomicrograph is taken through the opening in a black screen (S); the borders of the structures are not so sharply delineated as those of Fig. 2; bar: 3 mm.

Fig. 4. – Stereomicrograph of a living unincubated quail blastoderm *in situ* on its egg yolk ball after a single injection of 1 ml of 0.25% trypan blue solution during oogenesis, 4 days before

On Fig. 3 we see a similar quail germ without trypan-blue staining. The improvement of the visibility of the structural components after trypan blue yolk staining is very obvious. In Fig. 2 the RAUBER's sickle and the nucleus of PANDER are sharply visible in full contrast with the neighbourhood. So their eccentric localization indicates an early stage of primary (paradoxical) eccentricity, visible at the end of the intrauterine period and sometimes still found in just laid eggs (CALLEBAUT, 1993b). The extent of the narrow future caudal germ wall (behind RAUBER's sickle) and of the broader diametrically opposite cranial germ wall are also easily seen and also indicate a primary eccentricity. Most obvious in the trypan-blue-stained egg is the perigerminal ooplasm forming a dark halo around the sharply-defined blastoderm edge. The paragerminal ooplasm (rich in tubulin: CALLEBAUT *et al.*, 1996b) surrounds the perigerminal ooplasm forming a contrasting white halo. The latter peripheral structures are barely visible on the micrographs of unstained eggs (even after the use of a black screen). Fig. 4 represents an unincubated blastoderm *in situ* on the egg yolk ball of an egg laid 3 days after a single injection of 1 ml of 0.25% trypan blue solution to the mother. The staining is less pronounced and has a brown aspect, however it gives sufficient contrast for clear observation and photographing (compare with the unstained, unincubated quail blastoderm of Fig. 5). This intravital yolk staining with trypan blue permits us to localize and visualize much better small and narrow RAUBERIS sickles in unincubated blastoderms. Thus in 50 unincubated trypan-blue-stained blastoderms we could localize 30 RAUBER's sickles. This is a higher percentage (60 %) than could be observed in unstained blastoderms (30 % according to FARGEIX, 1964). When no RAUBER's sickle is visible, even after trypan-blue staining, usually a clear cut eccentricity can be seen. On sections of such blastoderms (stained or not) where only an eccentricity is visible from the surface, still the presence of a RAUBER's sickle as the cranial boundary of the caudal marginal zone (Fig. 1) can be demonstrated when sectioning is performed through regions where contrast is most pronounced (Figs 6, 7). Small dark zones in the germ walls seem to correspond, when sectioned, to voluminous yolk masses or «yolk islands» as described in the caudal marginal zone in the neighbourhood of RAUBER's sick-

---

laying; N: eccentric nucleus of PANDER; arrow head indicates narrow RAUBER's sickle, close to the caudal germ wall; arrows indicate the perigerminal ooplasm; bar: 2 mm.

Fig. 5. – Stereomicrograph of unstained living unincubated blastoderm *in situ* on its egg yolk ball; the micrograph is taken through the opening of a black screen (S): the different structures are not clearly visible through lack of contrast; bar: 2 mm.

Fig. 6. – Mediosagittal section through the caudal part of an unincubated quail blastoderm that, when alive, presented an eccentricity but no visible RAUBER's sickle (even after trypan-blue staining); RS: RAUBER's sickle, only visible on sections; CMZ: caudal marginal zone; A: axilla-shaped pocket of the subgerminal space; EN: endophyll sheet; note the localization of the nucleus of PANDER (NP), partially below RAUBER's sickle; CAGW: small caudal germ wall in intimate contact with the caudal peripheral subgerminal ooplasm (PSO); iron hematoxylin and eosin staining; bar: 100 µm.

Fig. 7. – The cranial part of the mediosagittal section through the same unincubated quail blastoderm as in Fig. 6; AS: anti-sickle in the cranial recessus (R) of the subgerminal space; CR: cranial germ wall loosely or not bound to the cranial peripheral subgerminal ooplasm (PSO); bar: 100 µm

le (CALLEBAUT, 1993a). The trypan blue *in ovo* staining of unincubated quail blastoderms also permits observations on the frequent individual variability in the volume or localization of the different structures. The nucleus of PANDER (1817) f.i. is often not found below the centre of the blastoderm but is localized more caudally (never cranially), sometimes very close or even partially below RAUBER's sickle (Fig. 6). This is obviously the result of the permanent eccentricity provoked by the inclination of the blastoderm on its egg yolk during the rotation in utero (CALLEBAUT, 1994). After a few hours of incubation the formation of the embryonic shield (first Anlage of the embryo) becomes visible (Fig. 8) in the blastoderms of trypan-blue-stained egg yolks. Often the first ingrowth of sickle endoblast, derived from RAUBER's sickle, into the endophyll of the area centralis (preceding the appearance of the primitive streak: CALLEBAUT & VAN NUETEN, 1994; CALLEBAUT *et al.*, 1997a,b) becomes visible. The initial appearance of the white-stained massive yolk endoblast forming part of a peripheral circle in the area opaca is, however, most prominent (Fig. 8). After approximately 12h of incubation the primitive streak becomes clearly visible (Fig. 9) by contrast with the dark background of the subgerminal ooplasm. In the blastoderms of unstained eggs the primitive streak is usually only faintly visible. On the sections through the germ disc, one sees clearly after fluorescence microscopy, the difference in distribution, volume and intensity of the fluorescence of the trypan-blue-stained yolk granules (Fig. 10). Seen from the surface in the living state this trypan-blue-stained yolk forms a brown, blue or black background from which no or less light is reflected. The more superficial parts of the blastoderm are less labelled and still transparent and so their structure can be better seen.

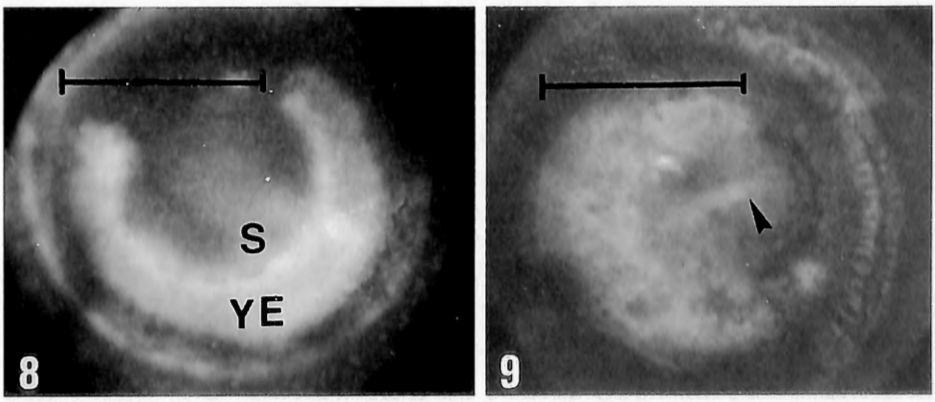


Fig. 8. – Stereomicrograph of a trypan blue stained (6 injections) quail blastoderm after 7h of incubation; an embryonic shield (S) appears in the concavity of RAUBER's sickle; also the white-stained horse-shoe-shaped massive yolk endoblast (YE) surrounding junctional endoblast appears in the area opaca; bar: 2 mm.

Fig. 9. – Stereomicrograph of a trypan-blue-stained quail blastoderm (after 11 injections during oogenesis) *in situ* on its egg yolk ball after 12h of incubation; the primitive streak (arrowhead) is clearly seen in contrast with the dark subgerminal background; bar: 2 mm.

Not withstanding the repeated i.m. injections of 1 ml (1% trypan blue in Ringer), the quails continued to lay eggs. The rate of egg laying somewhat decreased: instead of laying one egg every day, 2 eggs were usually laid every 3 days. However egg laying did not stop even after 1 month of treatment. The baby quails that hatched after the prolonged treatment with trypan blue were apparently normal.

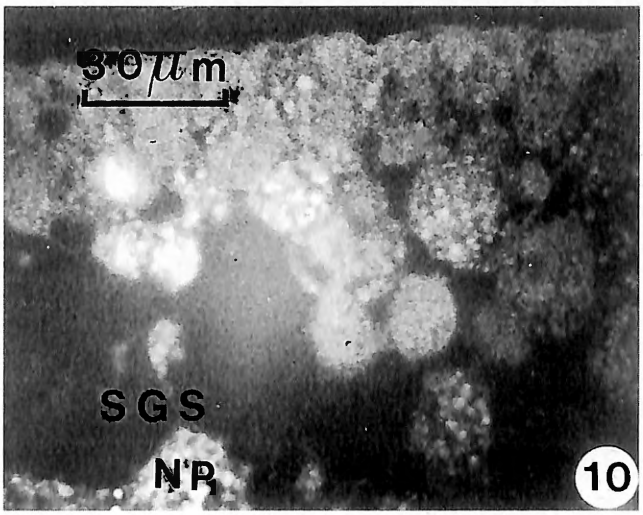


Fig. 10. – Fluorescence micrograph of a sagittal section through a quail germ, after oocytal labelling with trypan blue (6 days after an injection to the mother). The deeper layer of cells is strongly labelled (white aspect on the photograph), whilst the superficial layer contains less-labelled yolk granules. Below the subgerminal space (SGS) also part of the nucleus of PANDER (NP) contains numerous trypan-blue-labelled yolk granules. Seen from the surface in the living state the strongly labelled parts appear brown, blue or black and reflect less or no penetrating light. Thus the more superficial, more or less transparent parts become sharply visible; bar: 30 µm.

DISCUSSION

The technique described herein of intraoocytal trypan-blue staining allows us to more readily and accurately observe, focus on, and photograph the morphological features of living unincubated (represented on Fig. 1) and briefly incubated quail blastoderms. The trypan blue staining gives an impression of transparency to the blastoderm by forming a dark-field-like background in the subgerminal ooplasm. Since only the yolk is stained by trypan blue (CALLEBAUT & VAKAET, 1981; CALLEBAUT *et al.*, 1981; CALLEBAUT, 1983, 1987), the higher contrast between the blastodermal regions seems to be produced by the difference in content and volume of the trypan-blue-stained yolk granules (Fig. 10). This is also the case for the subgerminal, perigerminal and paragerminal ooplasm. Most ob-

vious in trypan-blue-stained blastoderms is the possibility to localize a higher percentage (both a quantitative as a qualitative improvement) of RAUBER's sickles than in unstained blastoderms. Our study suggests that RAUBER's sickle is nearly always present in a normal unincubated blastoderm but that its visibility seen from the surface in the living state, is sometimes hidden by lack of contrast with surrounding structures. Also the visual superposition of RAUBER's sickle sometimes localized above part of the nucleus of PANDER (Fig. 6) can have the same effect. Therefore the trypan-blue staining can be useful in expensive experimental studies to predict and to orient more precisely the future caudocephalic axis in order to make perfect mediosagittal and parasagittal sections of unincubated blastoderms. In the central subgerminal ooplasm, the nucleus of PANDER and its eventual eccentricity becomes clearly visible in contrast to the surrounding (Fig. 2) darker-staining bottom of the subgerminal cavity. The directly visible relationship between the living blastoderm and underlying subgerminal ooplasm is also one of the advantages of the trypan-blue staining. Very obvious in trypan-blue-stained egg yolks is the contrast between the perigerminal acellular tubulin-poor ooplasm (dark) and the rim of the blastoderm. On the other hand the acellular paragerminal ooplasm forms a broad white halo around the perigerminal ooplasm. This is probably due to the fact that in the paragerminal ooplasm numerous tubulin threads surround the local yolk granules (CALLEBAUT *et al.*, 1996b). The intravital trypan-blue staining described here also allows sequential observations on the evolution of the different components of the avian blastoderm and ooplasms *in situ* on their egg yolk ball by culture *in vitro* according to ROMANOFF (1943).

ACKNOWLEDGEMENTS

We thank Mr. F. De Bruyn for artwork and Mrs. V. De Maere for typing the manuscript. This study was supported by Grant 3.0029.93 of the National Fund of Scientific Research of Belgium and a Rafo fonds of RUCA.

REFERENCES

CALLEBAUT, M. (1974) – La formation de l'oocyte d'oiseau. *Arch. Biol.(Brux)*, **85**: 201-233.

CALLEBAUT, M. & L. VAKAET (1981) – Fluorescent yolk marking of the primary gonocytes in quail blastoderms by administration of trypan blue during late oogenesis. *I.R.C.S. Med. Sci.*, **9**: 458.

CALLEBAUT, M., F. HARRISSON & L. VAKAET (1981) – Peripheral avian yolk assemblage and its persistence in the blastoderm, studied by trypan blue-induced fluorescence. *Anat. Embryol.*, **163**: 55-69.

CALLEBAUT, M. (1983) – The constituent oocytal layers of the avian germ and the origin of the primordial germ cell yolk. *Arch. Anat. Micros.*, **72**(3): 199-214.

CALLEBAUT, M. & R.J. SIJENS (1985) – Trypan blue induced fluorescence in adult quail oocytes during the prelampbrush and early lampbrush stage. *I.R.C.S. Med. Sci.*, **13**: 920-921.

CALLEBAUT, M. (1987) – Ooplasmic localization and segregation in quail germs: fate of the four ooplasms. *Arch. Biol. (Brux.)*, **98**: 441-473.

CALLEBAUT, M. (1993 a) – Unequal caudocephalic ooplasmic uptake and eccentric formation of the subgerminal space below unincubated quail blastoderms presenting a Koller's sickle. *Belg. J. Zool.*, **123**(2): 107-112.

CALLEBAUT, M. (1993 b) – Early eccentricity in gravitationally oriented quail germs. *Eur. J. Morph.*, 31: 5-8.

CALLEBAUT, M. (1994) – Relationship between the avian blastoderm and the subgerminal ooplasm. *Eur. Arch. Biol.*, **105**: 111-123.

CALLEBAUT, M. & E. VAN NUETEN (1994) – Rauber's (Koller's) sickle: the early gastrulation organizer of the avian blastoderm. *Eur. J. Morph.*, **32**: 35-48.

CALLEBAUT, M., E. VAN NUETEN, H. BORTIER, F. HARRISSON & L. VAN NASSAUW (1996 a) – Map of the Anlage fields in the avian unincubated blastoderm. *Eur. J. Morph.*, **34**: 347-361.

CALLEBAUT, M., L. VAN NASSAUW, F. HARRISSON & A. SCHREVENS (1996 b) – Immunohistochemical localization of β-tubulin in the unincubated avian germ and in peri-, para- and subgerminal ooplasm: homology with meroblastic teleost embryos. *Belg. J. Zool.*, **129**: 169-176.

CALLEBAUT, M., E. VAN NUETEN, H. BORTIER, F. HARRISSON, L. VAN NASSAUW & A. SCHREVENS (1997 a) – Spatial relationship between endophyll, primordial germ cells, sickle endoblast and upper layer in cultured avian blastoderms. *Reprod. Nutr. Dev.*, **37**: 293-304.

CALLEBAUT, M., E. VAN NUETEN, F. HARRISSON, L. VAN NASSAUW, A. SCHREVENS & H. BORTIER (1997 b) – Avian gastrulation and neurulation are not impaired by the removal of the marginal zone at the unincubated blastoderm stage. *Eur. J. Morph.*, **35** (2): 69-77.

D'HERDE, K. & F. ROELS (1993) – Experimental modification of vitellogenesis in Japanese quail by trypan blue *in vivo*. *Reprod. Nutr. Dev.*, **33**: 597-608.

FARGEIX, N. (1964) – La caille domestique (*Coturnix coturnix japonica*). Ponte et étude de l'œuf au stade non incubé. *Arch. Anat. Histol. Embryol. et exp.*, **1**(4): 275-281.

HARRISSON, F., M. CALLEBAUT & L. VAKAET (1981) – Microspectrographic analysis of trypan blue-induced fluoresence in oocytes of the Japanese quail. *Histochemistry.*, **72**: 563-578.

KOLLER, C. (1882) – Untersuchungen über die Blätterbildung im Hühnerkeim. *Arch Mikrosk. Anat.*, **20**: 171-211.

LUTZ, H. (1964) – Le stade non incubé du blastoderme d'oiseau. *Arch. Anat. Histol. Embryol.*, **47**: 531-537.

NIEUWKOOP, N. (1973) – The organization center of the amphibian embryo; its spatial organization and morphogenetic action. *Adv. Morphogen.*, **10**: 1-39.

PANDER, C. (1817) – Historiam metamorphoseos quam ovum incubatio prioribus quinque diebus subit. *F. E. Nitribitt.* Ed. Wirceburgi (69 pp).

RAUBER, A. (1876) – *Uber die Stellung des Hühnchens im Entwicklungsplan.* W. Engelmann, Leipzig (28 pp).

ROMANOFF, A. (1943) – Cultivation of the early chick embryo *in vitro*. *Anat. Rec.*, **87**: 365-369.

ROMEIS, W. (1948) – *Mikroskopische Technik*, 15 Aufl. Leibnitz, München (695 pp).

Belg. J. Zool. - Volume 128 (1998) - issue 1 - pages 13-22 - Brussels 1998

Received: 30 April 1997

PONTONIINAE (DECAPODA, CARIDEA) ASSOCIATED WITH *HELIOFUNGIA ACTINIFORMIS* (SCLERACTINIA) FROM HANSA BAY, PAPUA NEW GUINEA)(1)

SAMMY DE GRAVE

Department of Invertebrates, Koninklijk Belgisch Instituut voor Natuurwetenschappen, Vautierstraat 29, B-1040 Brussels, Belgium (2)

Abstract. A small collection of Pontoniinae (Crustacea, Decapoda, Caridea) from Hansa Bay, northern Papua New Guinea associated with the diurnally active coral species, *Heliofungia actiniformis* is discussed. Four species are recorded as new to the Papua New Guinea fauna. Morphological details of *Periclimenes venustus and P. watamuae* are discussed and compared with previous records.

Key words: Pontoniinae, Caridea, Papua New Guinea.

INTRODUCTION

Although some of the earliest records of caridean shrimps came from the Papua New Guinea region (BORRADAILE, 1898; NOBILI, 1899), the shrimp fauna remains little studied. In a comparison of the Pontoniinae, one of the most speciose groups of Caridea, from Australia and neighbouring regions by BRUCE (1990a) many genera and species, although widespread in the Indo-West Pacific, were not recorded from Papua New Guinea. In recent decades, only very limited collecting has been carried out along the northern coastline of Papua New Guinea. MORGAN (1988) recorded only four species from Madang, which compares quite unfavourably with BRUCE (1981) who recorded 100 species from Heron Island in the southern part of the Great Barrier Reef. In view of the widespread nature of coral reefs along the northern coastline of Papua New Guinea (CLAEREBOUDT *et al.*, 1989) and the species richness of coral-biotope-associated Pontoniinae (BRUCE, 1976a), it is expected that many more species, especially the smaller or cryptic ones, await discovery.

This report deals with a small collection of Pontoniinae, found associated with the diurnally active coral, *Heliofungia actiniformis* (Quoy & Gaimard, 1833). *H. actiniformis* is one of only a few corals which have their polyps extended during the day. Five species of Pontoniinae are known to associate with *H. actiniformis*, only one of which, *Periclimenes holthuisi* Bruce, 1969 has been recorded previously from the northern coast of Papua New Guinea (BRUCE, 1977a).

(1) Contribution nr. 300 of the Leopold III Biological Station, Laing Island.

(2) Present address: Department of Zoology, University College Cork, Lee Maltings, Prospect Row, Cork, Ireland.

MATERIAL AND METHODS

Samples of *H. actiniformis* and its associated fauna were collected from Hansa Bay, about 10 km NW of Bogia (Madang Province) on the northern coast of Papua New Guinea during 1992 and 1993. For a map of the general area in which all sampling localities are listed see CLAEREBOUDT *et al.* (1989). Samples were obtained by placing the complete coral in a sealed plastic bag in situ. In the laboratory, extraction of the commensal shrimp fauna was achieved by dousing the coral with a weak formalin solution.

Post-orbital carapace lengths (cl.) are given in mm. All specimens have been deposited in the collections of the «Koninklijk Belgisch Instituut voor Natuurwetenschappen», registration numbers IG 27951 and IG 28056.

SYSTEMATICS

Family Palaemonidae Rafinesque

Subfamily Pontoniinae Kingsley

Genus *Hamopontonia* Bruce, 1970

Hamopontonia corallicola Bruce, 1970

Restricted synonymy

Hamopontonia corallicola BRUCE, 1970:37-48; Figs 1-4. – BRUCE, 1977b: 172-173; Fig. 4. – BRUCE, 1983a:896; Fig. 10g. – SUZUKI & HAYASHI, 1977: 195-196; Figs 1b, 2b; Plate 1.

Material examined

(i) 1 ovigerous (ov.) female cl. 4.1, 3 males cl. 1.8-1.9; Laing Island lagoon, NW side, 10m depth, from *H. actiniformis*; 15.09.1992; S. De Grave coll., field no. S92/58; KBIN IG 27951/NAT1. (ii) 1 female cl. 3.9, 1 male cl. 2.5; Davit wreck, Hansa Bay, 6mm depth, from *H. actiniformis*; 06.10.1992; S. De Grave coll., field no. S92/132; KBIN IG 27951/NAT2. (iii) 1 ov. female cl. 5.1; Laing Island lagoon, NW side, 6m depth, from *H. actiniformis*; 14.09.1994; H. Wilkins coll., field no. S92/54; KBIN IG 27951/NAT3.

Remarks

The specimens agree closely with previous descriptions (BRUCE, 1970, 1977b), although some differences were noted. As in the Indonesian material of BRUCE (1983a) the telson has three pairs of spines and the rostrum has seven dorsal spines in all specimens except the ovigerous female (i). A further difference was found in the structure of the second pereopods. All females and one male (ii) had the second pereopods of nearly equal size, in contrast to the type specimens (BRUCE, 1970). In nearly all specimens the ratio between the palm and fingers of the second pereopod approached 2:1 rather than 3:1.

Colour in life. Females with figure eight shaped white patch on gastric region, posterodorsal aspect of abdominal segments with transverse white bands. Colouration of males not noted.

Distribution

Papua New Guinea (this report), Hong Kong (BRUCE, 1970), Japan (SUZUKI & HAYASHI, 1977), Indonesia (BRUCE, 1983a), Australia (BRUCE, 1977b).

Hosts

Scleractinia: *H. actiniformis*, *Euphyllia glabrescens* (Chamisso & Eysenhardt, 1821), *Goniopora stokesi* Edwards & Haime, 1851; Actiniaria: *Entacmaea quadricolor* (Rüppel & Leuckart, 1828).

Genus *Periclimenes* Costa, 1844

Periclimenes kororensis Bruce, 1977

Restricted synonymy

Periclimenes kororensis BRUCE, 1977c: 33-43; Figs 1-4. – BRUCE, 1983b: 209. – BRUCE & SVOBODA, 1984: 94-96; Figs 5-6.

Material examined

1 ov. female cl. 4.5, 1 male cl. 2.9; Laing Island Reef, outer slope, 10m depth, from *H. actiniformis*; 14.10.1993; S. De Grave coll., field no. S93/116; KBIN IG 28056/NAT4.

Remarks

The specimens agree with previous descriptions (BRUCE, 1977c; BRUCE & SVOBODA, 1984) with some minor differences noted. The rostral dentition of the female was 7/4 and the male 6/3 with a distinct postrostral tubercle, the latter being completely absent in the female. The second pereopods are similar but unequal, with the minor one being 0.65 times as long as the major second pereopod.

Colour in life. Orbital region, rostrum and antennal peduncles white; remainder of carapace orange, although transparent; pleon segments and appendages transparent; joints of all pereopods blue; eye stalks with blue longitudinal stripes.

Distribution

Papua New Guinea (this report), Palau Islands (BRUCE, 1977c), Philippines (BRUCE & SVOBODA, 1984), Australia (BRUCE, 1983b).

Hosts

Scleractinia: *H. actiniformis*.

Periclimenes venustus Bruce, 1990

Restricted synonymy

Periclimenes venustus BRUCE, 1990b: 229-243; Figs. 1-6. – FRANSEN, 1989: 139-143 (partim, as *P.holthuisi*). – BRUCE, 1990a: 12. – BRUCE, 1989: 178.

Material examined

(i) 2 ov. females cl. 4.1-5.1, 2 males cl. 1.6-3.1; Laing Island Lagoon, NW reef slope, 6m depth, from *H. actiniformis*; 14.09.1992; coll. S. De Grave, field no. S92/54; KBIN IG 27951/NAT5. (ii) 1 ov. female cl. 4.1, 2 males cl. 2.1-2.2, 4 juveniles cl. 1.3-2.0; Laing Island Lagoon, NW reef slope, 10m depth, from *H. actiniformis*; 16.09.1992; coll. S. De Grave, field no. S92/58; KBIN IG 27951/NAT6. (iii) 2 females cl. 2.1-2.8, 2 males cl. 1.2-1.3, 3 juveniles cl. 1.0-1.1; Laing Island Lagoon, 6m depth, from *H. actiniformis*; 18.09.1993; coll. S. De Grave, field no. S93/132; KBIN IG 28056/NAT7.

Remarks

The specimens agree closely with the type description (BRUCE, 1990b). As already pointed out by BRUCE (1990b) the close similarity of *P. venustus* to *P. holthuisi* suggests that some of the records of the latter species probably belong to *P. venustus*. Indeed, some of the chelae of *P. holthuisi* illustrated by FRANSEN (1989) show a close similarity with *P. venustus*, and in all likelihood belong to the latter species. Although in fully grown individuals the characteristic dentition of the chelae (Fig. 1a, b) is the single most useful morphological difference with *P. holthuisi*; in juveniles (Fig. 1c, d) this dentition is largely absent. The only reliable morphological difference in small specimens between both species appears to be the spines on the propodus of the third pereopod, which in *P. venustus* only bears a few short ventrodistal spines (Fig. 1e, f, g) while in *P. holthuisi* more and longer spines are present.

The first record of this species is by BRUCE (1989), who lists the actual species description as in press. This was followed in 1990 (BRUCE, 1990b) by a more formal description, inclusive of the designation of type material and locality selection. Clearly the latter publication was intended to be the type description, its publication in all possibility being delayed. As volume 6 of Indo-Malayan Zoology was the 1989 volume, but only appeared in June 1990, it seems logical to maintain the authorship of *P. venustus* as BRUCE (1990).

Colour in life. Body and appendages transparent, white patch on third abdominal segment with two to four smaller pink or blue patches superimposed. The colouration of live *P. venustus* distinguishes this species immediately from *P. holthuisi*.

Distribution

Papua New Guinea (this report), Australia (BRUCE, 1990), Philippines (BRUCE, 1989), Indonesia (FRANSEN, 1989), Ryukyu Islands (CHACE & BRUCE, 1993).

Hosts

Actiniaria: unidentified sp.; Scleractinia: *H. actiniformis*.

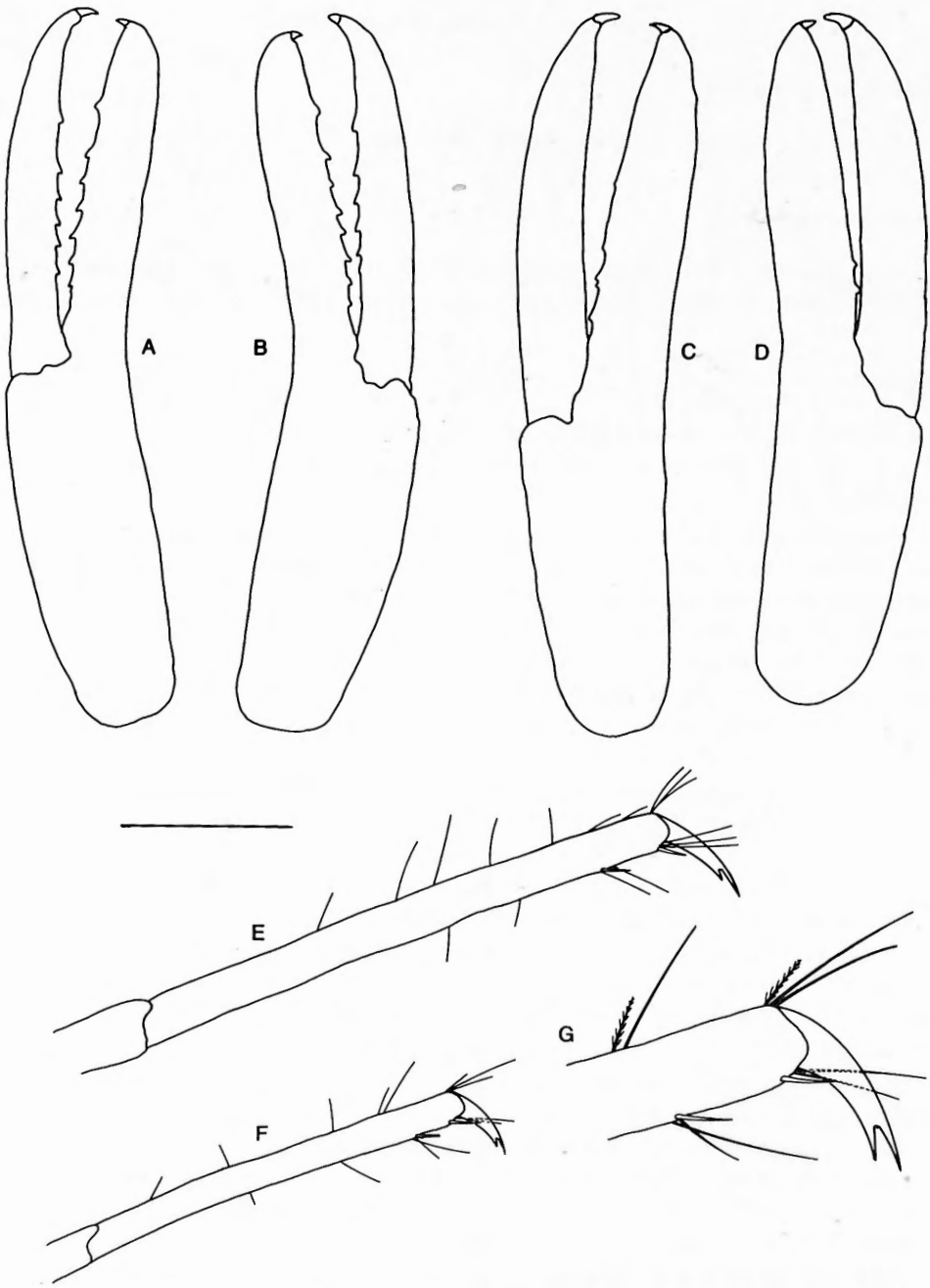


Fig. 1. – *Periclimenes venustus* Bruce, Laing Isl. Lagoon, Hansa Bay, ovigerous female (cl. 4.1mm): chelae of left (A) and right (B) second pereopod, third pereopod (E); juvenile male (cl. 2.1mm), chelae of left (C) and right (D) second pereopod, third pereopod (F), distal part of third pereopod (G). Scale bar indicates 0.1 mm (A, B, E) or 0.4 mm (C, D, F, G).

Periclimenes watamuae Bruce, 1976

Restricted synonymy

Periclimenes watamuae BRUCE, 1976b: 16-20, Figs. 5-6 – FRANSEN, 1994: 130.

Material examined

1 ov. female cl. 1.2, 2 males cl. 1.0-1.1; Purar-I Reef, Hansa Bay, 10m depth, from *H. actiniformis*; 11/10/1993; S. De Grave coll., field no. S93/95; KBIN IG 28056/NAT8.

Remarks

Rostrum slender, with rostral formula 5/1 in female, 5/0 and 4/1 in males. Third maxilliped with well developed exopod with 4 plumose, segmented terminal setae. Epipod well developed.

First pereopods slender (Fig. 2a, c, d). Chelae with subcylindrical palm, about twice as long as wide, with slender fingers, slightly gaping, not subspatulate, with entire blunt cutting edge and hooked tips, equal to approximately 0.7x length of palm. Distal part of both fixed finger and dactylus with numerous, finely-serrated long setae. Carpo-propodal region with numerous serrated stout setae. Carpus and merus slender, equal in length, approximately 1.5 times the length of the chela. Ischium and basis about 0.4x and 0.3x as long as merus, respectively. Coxa with small medial lobe, furnished with three long setae. Fourth thoracic sternite unarmed.

Second pereopods similar and equal (Fig. 2e, f), showing a resemblance to those of *P. diversipes* Kemp, 1922 form c. Chelae 5.2x as long as wide, palm gradually expanding distally. Fingers 2.4 times as long as palm, shallowly spatulate with entire cutting edges. Tip of dactylus blunt, curved. Tip of fixed finger with accessory blunt spine, so as to create bifid aspect in which dactylar tip fits. Carpus short, distally expanded, approximately 0.9 times as long as palm. Ischium and merus unarmed, slender, with ischium approximately 1.45 times as long as merus. Basis and coxa show no special features. The second pereopods of the males are similar to those of the female.

Ambulatory pereopods slender. Third pereopod dactylus slender (Fig. 2g, h), nearly five times longer than proximal width. Unguis well demarcated, equal to 0.75x length of corpus. No accessory spines, but one setae present on the lateral aspect of the corpus. Propodus approximately 8.7x longer than wide, with a single ventrodistal blunt spine. Two plumose setae present on the upper border, situated in the distal half. Carpus, merus and ischium unarmed. Merus approximately 1.75x as long as carpus, ischium subequal in length to carpus. Fourth and fifth pereopods similar.

Male first pleopod with endopod approximately 0.5x exopod length (Fig. 3a, b), distal part broadly expanded, with small lobe on medio-distal margin and with four short simple spines on proximal half, distal margin with one plumose seta. Second pleopod with endopod approximately 0.8x exopod length, with appendices at 0.4 along medial margin(Fig. 3c, d). Appendix masculina feebly tapering distally, with two long, equal ter-

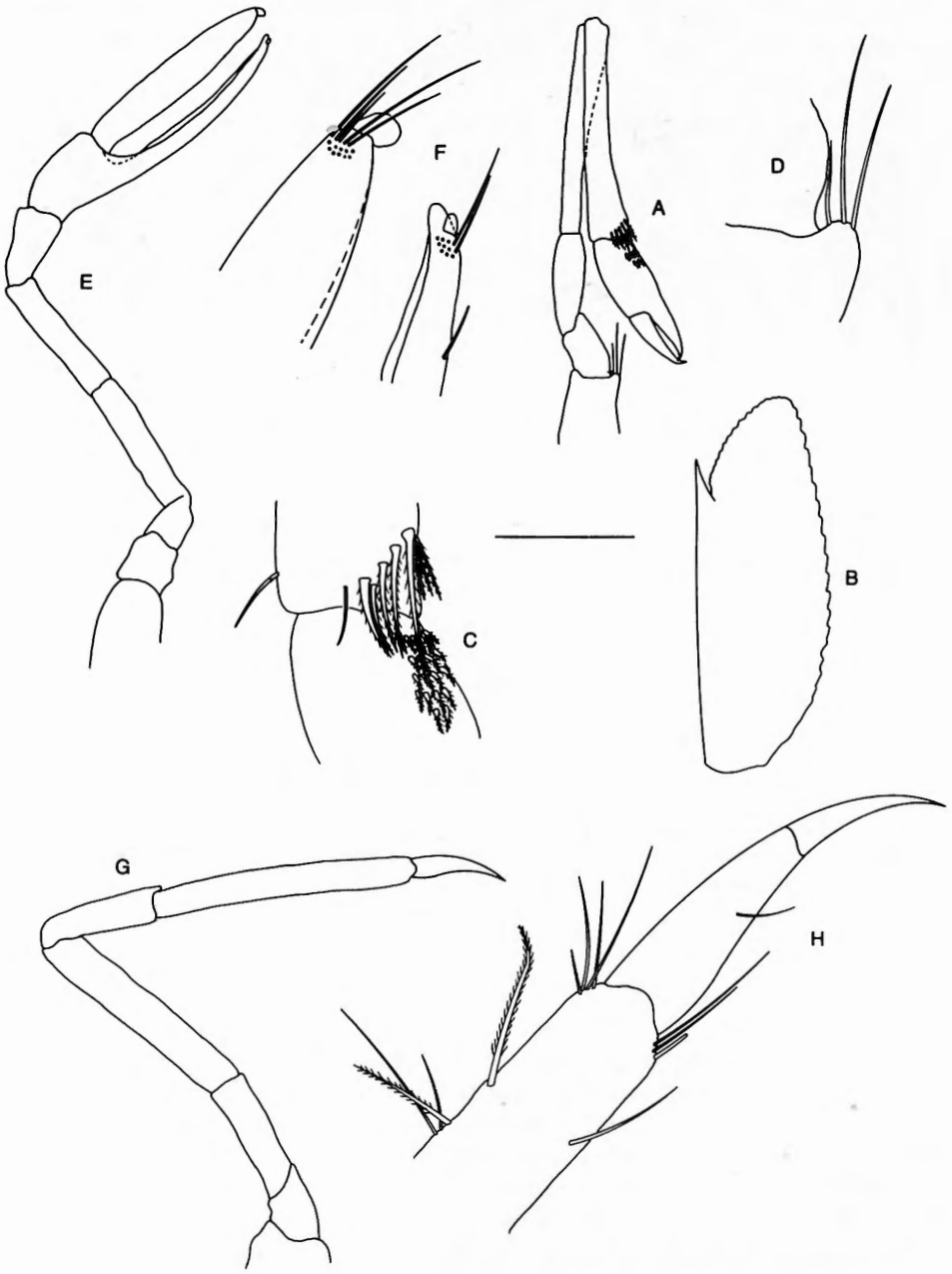


Fig. 2. – *Periclimenes watamuae* Bruce, Purar-I Reef, Hansa Bay, female: first pereopod (A), antennal scale (B), mesial aspect of carpo-propodal joint of first pereopod (C), coxal lobe of first pereopod (D); second pereopod (E), detail of chelae of second pereopod (F); third pereopod (G), dactylus of third pereopod (H). Scale bar indicates 0.1 mm (B, C, D, F, H) or 0.4 mm (A, E, G).

minal spines and six spines of decreasing length along lateral margin. Appendix interna approximately 1.3x as long as appendix masculina, with four subterminal cincinnuli.

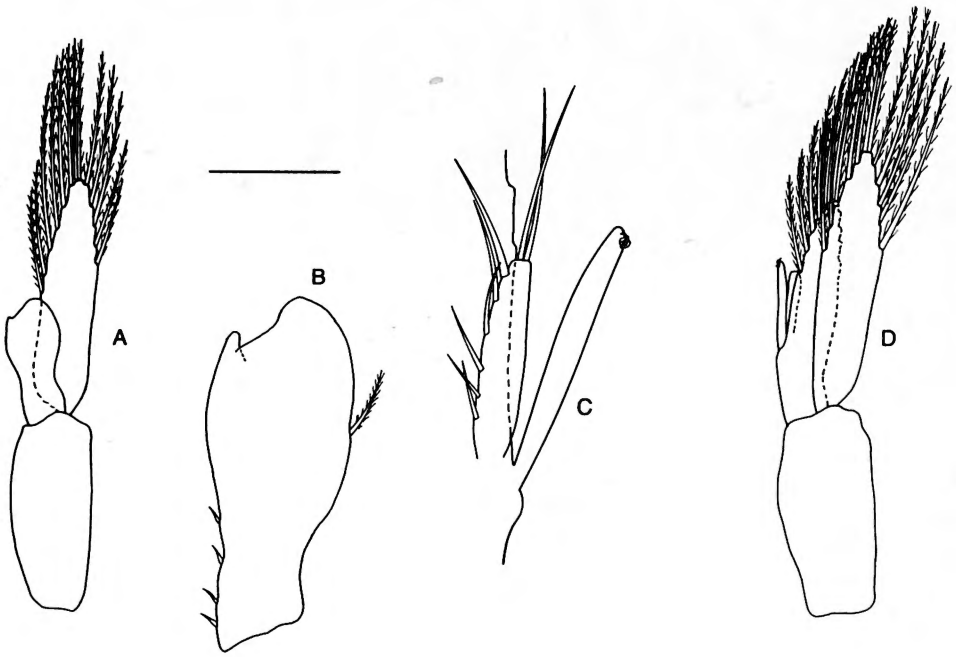


Fig. 3. – *Periclimenes watamuae* Bruce, Purar-I Reef, Hansa Bay, male: first pleopod (A), endopod of first pleopod (B), appendix masculina and appendix interna of second pleopod (C), second pleopod (D). Scale bar indicates 0.1 mm (B, C) or 0.3 mm (A, D).

Generally, the present specimens agree with the type description (BRUCE, 1976b) although some differences were noted, especially in the fingers of the second pereopod. The latter character is the single most useful interspecific character in the *P. diversipes* group, to which *P. watamuae* belongs. Although BRUCE (1976b) stated that the tip of the fingers are simple, Dr. C. H. J. M. Fransen on examination of the holotype and allotype found these to be bifid, as in the present specimens. Other minor differences, notably in the structure of the male secondary sexual appendages may be developmental in nature or simply structural variations in this poorly known species.

Colour in life. Body and appendages near totally transparent, with minute scattered red chromatophores over body

Distribution

Papua New Guinea (this report), Kenya (BRUCE, 1976), Seychelles (FRANSEN, 1994).

Hosts

Alcyonaria: unidentified sp., Gorgonacea: unidentified sp., Scleractinia: *H. actiniformis*, *Fungia* sp.

DISCUSSION

Five species of Pontoniinae were previously known to associate with *Heliofungia actiniformis*, and with *Periclimenes watamuae* this number is now raised to six. Of these, only *P. tenuipes* Borradaile, 1898, recorded by READ (1974) as occurring with *H. actiniformis*, has not yet been recorded from northern Papua New Guinea. As BRUCE (1983) regards *P. tenuipes* as a free-living species, its association may have been incidental.

It was noted in the field that the different shrimp species exhibited some form of habitat segregation on their host corals. Female *H. corallicola* were invariably perched on top of the mouth of *H. actiniformis* between the tentacles, while the smaller-sized males occurred between the tentacles. The cryptic colouration of this species, which resembles the tentacle tips of their hosts, renders them very inconspicuous. Individuals of *P. venustus* were much more mobile and roamed freely over the oral surface of the coral, darting around the tips of the tentacles.

The pair of *P. kororensis* were encountered between the tentacles, where their large size and bright colouration made them very conspicuous. In contrast to the other species, the specimens of *P. watamuae* were encountered on the aboral side of the coral, the side resting on the sand. *Metapontonia fungiacola* Bruce, 1967 occupies the latter habitat in parts of the Indian Ocean and the Ryukyu Islands, however this species has not been recorded in association with *H. actiniformis*, only with the coral genera *Fungia*, *Halomitra*, *Hydnophora* and *Goniastrea* (BRUCE, 1977b). On two occasions, individuals of *H. corallicola* and *P. venustus* were collected from the same host individual.

ACKNOWLEDGEMENTS

The author wishes to thank the Belgian Fund for Joint Basic Research and the King Leopold-III Fund for financial assistance and Dr. H. Wilkins for help with collecting. Dr. C.H.J.M. Fransen kindly examined the type material of *P. watamuae*.

REFERENCES

BORRADAILE, L.A. (1898) – A revision of the Pontoniidae. *Annals and Magazine of Natural History, series 7*, **2**: 376-391.

BRUCE, A.J. (1970) – Notes on some Indo-Pacific Pontoniinae. XV. *Hamopontonia corallicola* gen. nov., sp. nov., a new pontoniid shrimp from Hong Kong. *Crustaceana,* **18**(1): 37-48.

BRUCE, A.J. (1976a) – Shrimps and prawns of coral reefs, with special reference to commensalism. In: O. A. JONES & R. ENDEAN (eds.). *Biology and geology of coral reefs*, volume 3. Academic Press, New York: 37-94.

BRUCE, A.J. (1976b) – A report on a small collection of shrimps from the Kenya National Marine Parks at Malindi, with notes on selected species. *Zoologische Verhandelingen*, Leiden, **145**: 1-72.

BRUCE, A.J. (1977a) – The hosts of the coral-associated Indo-west-Pacific pontoniine shrimps. *Atoll Research Bulletin*, **205**: 1-19.

BRUCE, A.J. (1977b) – A report on a small collection of pontoniine shrimps from Queensland, Australia. *Crustaceana,* **33**(2): 167-181.

BRUCE, A.J. (1977c) – *Periclimenes kororensis* n. sp., an unusual shrimp associate of the fungiid coral, *Heliofungia actiniformis. Micronesica*, **13**(1): 33-43.

BRUCE, A.J. (1981) – Pontoniine shrimps of Heron Island. *Atoll Research Bulletin*, **245**: 1-33.

BRUCE, A.J. (1983a) – Expedition Rumphius II (1975). Crustaces parasites, commensaux, etc. (Th. MONOD ed.), IX: Crustaces Decapodes (1re partie: Natantia Pontoniinae. *Bulletin du Muséum National d'Histoire Naturelle*, Paris, series 4(5), section A, **3**: 871-902.

BRUCE, A.J. (1983b) – The pontoniine shrimp fauna of Australia. *Memoirs of the Australian Museum*, **18**: 195-218.

BRUCE, A.J. (1989) – A report on some coral reef shrimps from the Philippine Islands. *Asian Marine Biology*, **6**: 173-192.

BRUCE, A.J. (1990a) – Recent additions to the pontoniine shrimp fauna of Australia. *The Beagle*, **7**(2): 9-20.

BRUCE, A.J. (1990b) – A new cnidarian-associated shrimp from Port Essington, Cobourg Peninsula, Australia. *Indo-Malayan Zoology*, **6**: 229-243.

BRUCE, A.J. & A. SVOBODA (1984) – A report on a small collection of coelenterate-associated pontoniine shrimps from Cebu, Philippines Islands. *Asian Marine Biology*, **1**: 87-99.

CLAEREBOUDT, M., C. MASSIN & J. BOUILLON (1989) – A general survey of Laing Island environment (Papua New Guinea). *Indo-Malayan Zoology*, **6**: 1-23.

FRANSEN, C. H. J. M. (1989) – Notes on caridean shrimps collected during the Snellius-II expedition. I. Associates of Anthozoa. *Netherlands Journal of Sea Research,* **23**(2): 131-147.

FRANSEN, C.H.J.M. (1994) – Marine palaemonoid shrimps of the Netherlands Seychelles Expedition 1992-1993. *Zoologische Verhandelingen*, Leiden, **297**: 85-152.

MORGAN, G.J. (1988) – A checklist of decapod Crustacea from the Madang region, Papua New Guinea. *Science in New Guinea,* **14**(3): 124-139.

NOBILI, G. (1899) – Contribuzioni alla conoscenza della fauna carcinologica della Papuasia, delle Molucche e dell'Australia. *Annali del Museum Civico di Storia Naturale di Genova*, **20**(40): 473-523.

READ, K.R.H. (1974) – The rock islands of Palau. *Oceans,* **7**(6): 10-17.

SUZUKI, K. & K.-I. HAYASHI (1977) – Five Caridean shrimps associated with sea anemones in Central Japan. *Publications from the Seto Marine Biological Laboratory*, **24**(1/3): 193-208.

Belg. J. Zool. - Volume 128 (1998) - issue 1 - pages 23-34 - Brussels 1998

Received: 30 April 1997

MORPHOMETRIC AND ALLOZYME VARIATION IN NATURAL POPULATIONS AND CULTURED STRAINS OF THE NILE TILAPIA *OREOCHROMIS NILOTICUS* (TELEOSTEI, CICHLIDAE)

EMMANUEL J. VREVEN(1), BÉATRICE ADÉPO-GOURÈNE(2), JEAN-FRANÇOIS AGNÈSE(2,3) AND GUY G. TEUGELS(1)*

(1)Musée Royal de l'Afrique Centrale, Laboratoire d'Ichtyologie, B-3080 Tervuren, Belgium.
(2)Centre de Recherches Océanologiques, ORSTOM, Laboratoire de Génétique, BP V18, Abidjan, Cote d'Ivoire
(3)CNRS, UPR 9060, Université Montpellier II, F-34095 Montpellier cedex 5, France.
e-mail: teugels@africamuseum.be

Abstract. Morphometric and allozyme variation of nine natural populations and three cultured strains of *Oreochromis niloticus* has been studied. Natural populations from West Africa and the Nile, identified as the same subspecies, *O. niloticus niloticus*, differed significantly. The Nile populations are genetically closer to the population from Lake Edward, identified as *O. niloticus eduardianus*. Morphological differences were observed between natural populations and their cultured strains. These are undoubtedly related to ecophenotypic influences, because cultured strains are genetically related to their natural parental populations.

Key words: *Oreochromis niloticus*, natural populations, cultured strains, morphometry, allozymes, Africa.

INTRODUCTION

The Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) is endemic to Africa but has been introduced in many parts of the world for aquaculture. In particular the subspecies *O. niloticus niloticus* is one of the most cultured freshwater fishes with an estimated production of 426,773 mt in 1994 (Garibaldi, 1996).

The meristic and morphometric characters of this subspecies that are available in local faunal guides generally refer to local populations (e.g. DAGET, 1954 for specimens originating from the Upper Niger). TREWAVAS (1983) presented comparative data for specimens originating from throughout the major part of its distribution but these are based on relatively small samples. GOURÈNE & TEUGELS (1993) examined the morphometrics of cultured strains and noted differences between them. An overall comparison of the data published is difficult if not impossible as different characters were employed and their definition is not always identical.

Several authors have published on allozyme variation especially of cultured stocks of *O. niloticus* (e.g. BASIAO & TANIGUCHI, 1983; MCANDREW & MAJUMDAR, 1983; SEYOUM

& KORNFIELD, 1992). MACARANAS *et al.* (1995) compared allozymes of Asian farmed strains and several wild African populations. ROGNON *et al.* (1996) compared allozymes in cultured strains and in some natural West African populations. AGNÈSE *et al.* (1997) studied allozymes in natural populations from all over the distribution range of the species.

Herein is offered the first attempt to qualify and compare the morphometric and allozyme variation in the same material of *O. n. niloticus*. This forms part of an ongoing program on the characterization of natural populations and cultured strains of species used in aquaculture in West Africa and the Nile, in order to increase their production on the basis of rational use of genetic resources.

MATERIAL AND METHODS

Eight natural populations of *O.niloticus niloticus*, one natural population of *O. niloticus eduardianus* and three cultured strains were examined. All these were collected in Africa between August 1993 and December 1994 (Table I, Fig. 1). Natural populations were identified morphologically according to TREWAVAS (1983). The cultured strains originate from a natural population from the Volta basin (Volta strain), a crossbred of natural populations from the Volta basin and Lake Edward (Bouake strain) and natural populations from the Nile near Cairo and Lake Manzalla (Quarun strain).

TABLE I

List of natural populations and cultured strains examined of Oreochromis niloticus

	coordinates	*N (Morphometry)*	*Standard Length (mm)*	*N (allozyme study)*	*Abbreviation*
Natural Populations					
Dagana, Senegal	±16°31'N-15°30'E	18	77.4-252.6	63	DAG
Selingue, Mali	±11°37'N-8°14'E	24	95.0-138.5	58	SEL
Bamako, Mali	±12°39'N-8°00'E	17	73.5-219.4	22	BAM
Battor, Ghana	±6°04'N-0°25'E	7	180.0-252.4	7	BAT
Lake Chad, Chad	±13°20'N-14°00'E	20	140.6-279.3	22	CHA
N'Djamena, Chari, Chad	±12°07'N-15°03'E	17	97.9-156.9	30	SEL
Cairo, Nile, Egypt	±30°02'N-31°15'E	17	136.9-246.7	18	CAI
Lake Manzalla, Egypt	±31°15'N-32°00'E	16	122.1-178.0	30	MAN
Lake Edward, Uganda	±0°25'S-29°30'E	28	152.9-223.9	30	EDW
Cultured Strains					
Bouake strain		29	95.0-138.5	55	BKE
Volta strain		32	101.0-136.8	50	VOL
Quarun strain		20	154.7-225.5	20	QUA

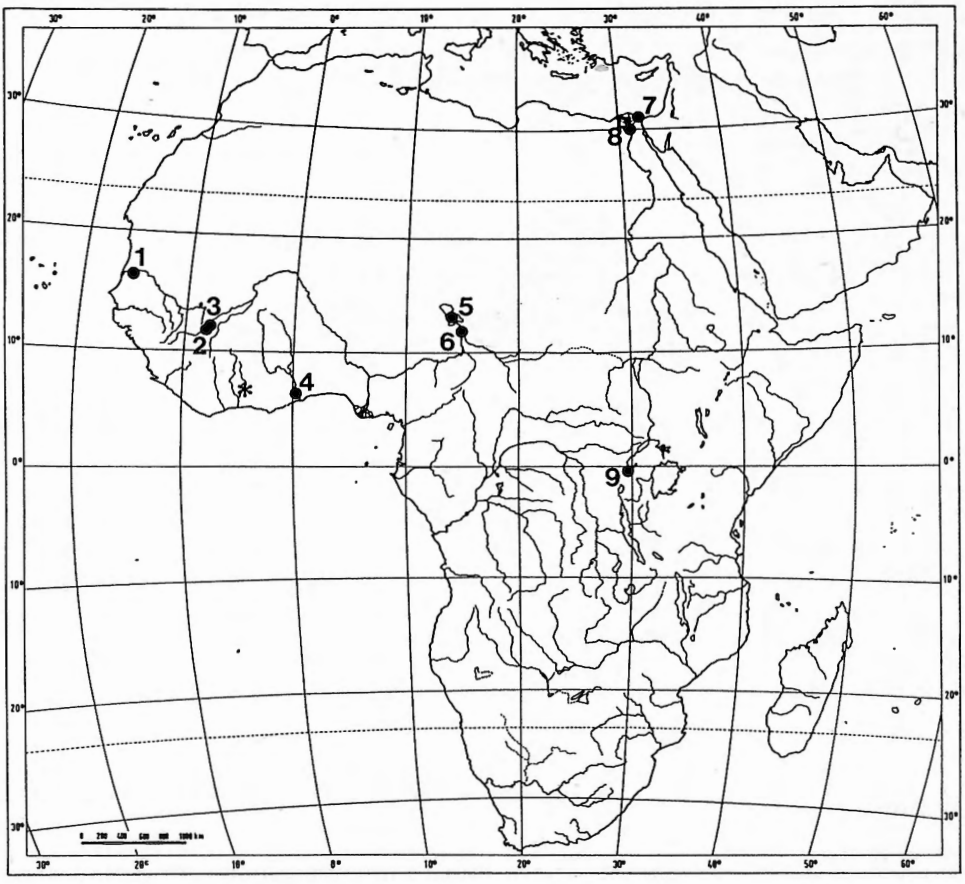


Fig. 1. – Geographical distribution of the natural populations and cultured strains examined of *O. niloticus*. 1 = Dagana (Senegal); 2 = Selingue (Mali); 3 = Bamako (Mali); 4 = Battor (Ghana); 5 = Lake Chad (Chad); 6 = N'Djamena (Chad); 7 = Lake Manzalla (Egypt); 8 = Cairo (Egypt); 9 = Lake Edward (Uganda). * = Bouake and Volta strains (= Quarun strain).

Twenty five measurements were taken on each specimen, for the morphometric analysis, using dial calipers (Fig. 2). Eight meristic counts were made on each fish: number of gill rakers on the lower part (cerato- + hypobranchial) of the first branchial arch; number of gill rakers on the complete first branchial arch; number of dorsal spines; number of soft dorsal-fin rays; number of anal spines; number of soft anal-fin rays; number of scales on the lower lateral line and number of scales on the upper lateral line. Due to preservation, it was not possible to obtain a complete data set for the meristic counts for some of the specimens. The results were evaluated by principal component analysis (PCA) using the CSS: STATISTICA package (Statsoft, versions 3.1 and 4.5). Only those specimens for which a complete data set was available were used in these analyses. Data were log transformed to fullfill the criteria of normality. The covariance matrix was used. As suggested

by HUMPHRIES *et al.* (1981) and BOOKSTEIN *et al.* (1985) the first principal component was interpretated as a size factor and the other components as shape factors, independent of size. Therefore the first principal component was not used.

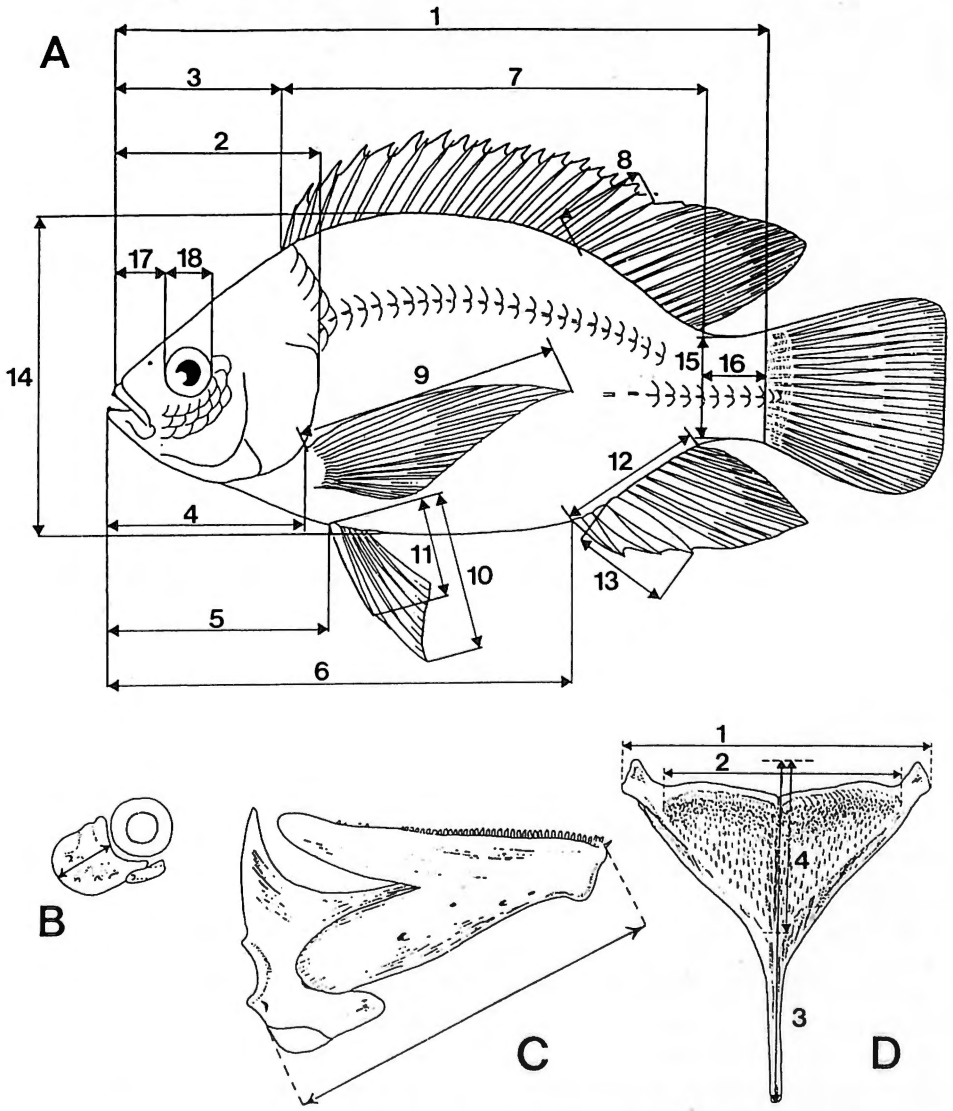


Fig. 2. – Measurements taken on the *O.niloticus* specimens. (A) 1. standard length (SL); 2. head length (HL); 3. predorsal length; 4. prepectoral length; 5. prepelvic length; 6. preanal length; 7. dorsal-fin length; 8. dorsal-spine length; 9. pectoral-fin length; 10. pelvic-fin length; 11. pelvic-spine length; 12. anal-fin length; 13. anal-spine length; 14. body depth; 15. caudal-peduncle depth; 16. caudal-peduncle length; 17. snout length and 18. eye diameter; (B) preorbital-bone depth; (C) lower-jaw length and (D) 1. total pharyngeal-bone width; 2. toothed pharyngeal-bone width; 3. total pharyngeal-bone length and 4. toothed pharyngeal-bone length.

For the allozyme variation, specimens were kept at -20°C for a few days and then stored at -80°C for later analysis. Standard horizontal starch gel (12%) electrophoresis was carried out to investigate the products of 25 loci. The stain protocols and buffers used were those described in POUYAUD & AGNÈSE (1995) and PASTEUR *et al.* (1987). The nomenclature is that proposed by SHAKLEE *et al.* (1990). The allozymic data were analysed with the phylogenetic software programme PHYLIP (PHYLIP software package, Felsenstein, version 3.5). A total of 100 randomly modified frequency matrices were obtained using the program SEQBOOT to build a genetic network. These matrices were then transformed into Nei's (1978) genetic distance matrices using the GENEDIST program. The corresponding trees were built by neighbourjoining by the program NEIGHBOR and summarized into a single tree using CONSENSE (bootstrapping).

RESULTS

Morphometric variation

Natural populations from West Africa, Egypt and Lake Edward

Principal component analysis on 25 metric variables performed on 142 specimens of *O.niloticus* belonging to 9 natural populations is illustrated in Fig. 3. PCI accounts for 96.0%, PCII for 1.0 % and PCIII for 0.6% of the observed variance. All specimens from Egypt (Cairo and Lake Manzalla) and Lake Edward, except one, are located on the negative sector of the second component, while the majority of specimens from West Africa are located on the positive sector of this component. The latter is defined mainly by the caudal peduncle length, the toothed pharyngeal bone length and width. Interestingly, the Bamako specimens hardly overlap with the specimens from Selingue; both localities are situated on the Upper Niger in Mali (Fig. 1). All other West African populations are largely overlapping and cannot be distinguished from each other on the second or the third component.

All natural populations and the Bouake strain

The plot of a principal component analysis on 25 metric variables for 169 specimens of *O.niloticus* belonging to 9 natural populations and the cultured Bouake strain is given in Fig. 4a. PCI accounts for 96.2%, PCII for 1.0% and PCIII for 0.8% of the observed variance. The cultured strain results from an interbreeding between specimens from the Volta basin and Lake Edward. This is however not discernible from the results obtained: a slight overlap is noted between the Bouake strain and the Battor (Volta) population, but the Bouake strain is completely separated from the population of Lake Edward. The latter is almost entirely located on the negative sector, while all Bouake specimens are situated on the positive sector of the second component, which is defined mainly by the toothed pharyngeal bone length, the anal spine length and the body depth.

All the natural populations, the Bouake and the Volta strains

Fig. 4b illustrates the plot of a principal component analysis on 25 metric variables for 200 specimens of *O.niloticus* belonging to 9 natural populations and two cultured strains.

PCI accounts for 95.8%, PCII for 1.0% and PCIII for 0.8% of the observed variance. The Volta strain, descending from a natural population of the Volta basin, only slightly overlaps with the Battor (=Volta) population. Interestingly, the Volta strain almost completely overlaps with the Bouake strain.

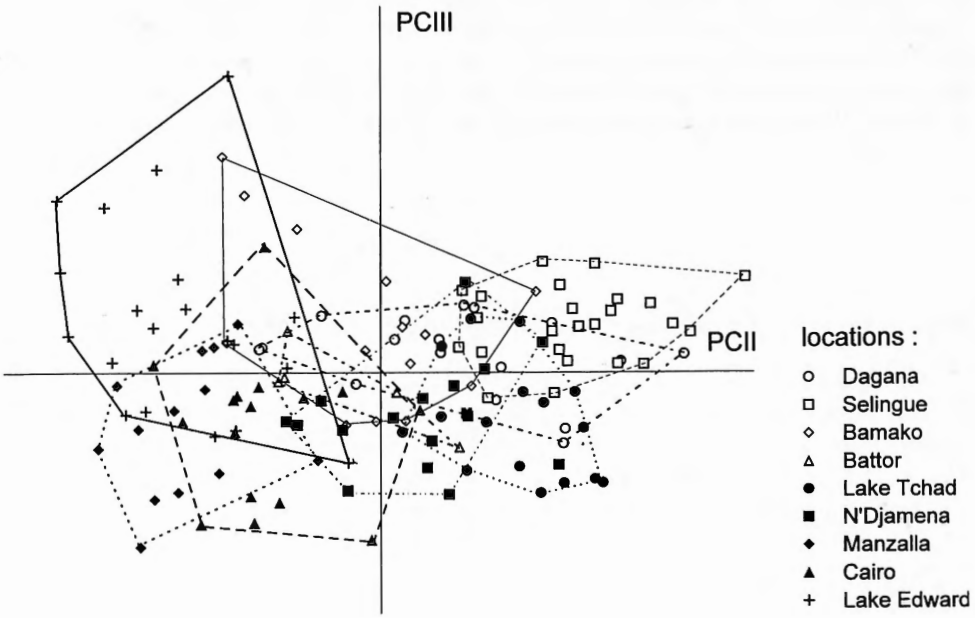


Fig. 3

Fig. 3. – Plot of a principal component analysis on log tranformed data of 25 metric variables for 142 specimens of *O.niloticus* originating from 9 natural populations from West Africa, the Nile and Lake Edward. For locality data see Table I.

Fig. 4. – (a) Plot of a principal component analysis (PCA) on log transformed data of 25 metric variables for 169 specimens of *O.niloticus* originating from 9 natural populations from West Africa, the Nile and Lake Edward and the cultured Bouake strain. The populations of Battor, Lake Edward (natural populations) and Bouake (cultured strain) are outlined. (b) Plot of a PCA on log transformed data of 25 metric variables for 200 specimens of *O.niloticus* from 9 natural populations from West Africa, the Nile and Lake Edward and the cultured Bouake and Volta strains. The populations of Battor (natural population) and Volta (cultured strain) are outlined. (c) Plot of a PCA on log transformed data of 25 metric variables for 220 specimens from 9 natural populations from West Africa, the Nile, Lake Edward and the cultured Bouake, Volta and Quarun strains. The populations of Cairo and Manzalla together (natural population) and Quarun (cultured strain) are outlined. Dagana (○); Selingue (□); Bamako (◇); Battor (△); Lake Tchad (●); N'Djamena (■); Manzalla (◆); Cairo (▲); Lake Edward (+); Bouake strain (✳); Volta strain (○) and Quarun strain (□). For locality data see Table I.

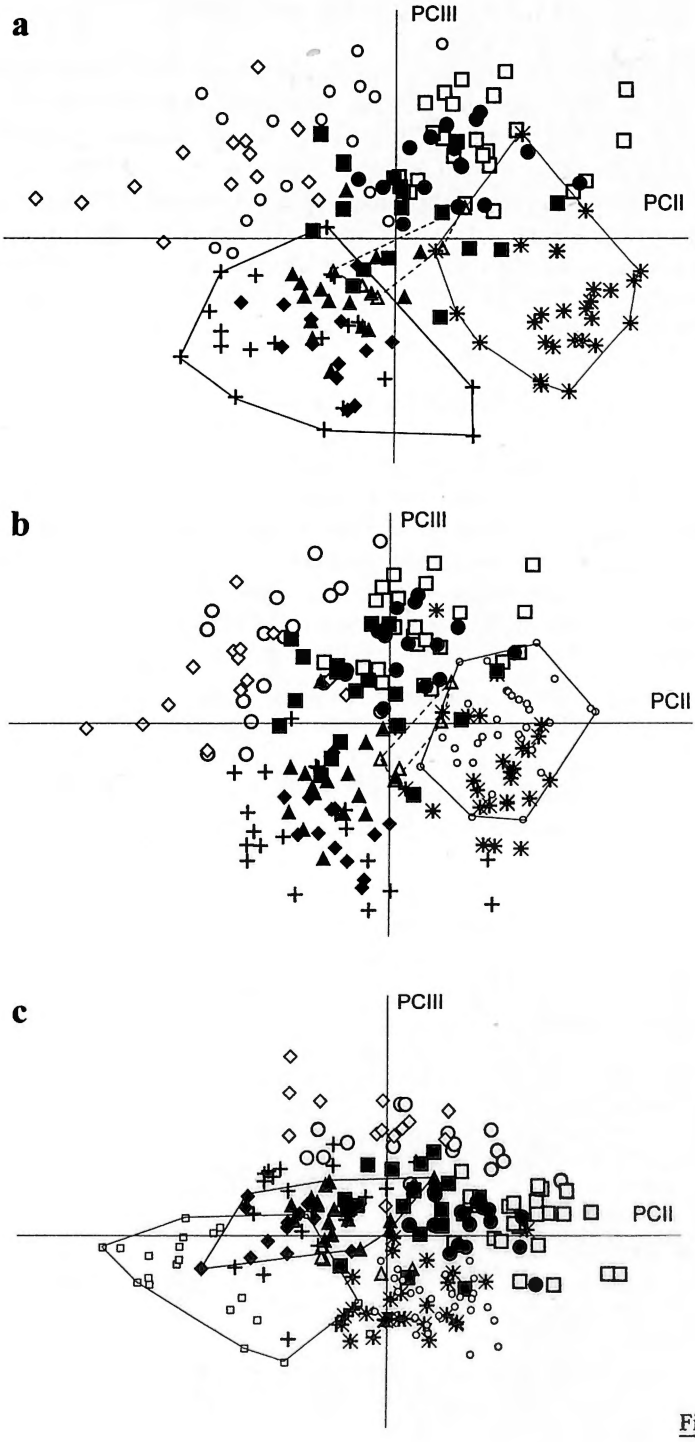


Fig. 4

All natural populations, the Bouake, Volta and Quarun strain

Fig. 4c shows the plot of a principal component analysis on 25 metric variables for 220 specimens of *O.niloticus* belonging to 9 natural populations and three cultured strains. PCI accounts for 95.1%, PCII for 1.1% and PCIII for 1.0% of the observed variance. Only a small part of the Lake Manzalla and Cairo specimens overlap with the Quarun strain polygone. Noteworthy in this figure is that most of the specimens belonging to cultured strains are located on the negative sector of the third component, while the majority of specimens from natural populations are situated on the positive sector of this component. The third component in this analysis is defined mainly by the lower jaw length, the body depth and the pelvic fin length.

Allozyme variation

Thirteen of the 25 loci studied were polymorphic (Table 2). The rate of observed heterozygosity (H) was between 0.01 (Lake Edward) and 0.047 (Quarun) and the rate of observed polymorphism (P95%) between 0.04 (Niger River at Selingue and Lake Edward) and 0.16 (Quarun). The values are comparable to those obtained in previous studies of natural *O. niloticus* populations (Seyoum & Kornfield, 1992) even if the loci analyzed were not the same as in the present study. Cultured strains did not show lower H and P values than natural populations, indicating that they did not loose genetic polymorphism.

The populations are clustered in two major genetic groups (Fig. 5). One cluster is composed of the natural populations from the Nile drainage (Manzalla, Cairo and Lake Edward) with two cultured strains (Quarun and Bouake). The second major group is composed of the West African natural populations (Dagana, Selingue, Bamako, N'djamena, Chad and Volta) with one cultured strain (Volta).

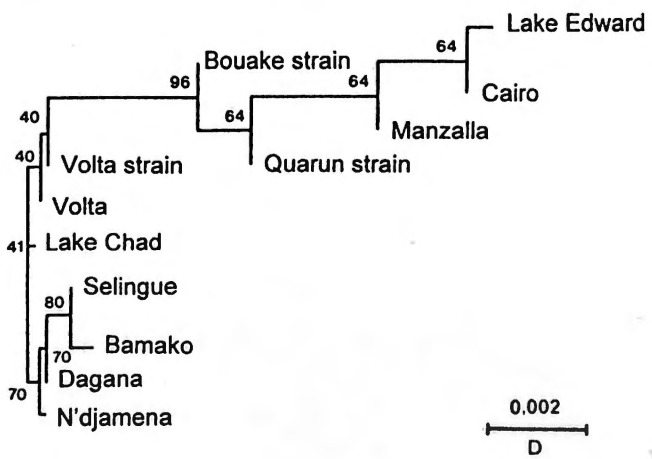


Fig. 5. – Consensus unrooted tree produced by neighbourjoining with the phylogenetic software PHYLIP for 9 natural populations and 3 cultured strains of *O. niloticus*. Number at each node indicates the percentage obtained using bootstrapping.

TABLE II

Allelic frequencies at polymorphic loci observed in 9 natural populations and 3 cultured strains of O. niloticus. *For abbreviations see Table I*

pop. locus	*DAG*	*SEL*	*BAM*	*BKE*	*VOL*	*BAT*	*CHA*	*NDJ*	*CAI*	*QUA*	*MAN*	*EDW*
AAT-2												
(N)	63	58	22	52	50	06	17	27	17	20	30	27
A	.52	.82	.93	.47	.34	.42	.44	.50	.06	.00	.02	
B	.48	.18	.07	.53	.66	.58	.56	.50	.94	1.00	.98	1.00
C												
AAT-3												
(N)	63	58	22	55	50	07	22	30	18	19	29	27
A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.97	.74	.88	1.00
B									.03	.26	.12	
ADH												
(N)	63	58	22	55	50	07	20	30	18	20	30	30
A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
B												
CK-1												
(N)	63	58	22	55	50	07	20	30	18	20	30	30
A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.98	1.00	1.00	1.00	1.00
B								.02				
CK-2												
(N)	63	58	22	55	50	07	20	28	18	20	30	30
A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.89	1.00	1.00	1.00	1.00
B								.11				
FBP-2												
(N)	63	57	22	55	50	07	21	30	18	20	30	28
A	1.00	1.00	1.00	.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.84
B				.01								.16
FH												
(N)	63	58	22	23	50	07	22	30	15	20	41	30
A	1.00	1.00	1.00	.44	1.00	1.00	1.00	1.00	.07	.55	.28	
B				.56					.93	.45	.72	1.00
IDHP-1												
(N)	62	58	22	55	50	07	22	30	18	20	30	30
A	.97	1.00	1.00	1.00	1.00	1.00	1.00	.98	1.00	1.00	1.00	1.00
B	.03							.02				
LDH-2												
(N)	62	58	22	55	50	07	20	30	18	20	30	30
A	1.00	1.00	1.00	1.00	.87	.86	1.00	1.00	1.00	1.00	1.00	1.00
B					.13	.14						
PGM												
(N)	62	58	22	55	50	07	20	30	17	20	30	30
A	1.00	1.00	1.00	1.00	1.00	1.00	.93	1.00	1.00	1.00	.97	1.00
B							.07				.03	
PT-1												
(N)	62	58	22	55	50	06	22	30	18	20	30	30
A	1.00	.99	.93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
B		.01	.07									
PT-2												
(N)	62	56	18	52	50	06	22	30	18	20	30	30
A	1.00	.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
B		.01										
SOD												
(N)	62	58	22	55	50	07	22	30	18	20	30	30
A	.78	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.97	.93	.97	1.00
B	.22								.03	.07	.03	
A	1.2	1.1	1.2	1.1	1.1	1.1	1.1	1.2	1.2	1.2	1.2	1.0
$P_{95\%}$	8.0	4.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	16.0	8.0	4.0
$P_{99\%}$	16	4.0	8.0	8.0	12	8.0	8.0	16	16	16	20	4.0
H%	3.6	1.2	1.1	3.7	2.6	4.5	2.7	3.3	1.4	4.7	3.0	1.0

DISCUSSION

The morphometric analysis of the natural populations originating from West Africa did not reveal clear differences between them. The separation of the Selingue and Bamako populations, both from the Upper Niger, and isolated by a relatively short geographical distance (±110 km), is explained by the isolated position of the former which lives in a man-made lake. All the natural populations from West Africa originate from Sahelo-Sudanian river systems (Senegal, Niger, Volta and Chad) (TREWAVAS, 1983; LÉVÊQUE *et al.*, 1991). Climatological (extension and recession of water bodies) and geological (tectonic activities such as earth movements, faulting, volcanism, and erosion) events during the Late Quaternary largely explain the similarities in faunal composition between them and, in this case, between the natural populations of *O. niloticus* (HUGUENY & LÉVÊQUE, 1994). It should also be noted that during the rainy season, the upper reaches of most of these basins are in contact and faunal exchanges can occur.

According to TREWAVAS (1983), natural populations from the Lower Nile system in Egypt belong to the same subspecies as those from West Africa, *O. niloticus niloticus*. Morphometrically, however, the majority of specimens of both geographic regions can be distinguished from each other. Moreover, the Nile specimens are morphologically closer to the Lake Edward specimens, which, following TREWAVAS (1983) belong to another subspecies *O. niloticus eduardianus*. This is confirmed by the allozyme study. Therefore the subspecific status for the Nile specimens as defined by TREWAVAS (1983) is called into question; further research is necessary.

Morphologically, the parental populations (Volta and Lake Edward, in particular the latter) are markedly different from the Bouake strain. Genetically however, the Bouake strain possesses two specific alleles of the Lake Edward population (FBP-2*B and FH*B) although the specific allele of the Volta (LDH-2*B) could not be detected. This clearly demonstrates that the morphology of the cultured strain has been seriously influenced by conditions in captivity, resulting in a different phenotype.

Concerning the Volta strain, only a small overlap in external morphology is found with the parental population. The small sample size of the latter however, does not enable firm final conclusions concerning the degree of difference in external morphology between them. Genetically, the Volta strain possesses the Volta specific LDH-2*B allele. Further, the Volta strain has a slightly higher number of alleles per locus when compared with the natural Battor (Volta) population, which is probably due to the small sample size of the latter. On the contrary, the H value of the Volta strain is lower. Those two results indicate that there is probably inbreeding, as the consanguinity within a population does not affect the number of alleles of the populations but decreases the number of heterozygotes.

Regarding the Quarun strain, only few specimens overlap with the Manzalla and Cairo specimens (parental populations). Again captive conditions resulted in a different ecophenotype. Genetically the Quarun population is close to the Lake Manzalla and Cairo populations. These three samples possess AAT-3*B, an allele which is characteristic (private) for the Nile populations.

Genetic polymorphism between the three cultured strains and their parental populations was comparable. ROGNON *et al.*, 1996 observed that cultured strains of tilapia have

sometimes higher H and P values compared to wild populations. We suspect that they erroneously mixed the origin of cultured strains and wild populations because no alien allele has been found in these populations, which excludes the possibility of interbreeding.

Morphological differences between cultured strains of *O. niloticus* were already reported by GOURENE &TEUGELS (1993). An overall comparison of all natural populations and all cultured strains examined, showed also important morphological differences between both. It is thus obvious that in captivity the external morphology will be considerably influenced by environmental conditions. MEYER (1987) reported on phenotypic plasticity in a Neotropical cichlid, *Cichlasoma managuense* caused by differences in diet and possibly in feeding mode during ontogeny. Other factors however, such as the lack of currents in culture ponds, undoubtedly affect the external morphology; this is expressed, for example, in the difference in body depth. The degree to which captivity conditions affect the growth rate and thus the aquaculture productivity is presently being studied.

ACKNOWLEDGEMENTS

The authors wish to thank R. Berthonnet, S. Gilles, J. Lemoalle, A. Pariselle, L. Pouyaud, J.C. Thouvenel and J. L. Zeddam for their invaluable help in collecting the material, as well as S. Norris and two anonymous referees for reading and commenting on the manuscript. This work was supported by the European Community (contract n° ERBTS3*CT920079) and ORSTOM (Institut Français de Recherche pour le Développement en Coopération).

REFERENCES

AGNÈSE, J.-F., B. ADÉPO-GOURÈNE, E.K. ABBAN & Y. FERMON (1997) – Genetic differentiation among natural populations of the Nile tilapia *Oreochromis niloticus* (Teleostei, Cichlidae). *Heredity*, **79**: 88-96.

BASIO, Z. U. & N. TANIGUCHI (1983) – An investigation of enzyme and other protein polymorphisms in Japanese stocks of the tilapias *Oreochromis niloticus* and *Tilapia zillii*. *Aquaculture*, **38**: 335-345.

BOOKSTEIN, F., B. CHERNOFF, R. ELDER, J. HUMPHRIES, G. SMITH & R. STRAUSS (1985) – Morphometrics in evolutionary biology. *Acad. Nat. Sci. Philadelphia*, Special Publication **15**: 1-277.

DAGET, J. (1954) – Les poissons du Niger supérieur. *Mém. I.F.A.N.*, **36**: 1-391.

GARIBALDI, L. (1996) – List of animal species used in aquaculture. *FAO Fisheries Circular*, **914**: 38 p.

GOURÈNE, G. & G.G. TEUGELS (1993) – Différenciation morphologique de souches des tilapias *Oreochromis niloticus* et *O. aureus* (Teleostei; Cichlidae) utilisées en pisciculture. *Cybium*, **17**: 343-355.

HUGUENY, B. & C. LÉVÊQUE (1994). – Freshwater fish zoogeography in West Africa: faunal similarities between river basins. *Environmental Biology of fishes*, **39**: 365-380.

HUMPHRIES, J.M., F.L. BOOKSTEIN, B. CHERNOFF, G.R. SMITH, R.L. ELDER & S.G. POSS (1981) – Multivariate discrimination by shape in relation to size. *Syst. Zool.*, **30**: 291-308.

LÉVÊQUE, C., D. PAUGY & G.G. TEUGELS (1991) – Annotated check-list of the freshwater fishes of the Nilo-sudan river basins, in Africa. *Rev. Hydrobiol. trop.*, **24**: 131-154.

MACARANAS, J.M., L.Q. AGUSTIN, MA. C. A. ABLAN, MA. J.R. PANTE, A.A. EKNATH & R.S.V. PULLIN (1995) – Genetic improvement of farmed tilapias: biochemical characterisation of strain differences in *Tilapia. Aquaculture international*, **3**: 43-54.

MCANDREW, B. J. & K.C. MAJUMDAR (1983) – Tilapia stock identification using electrophoretic markers. *Aquaculture*, **30**: 249-261.

MEYER, A. (1987) – Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. *Evolution*, **41**: 1357-1369.

NEI, M. (1978) – The theory of genetic distance and evolution of human race. *Jap. J. Hum. Genet.*, **23**: 341-369.

PASTEUR, N., G. PASTEUR, F. BONHOMME, J. CATALAN, & J. BRITTON-DAVIDIAN (1987) – Practical Isozyme genetics. Hellis Horwood Ltd., Chichester, 215 pp.

POUYAUD, L. & J.-F. AGNÈSE (1995) – Phylogenetic relationships between 21 species of three tilapiine genera *Tilapia*, *Sarotherodon* and *Oreochromis* using allozyme data. *J. Fish Biol.*, **47**: 26-38.

ROGNON, X., M. ANDRIAMANGA, B. MCANDREW, & R. GUYOMARD (1996) – Allozyme variation in natural and cultured populations in two Tilapia species: *Oreochromis niloticus* and *Tilapia zillii*. *Heredity*, **76**:640-650.

SEYOUM, S. & I. KORNFIELD (1992) – Taxonomic notes on the *Oreochromis niloticus* subspecies-complex (Pisces:Cichlidae), with a description of a new subspecies. *Can. J. Zool.*, **70**: 2161-2165.

SHAKLEE, J. B., F.W. ALLENDORF, D.C. MORIZOT & G.S. WHITT (1990) – Gene nomenclature for protein coding loci in fish. *Trans. Amer. Fish. Soc.*, **119**: 2-15.

TREWAVAS, E. (1983) – *Tilapiine fishes of the genera* Sarotherodon, Oreochromis *and* Danakilia. British Museum (Natural History), London.

Belg. J. Zool. - Volume 128 (1998) - issue 1 - pages 35-47 - Brussels 1998

Received: 3 December 1997

NOTES ON THE TAXONOMY AND DISTRIBUTION OF THE INTERSTITIAL ROTIFERA FROM A DUNE POOL

HENDRIK SEGERS

Laboratory of Animal Ecology, Zoogeography and Nature Conservation
Department of Biology, University of Gent
K.L. Ledeganckstraat 35, B - 9000 Gent
e-mail: Hendrik.Segers@rug.ac.be

Abstract. A preliminary study of the interstitial Rotifera from a dune pool at the Belgian coast yielded several rare and insufficiently known species, namely *Colurella salina* Althaus, *Encentrum villosum* Harring & Myers and *Lecane psammophila* (Wiszniewski). Brief accounts are presented on these and on *Colurella hindenburgi* Steinecke (new synonym: *C. geophila* Donner) and *Trichocerca taurocephala* (Hauer). Of the twenty-five morphotaxa recorded, nine, all of which are psammobionts, are new to the Belgian fauna. Since, the rotifers inhabiting the psammon have not been studied sufficiently, their contribution to species diversity in freshwater habitats is not recognised.

Key words: psammon Rotifera, taxonomy, distribution, new records.

INTRODUCTION

Rotifera inhabiting the psammon of stagnant waters have been studied only rarely since the 1930s, when WISZNIEWSKI (1934a, b, 1935, 1936) and NEISWESTNOWA-SHADINA (1935) in Europe, and MYERS (1936) in North America conducted extensive researches on the rotifer fauna living in the interstices of sand grains. More recently, interstitial rotifers of marine and brackish water habitats have been studied by ALTHAUS (1957a) and TZSCHASCHEL (1979, 1980), while SCHMID-ARAYA (1995a, 1995b) worked on rotifers in river bed sediments and TURNER (1990, 1993, 1995) and TURNER & PALMER (1996) published contributions on interstitial rotifers of both fresh and saline waters in North America (For a review see SCHMID-ARAYA, in press). These studies revealed that the rotifer fauna of the psammon habitat consists of a diverse taxocoenosis which includes a number of specialised taxa (TURNER & DISTLER, 1995).

Interstitial rotifers of dune pools have not been studied so far. In this paper results are presented of a preliminary study of such rotifers, sampled from the hygropsammon (*sensu* WISZNIEWSKI, 1934c) of a dune pool («Eendeput») in the nature reserve «De Fonteintjes» in Zeebrugge, Belgium. The rotifer content of these samples, and some comments regarding the occurrence of interstitial rotifers are presented here.

MATERIAL AND METHODS

The samples studied consist of ca. 50 cc of sand of the top 0.5 cm hygropsammon of the «Eendeput», a dune pool in the nature reserve «De Fonteintjes», Zeebrugge, Belgium. They were collected on 22 April and 12 July 1997, and fixed in formaldehyde (4%). Animals were selected under a Wild M10 dissection microscope, and examined and drawn using an Olympus CH2 research microscope with drawing tube. Permanent slides containing reference specimens are deposited in the collection of the Laboratory of Animal Ecology, University of Gent. Scanning electron microscopy was carried out using a JEOL JSM-840 microscope on trophi material processed following SEGERS (1993) and SEGERS & DUMONT (1993).

TAXONOMY

A list of the Rotifera identified from the psammon samples is given in Table 1. Of the 25 morphotaxa found, nine are new to the Belgian fauna (see DE RIDDER, 1989, 1992). Some of these, treated below, are particularly noteworthy on account of their rarity, or require taxonomic treatment.

TABLE 1

List of Rotifera from the psammon of De Eendeput, Fonteintjes Nature reserve, Zeebrugge, Belgium

*: new to the Belgian fauna; 1: 22 April 1997; 2: 12 July 1997; a: abundant (>20 specimens), c: common (6-20 specimens), r: rare (2-5 specimens), s: single specimen.

Asplanchna girodi (De Guerne, 1850): 2c
Colurella adriatica Ehrenberg, 1831: 1r (fig. 1)
**C.* cf. *anodonta* Carlin, 1939: 1s (figs 2-4)
C. colurus (Ehrenberg, 1830): 1c, 2c (figs 5-7)
C. hindenburgi Steinecke, 1917: 1r, 2c (figs 8-10)
**C. salina* Althaus, 1957: 1c (figs 11-12)
**C. sinistra* Carlin, 1939: 1s (fig. 13)
Cephalodella catellina (Müller, 1786): 2c
C. exigua (Gosse, 1886): 1a
C. gibba (Ehrenberg, 1832: 1c
**C. gibba microdactyla* Koch-Althaus, 1963: 2c
C. gracilis (Ehrenberg, 1832): 1s
**C. megalocephala* (Glascott, 1893): 1s, 2r
**C. sterea* (Gosse, 1887): 2r
**Encentrum villosum* Harring & Myers, 1928: 1c, 2r (fig. 27-28)
Euchlanis dilatata Ehrenberg, 1832: 2r
Keratella cochlearis (Gosse, 1851)(incl. f. *tecta* (Gosse, 1851)): 2a
Lecane closterocerca (Schmarda, 1859): 1r, 2a
L. lunaris (Ehrenberg, 1832): 2s
**L. psammophila* (Wiszniewski, 1932): 1a, 2a (figs 14-16)
Lepadella patella (Müller, 1773): 2r
Lindia torulosa Dujardin, 1841: 2r
Pompholyx sulcata Hudson, 1885: 2a
**Trichocerca taurocephala* (Hauer, 1931): 1a, 2a (figs 17-21, 29-31)
T. tenuior (Gosse, 1886): 1r

Family Colurellidae

Of the four genera belonging to Colurellidae, the taxonomy and, consequently, identification of members of *Colurella* Bory de St. Vincent, 1824 is by far the most difficult. This is because of their generally similar body (lorica) morphology and high intraspecific variability. Specimens of *Colurella* species are rarely found, and in many published records are mistakenly identified. As a contribution to the knowledge of the genus, illustrations of all members of *Colurella* encountered during this survey are given.

Colurella hindenburgi Steinecke, 1917
(Figs 8-10)

Synonyms: *C. gastracantha* Hauer, 1924
C. hindenburgi gastracantha: Wiszniewski (1953)
C. hindenburgi f. *gastracantha*: Koste (1978)
C. geophila Donner, 1951 (new synonym)
C. geophila hallensis Althaus, 1957
C. geophila f. *hallensis*: Koste (1978)

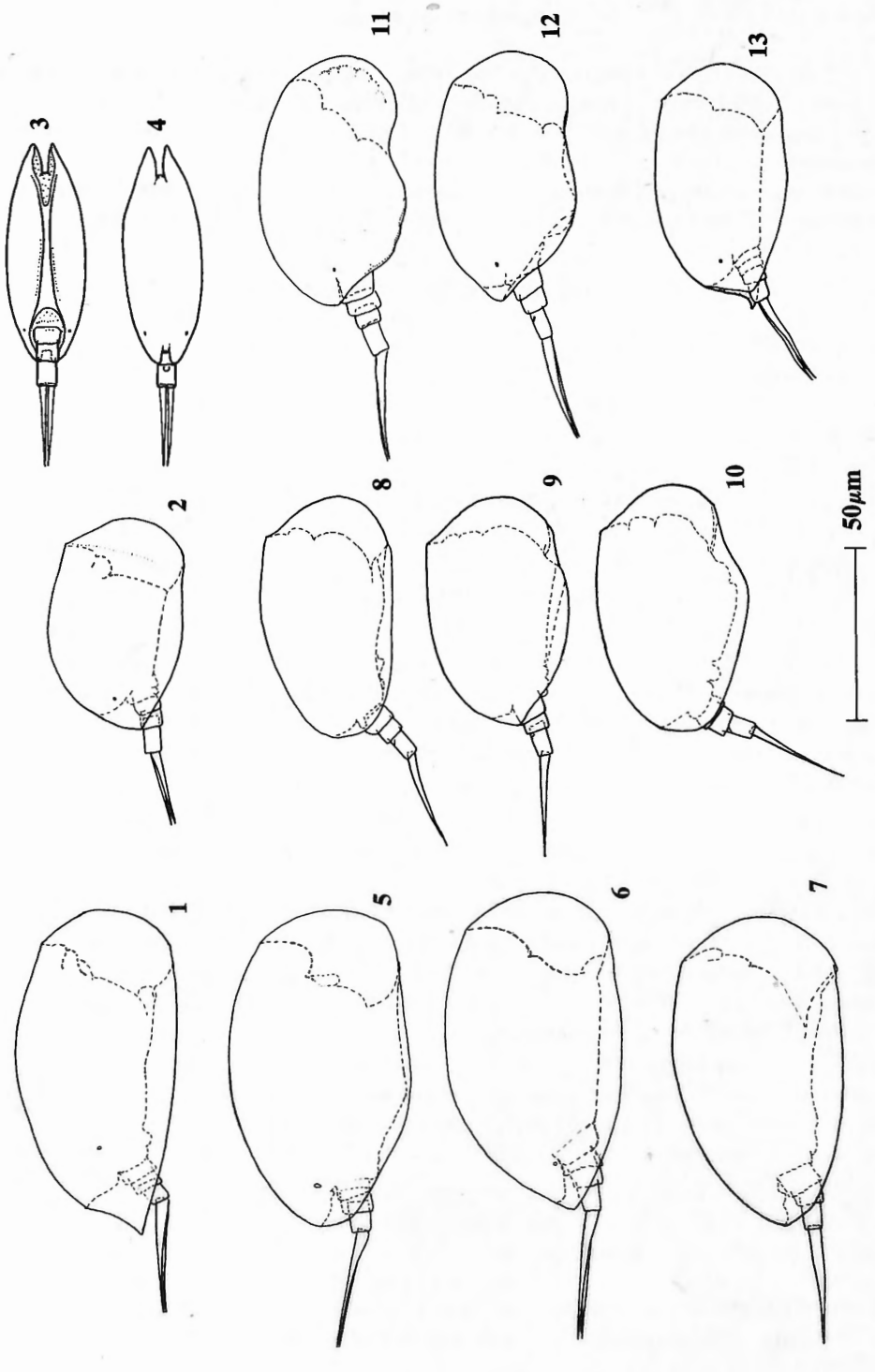
Steinecke (1917) p. 90, 97 figs 4a-b; Hauer (1924) p. 177-180 figs 1, 2; Donner (1951) p. 637-638 fig. 27; Wiszniewski (1954) p. 38; Althaus (1957a) p. 132 fig. 21e, f; Koste (1978) p. 167-170.

Differential diagnosis: *Colurella hindenburgi* has relatively long lorica and toes, when compared to *C. obtusa* (Gosse). The dorsal margin of the lorica is straight anteriorly, curved posteriorly (evenly curved in *C. colurus* (Ehrenberg)). The head aperture is dorsally marked by a notch, the foot groove extends to dorsally.

Measurements: Lorica length 70-75 µm, height 38-44 µm, toe length 30-33 µm.

Comments: *Colurella hindenburgi* is a variable species with respect to size and certain aspects of lorica shape (see Donner, 1970; Koste, 1978). Notwithstanding the fact that the synonymy of *C. hindenburgi* and *C. gastracantha* has long been recognised, the latter name has remained in use to denote an infrasubspecific variant of the former, characterised by the presence of a projection anterior to the insertion of the foot. The presence of such a projection, however, appears to result from the formation of transverse folds of the relatively soft membrane of the ventral sulcus, in conjunction with mobility of the foot. As such, it has no ecological or taxonomical relevance. Although it may be informative to separate ecologically relevant forms of polymorphous species (*e.g.*, environmentally-induced spined forms of *Brachionus* species) by using infrasubspecific names, this is not the case here. I therefore suggest that the use of f. *gastracantha*, for the above-mentioned variant be abandoned.

According to Donner (1951), *C. geophila* differs from the *C. hindenburgi-C. gastracantha* group by the uniformly high dorsal margin (anteriorly higher in *C. gastracantha*), and by the deep head and foot apertures. As can be judged from Figs 8-10, these features are subject to variation within the same population. In fact, the specimen illustrated in Fig. 8 strikingly resembles Donner's (1951) drawing of *C. geophila*, while Figs 9-10 correspond to *C. hindenburgi*. This suggests that the two are synonyms. The *C. geophila hal-*


1
2
3
4
5
6
7
8
9
10
11
12
13
50µm

lensis of ALTHAUS (1957a), considered a variant of *C. geophila* by KOSTE (1978), indeed appears to be nothing more than that. *C. geophila limnetica* Althaus, 1957a probably belongs to *C. colurus*, as far as can be judged from the poor description while *C. subtilis* Althaus, 1957c (*nom. nov.* for *C. gracilis* Althaus, 1957a *non* (Hilgendorf, 1898); *C. geophila* f. *gracilis* after Koste, 1978) is unidentifiable and may represent an artifact: the description of the single specimen known is reminiscent of specimens of *C. colurus* that have been rolled around their longitudinal axis under slight compression.

Colurella salina Althaus, 1957
(Figs 11-12)

Synonym: ?*Colurella colurus* (Ehrenberg) after Althaus, 1957b (partim: fig. 2, microphotograph)

ALTHAUS (1957a) p. 134 figs 25a-h; KOSTE (1978) p. 173 plate 54 figs 1a-f.

Differential diagnosis: *C. salina* is a medium-sized *Colurella* spcies that can be distinguished from congeners by the absence of a deep ventral furrow, by the characteristic ventral margin with clear postoral concavity and medially convex margin, and by being relatively broad.

Measurements: Lorica length 70-72 µm, height 39-42 µm, toe length 29-32 µm.

Comments: After its description from Germany, no subsequent illustrated records of *C. salina* were published although the animal has been reported from New Zealand (RUSSELL, 1960), Spain (VELASCO, 1990) and Florida, U.S.A. (TURNER, 1993). A microphotograph of a Black Sea specimen, identified as *C. colurus* (Ehrenberg) by ALTHAUS (1957b) may also refer to this species. The distribution of this evidently rare animal is insufficiently documented, and the New Zealand record especially needs confirmation.

Family Dicranophoridae

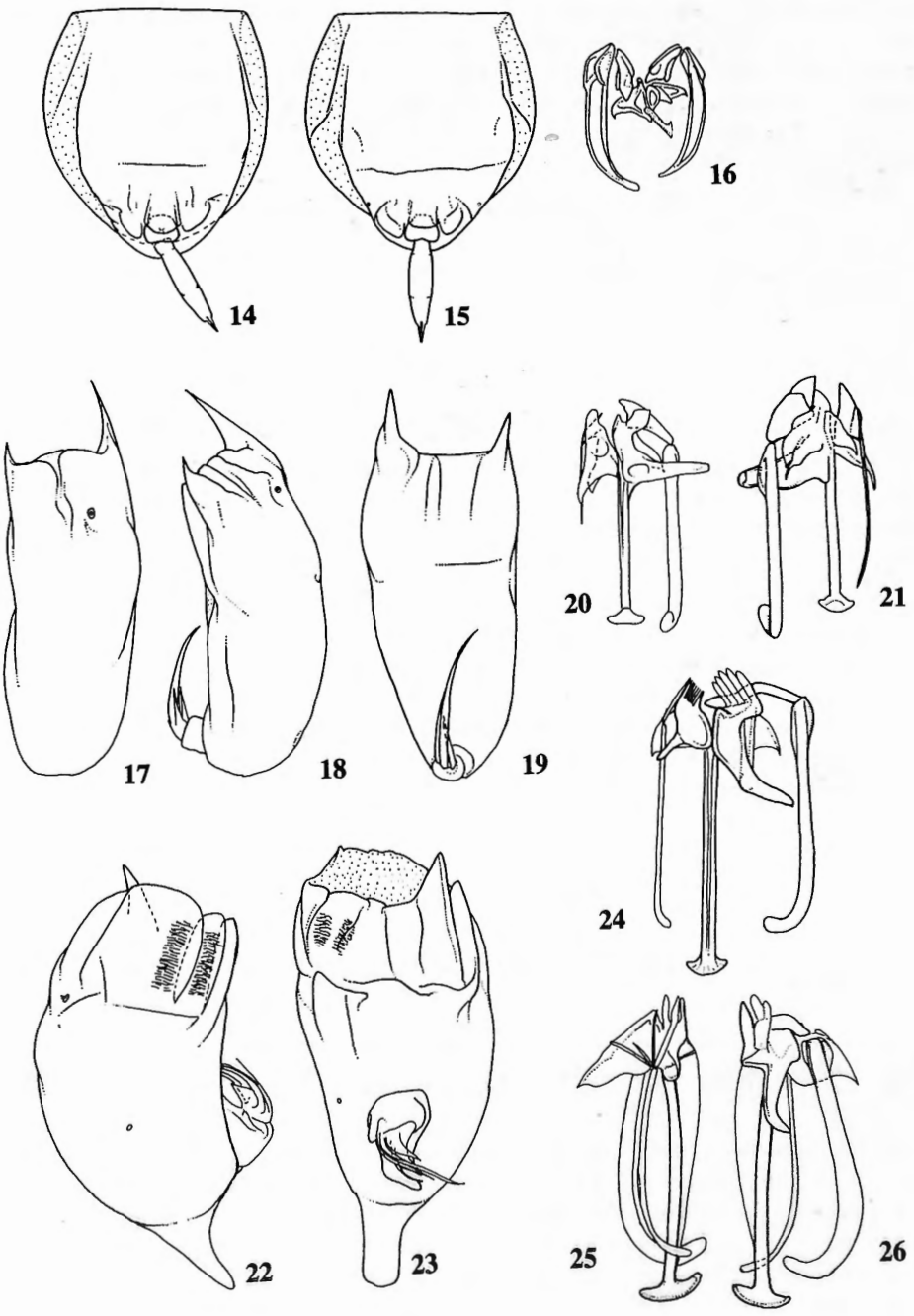
Encentrum villosum Harring & Myers, 1928
(Figs 27-30)

Synonym: *Encentrum glaucum* Wulfert after Althaus (1957a)

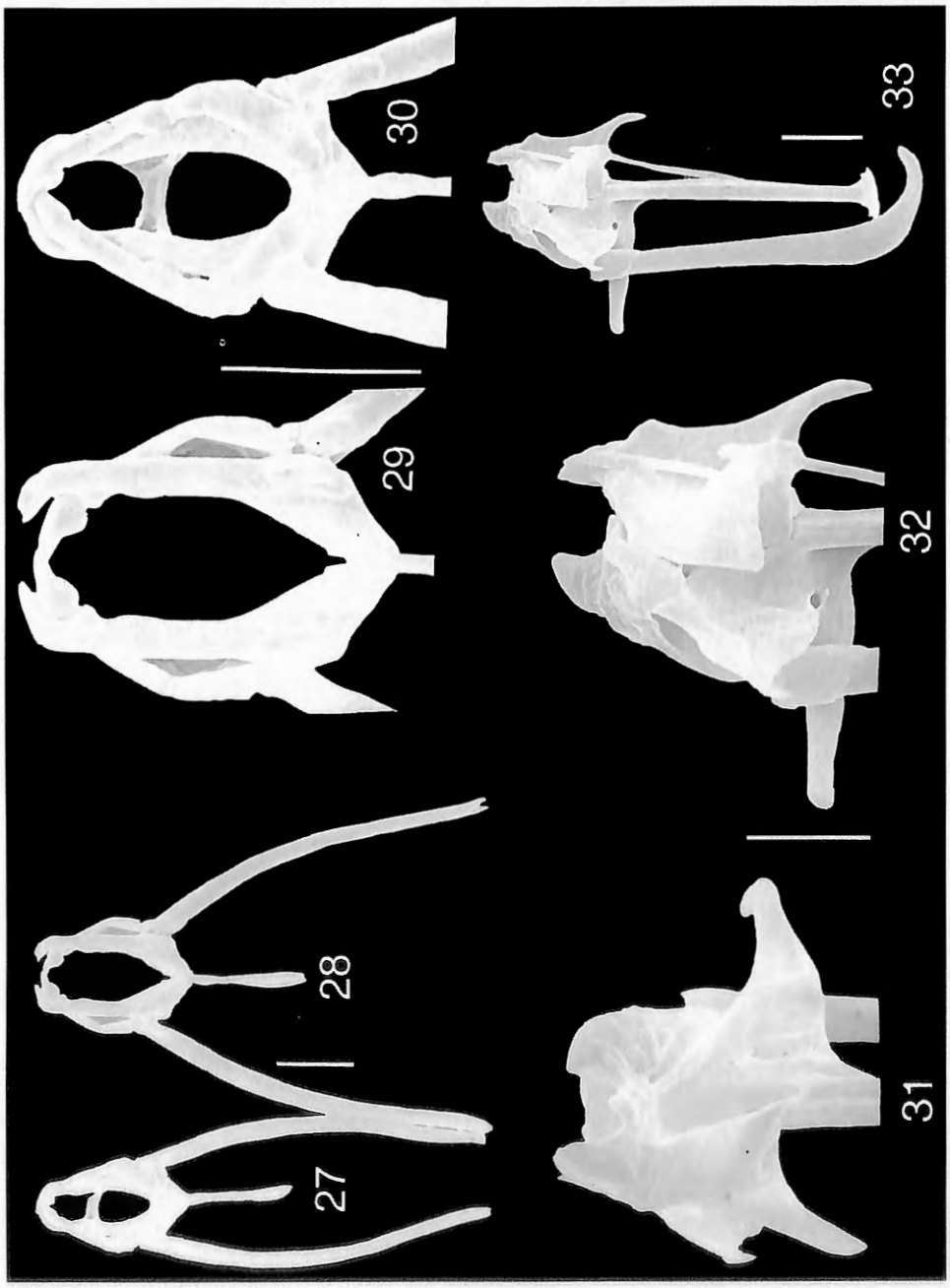
HARRING & MYERS (1928) p. 772-773 plate 43 figs 3, 4; DE SMET (1997) p. 222 figs 648-649.

Differential diagnosis: *E. villosum* resembles *E. rousseleti* Lie-Pettersen and *E. salsum* Myers by their similar rami and unci, a basally-dilated fulcrum and outcurved manubria tips. The low number of vitellarium nuclei (18-20 in *E. rousseleti*, 4-8 in the two others) and red-pigmented eyespot and granules in the retrocerebral sac (colourless in *E. salsum*) readily distinguish the morphospecies.

Figs 1-13. – *Colurella* spp. – 1: *C. adriatica* – 2-4: *C.* cf. *anodonta* – 5-7: *C. colurus* – 8-10: *C. hindenburgi* – 11-12: *C. salina* – 13: *C. sinistra*. (1-2, 5-13: lateral, 3: ventral, 4: dorsal).



50μm (figs 14-15, 17-19, 22-23)
25μm (figs 16, 20-21, 24-26)


27
28
29
30
31
32
33

Measurements: trophi length 27 µm, fulcrum 8 µm, incus width 5 µm, uncus length 4 µm, manubrium length 23 µm.

Comments: *E. villosum* inhabits coastal sand pools and has been recorded from North America (Atlantic: HARRING & MYERS, 1928) and Europe (W. Mediterranean: DE SMET, 1997; Baltic Sea: THANE-FENCHEL, 1968; North Sea). ALTHAUS' (1957a) record is from athallassic saline waters in Germany.

Family Lecanidae

Lecane psammophila (Wiszniewski, 1932)
(Figs 14-16)

WISZNIEWSKI (1932) p. 97 plate 4 figs 18-20; SEGERS (1995) p. 145-146 figs 368-369, 514.

Differential diagnosis: *L. psammophila* is readily recognised by its dorsal lorica plate being consistently broader than the ventral, and by its bulged toe bearing two short pseudoclaws. The species can be confused with the North American *L. gallagherorum* Segers. This latter species is larger, and has a longer, less-bulged toe bearing pseudoclaws and accessory claws. Both *L. psammophila* and *L. gallagherorum* differ from *L. obtusa* (Murray, 1913) by a difference in type of prepedal fold: it is narrow and bears a median projection distally in L. obtusa, and is wide and smoothly rounded distally in the two others.

Measurements: Dorsal plate length 56-62 µm, width 59-60 µm, ventral plate length 63-65 µm, width 45-46 µm. Toe length 12-13, width 5-6 µm, pseudoclaw length 4-5 µm. Trophi length 19 µm, fulcrum 4.5 µm, incus width 10 µm, uncus length 6-7 µm.

Comments: There are several recent records of *L. psammophila*, all of which are from Central Europe (Romania, Estonia, Poland, Russian Federation, North East Germany, Macedonia: see DE RIDDER & SEGERS, 1997). The species has been reported from the U.S.A. (*e.g.*, MYERS, 1942; EVANS, 1984), but it is likely that these records concern the recently recognised *L. gallagherorum*, as was shown for Myers' records (SEGERS, 1997). The present record is the first for Western Europe. Both the European *L. psammophila* and its North American relative *L. gallagherorum* are exclusively psammobiontic, and occur outside the psammon only during periods of mass abundance. They probably constitute a pair of vicariant sister taxa.

Legend to the figures (see pages 40-41)

Figs 14-16. – *Lecane psammophila*. – 14-15: habitus ventral – 16: trophi ventral.

Figs 17-21. – *Trichocerca taurocephala*. – 17-19: habitus – 17: dorsal – 18: lateral – 19: ventral – 20-21: trophi – 20: ventral – 21: dorsal.

Figs 22-26. – *Trichocerca insolens*. – 22-23: habitus – 22: lateral – 23: ventral – 24-26: trophi – 24: ventral – 25-26: lateral. (19, 21-26: Lenape Lake, 25 August 1997. New Jersey, USA)

Figs 27-30. – *Encentrum villosum*, trophi.

Figs 31-33. – *Trichocerca taurocephala*, trophi.

Scale bars: 5µm.

Family Trichocercidae

***Trichocerca taurocephala* (Hauer, 1931)**
(Figs 17-21, 31-33)

Recently, KOSTE & ZHUGE (1996) suggested a synonymy between *T. taurocephala* (Hauer), *T. pygocera* (Wiszniewski, 1932) and *T. insolens* (Myers, 1936) and attributed priority to the name *T. pygocera*. Apart from the fact that *T. taurocephala* is the senior synonym, a comparison of *T. taurocephala* from Europe (present material, figs 17-18, 20, 31-33) with co-occurring *T. taurocephala* (figs 19, 21) and *T. pygocera* (figs 22-26) from North America (Lenape lake, NJ, coll. 6 July 1996), reveals that at least this synonymy is questionable. As can be seen from the figures, the two have little in common besides a weak superficial resemblance and the fact that both are psammobionts. WISZNIEWSKI (1934a) provides a detailed comparison between *T. taurocephala* and *T. pygocera*, in which the large similarity between the two is commented upon, and the diagnostic features outlined. In fact, it is unlikely that KOSTE & ZHUGE'S (1996) record concerns either *T. taurocephala*, *T. pygocera* or *T. insolens*, considering the deviating trophi of the specimen as represented in their figure 15c (crescent-shape of left alulus and large apophysis on posterior edge of left ramus). Their material may belong to an unnamed taxon belonging to a group of *Trichocerca* species with similar lorica morphology but different trophus, containing the above-mentioned species and *T. pediculus* Remane, 1949.

DISTRIBUTION

As previously mentioned, 25 rotifer morphotaxa were found in the two samples examined. Most represented are the genera *Cephalodella* (7 species) and *Colurella* (6 species). The former genus is known to contain many psammon species. Other distinctly interstitial rotifers are *Trichocerca taurocephala* and *Lecane psammophila*. The report of a large number of *Colurella* species is in contrast with reports on both freshwater (WISZNIEWSKI, 1934a; MYERS, 1936) and marine (TZSCHASCHEL, 1979) psammon rotifers, but agrees well with ALTHAUS' (1957a) reports on brackish-water psammon rotifers. Some of the animals recorded are noteworthy for their rarity, especially *L. psammophila* which has so far been recorded from Central Europe, and the brackish-water *Colurella salina* and *Encentrum villosum* which are known only from few localities. The influence of the sea as evidenced by the presence of brackish-water animals is important, but is not surprising in the case of a coastal dune pool. To my knowledge, there are no further literature records signalling the existence of a psammon rotifer community similar to the one reported here, although it is clear that it must exist elsewhere. Factors affecting the occurrence of psammon rotifers are well-known, and include grain size distribution, oxygen concentration and detritus content (see RUTTNER-KOLISKO, 1955; 1961). Generally, psammon rotifers can only survive in areas with exposed sand in the contact zone between land and water. Some types of dune pool naturally contain such biotopes, as a result of the dynamics of sand due to wind action and human trampling, and water level fluctuations. At present, the

available habitat for an interstitial rotifer community in the Eendeput is reduced by the stabilisation of the sandy shore and corresponding development of a reed belt.

Another component of the rotifer fauna of the Eendeput, is formed by taxa such as *Asplanchna girodi*, *Euchlanis dilatata*, *Keratella cochlearis* and *Pompholyx sulcata* which form a species-poor pelagic community of common, widely distributed species known to occur in many types of water, including polluted ones. In addition, a bloom of cyanobacteria (*Anabaena* sp., det. K. Sabbe) was observed in the pelagic zone of the pool on 12 July 1997. A pelagic rotifer community consisting of the above-mentioned taxa, and a bloom of cyanobacteria are indications that the ecosystem of the pond may be disturbed. The present, limited data do not permit any assessment of the impact or causes of this disturbance. However, DENYS (1996, unpublished report*) recently cautioned against the further deterioration of the water quality in the nature reserve, and suggested that organic pollution by overwintering water birds may contribute significantly to this.

Considering the above, it appears that the interstitial rotifer community of the pond is threatened, but it is not possible to assess the importance of this as our knowledge on the distribution on these animals is very limited. It does illustrate, however, that it may be relevant to incorporate data on this, and other similarly cryptic taxonomic groups in the assessment of nature conservation priorities and measures.

CONCLUSIONS

Twenty-five rotifer species are registered from the psammon of the Eendeput, in the Fonteintjes nature reserve, Zeebrugge, Belgium. Several of the taxa found are of special faunistic importance including nine species that are new to the Belgian fauna. *Lecane psammophila* had previously been recorded from Central Europe, while both *Colurella salina* and *Encentrum villosum* are only known from a few localities worldwide. The synonymy of *Colurella hindenburgi* and of *Trichocerca taurocephala* is discussed.

The interstitial rotifer community of the nature reserve is threatened by the stabilisation of the sandy shore and extension of a reed belt, and by the apparent deterioration of water quality. It is suggested that more research effort is necessary, directed toward the collection of data on the distribution of this and similar taxonomic groups, before a reliable assessment can be made of the significance of the present observations.

ACKNOWLEDGEMENTS

Prof. Dr. E. Kuijken suggested this study and provided enthusiastic support, while Mr. J. Van Gompel (Curator of the Nature Reserve «De Fonteintjes») is thanked for help in the field and for granting permission to study the rotifers of this nature reserve. Dr. K. Sabbe identified the cyanobac-

*DENYS, L. (1996) – Algemeen historisch referentiekader t.b.v. natuurontwikkelingsprojecten in stilstaande zoete waters: samenstelling van diatomeeengemeenschappen in Vlaanderen voor de tweede wereldoorlog als ecologisch referentiekader voor stilstaande zoete waters. II. Enkele gevalstudies. Departement Biologie, Universitair Centrum Antwerpen, 43 pp., annex.

teria. Mr. M. Bruyneel is gratefully acknowledged for technical support. Prof. Dr. B. Pejler and one anonymous referee are thanked for critically reviewing the manuscript.

REFERENCES

ALTHAUS, B. (1957a) – Faunistisch-ökologische Studien an Rotatorien salzhaltiger Gewässer Mitteldeutschlands. *Wiss. Z. Univ. Halle, Math.-Nat.*, **VI/1**: 117-158.

ALTHAUS, B. (1957b) – Neue Sandbodenrotatorien aus dem Schwarzen Meer. *Wiss. Z. Univ. Halle, Math.-Nat.*, **VI/3**: 445-458.

ALTHAUS, B. (1957c) – Faunistisch-ökologische Studien an Rotatorien salzhaltiger Gewässer Mitteldeutschlands (Nachtrag). *Wiss. Z. Univ. Halle, Math.-Nat.*, **VI/3**: 459-460.

DE RIDDER, M. (1989) – De huidige stand van het raderdieronderzoek in België. *Verh. Symp «Invertebraten van België»*, Brussels, 1989: 31-41.

DE RIDDER, M. (1992) – Distribution of Belgian Rotifera. *Proc. 8th Int. Coll. Europ. Invert. Survey*, Brussels, 1991: 199-212.

DE RIDDER, M. & H. SEGERS (1997) –Rotifera Monogononta in six zoogeographical regions after publications between 1960-1992. *Studiedocumenten K.B.I.N.*, **87**: 1-481.

DE SMET, W.H. (1997) – Rotifera vol. 5: The Dicranophoridae. *Guides to the Identification of the Microinvertebrates of the Continental Waters of the World,* **12** (H.J. Dumont & T. Nogrady eds). SPB Academic Publishing bv, 1997: 1-325.

DONNER, J. (1951) – Rotatorien der Humusböden. III. Teil. *Zool. Jahrb. Syst.*, **79**: 615-638.

DONNER, J. (1970) – Die Rädertierbestände submerser Moose der Salzach und anderer Wasser-Biotope des Flubgebietes. *Arch. Hydrobiol.*, suppl. **36**(2/3): 109-254.

EVANS, W. (1984) – Seasonal abundances of the psammic rotifers of a physically controlled stream. *Hydrobiologia*, 108: 105-114.

HARRING, H.K. & F.J. MYERS (1928) – The rotifer fauna of Wisconsin. IV. The Dicranophoridae. *Trans. Wisconsin Acad. Sci., Arts & Letters*, **23**: 667-808.

HAUER, J. (1924) – Zur Kenntnis des Rotatorien-Genus Colurella Bory de St. Vincent. *Zool. Anz.*, **59**(7/8): 177-189.

KOSTE, W. (1978) – *Rotatoria. Die Rädertiere Mitteleuropas*. Borntraeger, Berlin, Stuttgart, 2 vols: 673 pp., 234 plates.

KOSTE, W. & Y. ZHUGE (1996) – A preliminary report on the occurrence of Rotifera in Hainan. *Quekett J. Microsc.*, **37**: 666-883.

MYERS, F.J. (1936) – Psammolittoral rotifers of Lenape and Union lakes, New Jersey. *American Museum Novitates*, **830**: 1-21.

MYERS, F.J. (1942) – The rotatorian fana of the Pocono Plateau and environs. *Proc. Acad. Nat. Sci. Philad.*, **44**: 251-285.

NEISWESTNOWA-SHADINA, K. (1935) – Zur Kenntnis des rheophilen Mikrobenthos. *Arch. Hydrobiol.*, **28**: 555-582.

RUSSELL, C.R. (1960) – An index to the Rotatoria of New Zealand and outlying islands from 1859-1959. *Trans. Roy. Soc. New Zeal.*, **n.s. 1**: 235-239.

RUTTNER-KOLISKO, A. (1955) – Einige Beispiele für die unmittelbare Auswirkung des Wetters auf die Lebensbedingungen im feuchten Sand. *Wetter und Leben,* **7**: 16-22.

RUTTNER-KOLISKO, A. (1961) – Biotop und Biozönose des Sandufers einiger österreichischer Flüsse. *Verh. Internat. Verein. Limnol.*, **14**: 362-338.

SCHMID-ARAYA, J.M. (1995a) – Distubance and population dynamics of rotifers in bed sediments. *Hydrobiologia*, **313/314**: 279-290.

SCHMID-ARAYA, J.M. (1995b) – New records of rare Bdelloidea and Monogononta rotifers in gravel streams. *Arch. Hydrobiol.*, **135**: 129-143.

SCHMID-ARAYA, J.M., in press. Rotifers in interstitial sediments. *Hydrobiologia.*

SEGERS, H. (1993) – Rotifera of some lakes in the floodplain of the River Niger (Imo State, Nigeria). I. New species and other taxonomic considerations. *Hydrobiologia,* **250**: 39-61.

SEGERS, H. (1995) – Rotifera vol. 2: The Lecanidae (Monogononta). *Guides to the Identification of the Microinvertebrates of the Continental Waters of the World,* **6** (H.J. Dumont & T. Nogrady eds). SPB Academic Publishing bv, 1995: 1-226.

SEGERS, H. (1997) – Some Rotifera from the collection of the Academy of Natural Sciences of Philadelphia, including new species and new records. *Proc. Acad. Nat. Sci. Philad.*, **148**: 147-156.

SEGERS, H & H.J. DUMONT (1993) – Rotifera from Arabia, with descriptions of two new species. *Fauna of Saudi Arabia*, **13**: 3-26.

STEINECKE, F. (1917) – Die Rotatorien und Gastrotrichen des Zehlaubruckes. *Schrift Phys.-Ökonom. Gesell. Köningsberg*, **57** (1916): 84-100.

THANE-FENCHEL, A. (1968) – Distribution and ecology of non-planktonic brackish-water rotifers from Scandinavian waters. *Ophelia*, **5**: 276-297.

TURNER, P. (1990) – Some Insterstitial Rotifer from a Florida, U.S.A., *Beach. Trans. Am. Microsc. Soc.*, **109**: 417-421.

TURNER, P. (1993) – Distribution of rotifer in a Floridian saltwater beach, with a note on rotifer dispersal. *Hydrobiologia,* **255/256**: 435-439.

TURNER, P. (1995) – Rotifer look-alikes: two species of *Colurella* are ciliated protozoans. *Invertebrate Biology*, **114**: 202-204.

TURNER, P. & D.A. DISTLER (1995) – Notes on the Hyporheic Rotifer of the Ninnescah River, Kansas, USA. *Trans. Kansas Acad. Science*, **98**: 92-101.

TURNER, P., & M.A. PALMER (1996) – Notes on the species composition of the rotifer community inhabiting the interstitial sands of Goose Creek, Virginia with comments on habitat preferences. *Quekett J. Microsc.*, **37**: 552-565.

TZSCHASCHEL, G. (1979) – Marine Rotatoria aus dem Interstitial der Nordseeinsel Sylt. Mikrofauna des Meeresboden, **71**: 1-64.

Tzschaschel, G. (1980) – Verteilung, abundanzdynamik und biologie mariner interstitieller Rotatoria. *Mikrofauna des Meerseboden*, 81: 1-56

VELASCO, J. L. (1990) – Lista faunistica & bibliographia de los Rotiferos (Rotatoria) de la Peninsula Iberica e Islas Baleares & Canarias. *AEL, Publ. 8.,* 195 pp.

WISZNIEWSKI, J. (1932) – Les rotifères des rives sablonneuses du lac Wigry. *Ach. Hydrobiol. Ryb.*, **6**: 86-100.

WISZNIEWSKI, J. (1934a) – Les rotifères psammiques. *Annales Musei Zoologici Polonici*, **10**: 339-399.

WISZNIEWSKI, J. (1934b) – Les Mâles des Rotifères psammiques. *Mémoires de l'Académie Polonaise des Sciences et des Lettres, Sciences Mathématiques et Naturelles*, Série B: 143-165.

WISZNIEWSKI, J. (1934c) – Recherches ecologiques sur le psammon et specialement sur ler Rotiféres psammiques. *Arch. Hydrobiol. Ryb.*, **8**: 149-271.

WISZNIEWSKI, J. (1935) – Note sur le psammon du lac Ohrid. Verhandl. Intern. *Ver. f. Limnologie*, **7**: 238-244.

WISZNIEWSKI, J. (1936) – Notes sur le psammon. Deux tourbières aux environs de Varsovie. *Arch. Hydrobiol. Ryb.*, **10**: 173-187.

WISZNIEWSKI, J. (1954) – Matériaux relatifs à la nomenclature et à la bibliographie des Rotifères. *Polski Arch. Hydr.*, **2/15**: 7-249.

Belg. J. Zool. - Volume 128 (1998) - issue 1 - pages 49-54 - Brussels 1998

Received: 27 February 1998

NUTHATCHES *SITTA EUROPAEA* DO NOT DELAY POSTFLEDGING DISPERSAL IN ISOLATED FOREST FRAGMENTS

DAVID CURRIE (1) AND ERIK MATTHYSEN
Department of Biology (UIA), University of Antwerp, B-2610 Wilrijk, Belgium
e-mail: matthys@uia.ua.ac.be

Abstract. We followed the fate of colour-ringed Nuthatch *Sitta europaea* fledglings in six territories within a large forest and eight territories within small (<15 ha) forest fragments. Almost half of the young birds had disappeared by day 8 after fledging, before any dispersal was expected to have occurred. This high level of mortality was probably mainly due to predation. After day 15 the rate of disappearance increased markedly due to dispersal, and all young had left the study areas by day 28. The pattern of disappearance was highly similar in both study areas, suggesting that dispersal was not delayed in forest fragments, nor was the pattern of early fledgling mortality different from a large forest.

Key words: habitat fragmentation, dispersal, juvenile mortality, Nuthatch.

INTRODUCTION

Natal dispersal of birds and other animals is commonly defined as the movement between the place of birth and the site of first reproduction (GREENWOOD, 1980). Dispersal involves a behavioural sequence of acts or decisions, most of which are still poorly understood. For instance, it is possible to distinguish between a «leaving», a «transfer» and a «settling» phase (cf. SMALL *et al.*, 1993; IMS & YOCCOZ, 1997). Although an increasing number of studies have attempted to follow the entire dispersal sequence by radiotracking (see BELTHOFF & RITCHISON, 1989; GONZALEZ *et al.*, 1989; SMALL *et al.*, 1993 for avian examples), information on dispersal patterns remains largely based on separate observations per phase.

Recently the study of dispersal has acquired additional relevance in the context of habitat fragmentation. Conservation biologists want to know, for instance, to what extent dispersal flows are channeled or arrested by landscape elements (MADER, 1984; Saunders & HOBBS; 1991, ROSENBERG *et al.*, 1997), or how isolation of habitat patches affects gene flow, immi/emigration balance and recolonization probability (WAUTERS *et al.*, 1994; HANSKI & GILPIN, 1997). The magnitude of these landscape effects probably depends on

(1) Present address: Department of Zoology, University of Turku, FIN-20014 TURKU, Finland

the nature of the costs and benefits of dispersal, which vary across species (JOHNSON & GAINES, 1990; MATTHYSEN, 1994). In addition there may be differential effects in each phase.

Nuthatches (*Sitta europaea*: Aves) have repeatedly been shown to be less abundant in more isolated forest fragments, suggesting that dispersal is insufficient to overcome isolation (OPDAM & SCHOTMAN, 1987; VERBOOM *et al.*, 1991). In previous papers we have presented information on the «transfer» and «settling» phases in a highly fragmented habitat (MATTHYSEN *et al.*, 1995; MATTHYSEN & CURRIE, 1996). These studies showed that almost all Nuthatches leave the natal patch to settle elsewhere, but also suggested a high cost related to dispersal. Here we present more detailed observations on the «leaving» phase. In particular, we test the prediction that if there is a barrier effect associated with isolation of forest fragments (STAMPS *et al.*, 1987), Nuthatch fledglings should disperse later in fragments compared to a large forest.

METHODS

In the summer of 1994 we monitored the rate of disappearance of colour-ringed juveniles from their natal territories in 14 broods. Six territories were chosen within a 30 ha study plot in a forest of more than 200 ha (Peerdsbos). The eight remaining territories were located in six different forest fragments within a 4x4 km area in a highly fragmented landscape, described in more detail elsewhere (MATTHYSEN *et al.*, 1995; MATTHYSEN & CURRIE, 1996). Five fragments were small (2 to 12 ha) forest stands dominated by mature Common Oak *Quercus robur* with some Beech *Fagus sylvatica*, each containing one or two breeding pairs, and separated from one another by at least 500 m of agricultural land. The sixth fragment was a narrow belt of ornamental trees (mainly Beech) in a small town, almost 2 km in length and 10 ha in size. Here two territories out of the four present were chosen for observation.

All nestlings were individually colour-ringed, weighed and sexed when 10 to 15 days old (MATTHYSEN *et al.*, 1995). Brood sizes varied from six to eight except for one fragment brood with two nestlings only. Nestlings fledged when they were approximately 22 days old. It is assumed that all ringed nestlings survived to fledging; nestling loss at this stage is unusual in Nuthatches (MATTHYSEN, 1998). All territories were checked at least once during the first week (day 2-8) after fledging, and subsequently all were checked about twice a week and the number of fledglings recorded, and, if possible, their identity. Parent-offspring interactions were also noted. Fledglings were initially located by their conspicuous calls but as they became more mobile, playback was used to locate adults as fledglings associated with their parents in relatively cohesive family groups until they disappeared. Fledglings never responded to the playback. Territories were monitored until all the fledglings were found to be absent on several successive visits.

We characterized between-brood variation in dispersal by maximum dispersal date per brood, which is the day the last fledgling was seen on territory (range: 10 to 27 days after fledging). We also computed a median date of disappearance of the fledglings still alive on day 8, assuming that no dispersal occurred before this age (see further). Since this parameter was closely correlated with maximum dispersal date ($r=0.82$, $n=14$) but probably

more subjected to observational errors since it is based on counts rather than presence/absence, we only present data on maximum dispersal date. Since we predicted that dispersal would, if anything, be delayed in fragments, one-sided tests were used to compare timing in the two study areas.

RESULTS

All fledglings disappeared from their natal territories within 28 days after fledging. During observations for another purpose in neighbouring fragments (MATTHYSEN & CURRIE, 1996), two fledglings were resighted on days 14 and 17 at distances of 1.7 and 2.8 km, in both cases with at least one sibling still on the natal territory. Parents were no longer observed to feed fledglings after day 11 although begging continued to day 18. In this period we frequently saw fledglings chase their parents and beg intensely for food, which in two cases elicited aggressive responses from adults (on days 17 and 18).

Maximum dispersal dates were very similar in the large forest (mean $\pm$S.D.: 21.0$\pm$5.9 days, N=8) and in the forest fragments (20.3$\pm$4.5 days, N=6; U=22, one-sided P>0.5). The difference in median date was even opposed to the one-sided prediction (means: 19.8 and 20.3 days, respectively). With the two study areas pooled, there was no correlation between maximum dispersal time and brood size (Spearman rank correlation, n=14, R_s=0.34), fledging date (R_s=-0.16, n=14) or mean chick weight (R_s=0.28, n=13) (all P>0.1). Using the pooled standard error of the two samples we calculated a one-sided confidence interval for the difference in maximum dispersal time between group means. The interval allows us to conclude with 95% confidence that fragment broods dispersed not more than 3.8 days later than broods from the large forest.

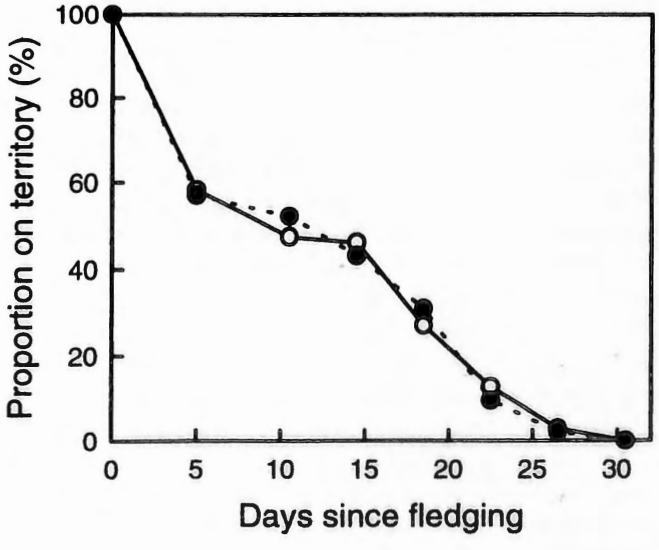


Fig. 1. – Mean proportion of fledglings per brood remaining on territory in a large forest (filled circles, n=6 families) and forest fragments (open circles, n=8 families). Data points are means for four-day periods, except for the first point giving a mean for day 2 to 8 (mean observation date in this interval=day 5.3 in both areas).

In both study areas, fledglings disappeared from the territory at a very similar rate which appeared to increase after day 15 (Fig. 1). This is illustrated by mean loss rates per 8-day period: 42% disappeared between days 0 and 8, 33% from day 8 to 16, 70% from day 16 to 24 and 100% from day 24 to 32 (only taking into account broods counted on or near days 8, 16 and 24). The difference between the first three periods is nearly significant (Kruskal-Wallis analysis of variance, $H=5.3$, $P=0.07$, total $n=21$ territory/period combinations).

DISCUSSION

In this study, Nuthatches born in isolated forest fragments disappeared from their natal territories at a similar rate as those born in a larger forest. We estimate that the true difference did not exceed a 3.8-day delay in fragments. This is much smaller than the natural variation within years and areas which easily covers two to three weeks (MATTHYSEN, 1989). A potential critique is that we sampled broods in a single large forest only. An obvious reason is the scarcity of large forests in our landscape and the practicalities of working in distant study areas at the same time (cf. LENS & DHONDT, 1994). We believe that our results are still of general significance because (i) the large forest and fragments differed little except in the variable of interest, *i.e.* the spatial isolation of territories; they were geographically close (<20 km), very similar in habitat and also in reproductive parameters of the Nuthatch population (MATTHYSEN & ADRIAENSEN, in press); and (ii) the single large forest appeared sufficiently representative of the «unfragmented» condition, since it differed little in population density and reproductive parameters from oak stands within two more distant large forests (MATTHYSEN & ADRIAENSEN, in press; MATTHYSEN, unpubl.).

The earliest known age for Nuthatch dispersal is 8 days after fledging for a bird settling close to the natal territory (MATTHYSEN, 1998) and 10 days for longer dispersal (STECHOW, 1937). This coincides with a phase of restlessness observed in captive young from around day 14 (LÖHRL, 1958). Therefore losses before day 10 are best regarded as early mortality. The extended length of stay of at least some young, up to two weeks after cessation of feeding, suggests a limited role for parental aggression to force juveniles to disperse (HOLLEBACK, 1974; DAVIES, 1978). The proportion of Nuthatches that disappeared by day 8 (nearly 50%) is much higher than reported on Marsh Tits *Parus palustris* where only 4% disappeared in the first 11 days (SMITH, 1967), and Black-capped Chickadees *Parus atricapillus* where 12% disappeared in the three to four weeks preceding family break-up (NILSSON & SMITH, 1985). Predation is probably a major cause of disappearance, as we witnessed attacks by both Jay *Garrulus glandarius* and Sparrowhawk *Accipiter nisus* (on days 2 and 20, respectively) as well as several chases by Jays. Jays and other corvids were very common in all study areas. However, contrary to many studies on nest predation, we found no suggestion for a higher predation rate in fragments (*e.g.* ANDRÉN & ANGELSTAM, 1988; NOUR *et al.*, 1993).

Variation between broods in maximum dispersal time could not be explained by fledging date, brood size or mean nestling weight. condition. A comparable study on Crested Tits *Parus cristatus* found a seven-day delay in pine forest fragments of 10-50 ha compared to a large forest (LENS & DHONDT, 1994). When comparing their original data

(L. LENS, *in litt.*) with our results, we found a significant species x area interaction (ANOVA, $F_{1,20}=8.3$, $P<0.01$) suggesting that the different results are not due to lack of statistical power in our own analysis. Lens & Dhondt (1994) suggested that Crested Tits dispersed later in fragments because of their lower body mass at fledging. In our case mean nestling body mass did not differ between fragments (24.3 ± 0.8g) and large forests (22.6 ± 0.7g; Mann-Whitney U-test, $U=12$, $P>0.1$), and an even smaller difference was found in a more extensive comparison over several years (MATTHYSEN & ADRIAENSEN, in press). Thus, the most parsimonious explanation for the difference between the two studies is that there is no barrier effect delaying dispersal from fragments, and that the delay in Crested Tits is caused by a lower fledgling weight. The conclusion that there is no barrier effect, is consistent with the long dispersal distances travelled by at least some Nuthatches and the low rate of local recruitment in both studies (LENS & DHONDT, 1994; MATTHYSEN *et al.*, 1995). However, the absence of a barrier effect does not preclude that disperser success is affected by habitat fragmentation (see MATTHYSEN & CURRIE, 1996).

ACKNOWLEDGEMENTS

We thank Frank Adriaensen for assistance in ringing nestlings, and André Dhondt and anonymous referees for useful suggestions on an earlier manuscript. This study was supported by an EC fellowship to DC (HCM network CEE CHRX-CT93-0187). EM is research associate with the National Fund for Scientific Research, Flanders (Belgium). More information on this and related research is available at http://alt-www.uia.ac.be/u/matthys/ownwork.html.

REFERENCES

ANDRÉN, H. & P. ANGELSTAM (1988) und for Scientific Research. More information on this and related research is available *Ecology,* **69**: 544-547.

BELTHOFF, J.R. & G. RITCHISON (1989) – Natal dispersal of Eastern Screech-Owls. *Condor,* **91**: 254-265.

DAVIES, N.B. (1978) – Parental meanness and offspring independence: an experiment with hand-reared Great Tits *Parus major*. *Ibis,* **120**: 509-514.

GONZALEZ, L.M., B. HEREDIA, J.L. GONZALEZ & J.C. ALONSO (1989) – Juvenile dispersal of Spanish Imperial Eagles. *J. Field. Ornithol.* **60**: 369-379.

GREENWOOD, P. J., P. H. HARVEY & C.M. PERRINS (1979) – The role of dispersal in the Great Tit (*Parus major*): the causes, consequences and heritability of natal dispersal. *J. Anim. Ecol.* **48**: 123-142.

GREENWOOD, P.J (1980) – Mating systems, philopatry and dispersal in birds and mammals. *Anim. Behav.,* **28**: 1140-1162.

HANSKI, I. & M.E. GILPIN (1997) – *Metapopulation biology: ecology, genetics, and evolution.* Academic Press, London.

HOLLEBACK, M. (1974) – Behavioral interactions and the dispersal of the family in Black-capped Chickadees. *Wilson Bull.*, **86**: 466-468.

IMS, R.A. & N.G. YOCCOZ (1997) – Studying transfer processes in metapopulations: emigration, migration, and colonization. In: HANSKI, I. & M.E. GILPIN (Eds.) *Metapopulation biology: ecology, genetics, and evolution*. Academic Press, London: 247-265.

JOHNSON, M.L. & M.S. GAINES (1990) – Evolution of dispersal: theoretical models and empirical tests using birds and mammals. *Ann. Rev. Ecol. Syst.*, **21**: 449-480.

LENS, L. & A.A. DHONDT (1994) – Effects of habitat fragmentation on the timing of Crested Tit *Parus cristatus* natal dispersal. *Ibis*, **136**: 147-152.

LÖHRL, H. (1958) – Das Verhalten des Kleibers (*Sitta europaea caesia* Wolf). *Z. Tierpsychol.* **15**: 191-252.

MADER, H.J. (1984) – Animal habitat isolation by roads and agricultural fields. *Biol. Conserv.* **29**: 81-96.

MATTHYSEN, E. (1989) – Fledging dates of Nuthatches *Sitta europaea* in relation to age, territory and individual variation. *Bird Study*, **36**: 134-140.

MATTHYSEN, E. (1994) – Dispersal as the key process in fragmented landscapes. In: HAGEMEIJER, E.J.M. & T.J. VERSTRAEL (Eds.) *Bird Numbers 1992. Distribution, monitoring and ecological aspects*. Voorburg/Heerlen: Statistics Netherlands & Beek-Ubbergen, Netherlands: SOVON: 111-116.

MATTHYSEN, E. (1998) – *The Nuthatches*. T & AD Poyser, London.

MATTHYSEN, E. & F. ADRIAENSEN (in press). Forest size and isolation have no effect on the reproductive success of the Eurasian Nuthatch (*Sitta europaea*). *The Auk.*

MATTHYSEN, E., F. ADRIAENSEN & A.A. DHONDT (1995) – Dispersal distances of Nuthatches, *Sitta europaea*, in a highly fragmented forest habitat. *Oikos*, **72**: 375-381.

MATTHYSEN, E. & D. CURRIE (1996) – Habitat fragmentation reduces disperser success in juvenile Nuthatches *Sitta europaea*: evidence from patterns of territory establishment. *Ecography*, **19**: 67-72.

NILSSON, J.-A. & H.G. SMITH (1985) – Early fledging mortality and the timing of juvenile dispersal in the Marsh Tit *Parus palustris*. *Ornis Scand.*, **16**: 293-298.

NOUR, N., E. MATTHYSEN & A.A. DHONDT (1993) – Artificial nest predation and habitat fragmentation: different trends in bird and mammal predators. *Ecography*, **16**: 111-116.

OPDAM, P. & A. Schotman (1987) – Small woods in rural landscape as habitat islands for woodland birds. *Acta Oecol./Oecol. Gen.*, **8**: 269-274.

ROSENBERG, D.K., B.R. NOON & E.C. MESLOW (1997) – Biological corridors: form, function, and efficacy. *BioScience*, **47**: 677-687.

SAUNDERS, D.A. & R.J. HOBBS (1991) – *Nature conservation 2: the role of corridors*. Chipping Norton, Australia: Surrey Beatty & Sons.

SMALL, R.J., J.C. HOLZWART & D.H. RUSCH (1993) – Are Ruffed Grouse more vulnerable to mortality during dispersal? *Ecology*, **74**: 2020-2036.

SMITH, S.M (1967). Seasonal changes in the survival of the Black-capped Chickadee. *Condor*, **69**: 344-359.

STAMPS, J.A., M. BUECHNER & V. KRISHNAN (1987) – The effects of edge permeability and habitat geometry on emigration from patches of habitat. *Am. Nat.*, **129**: 533-552.

STECHOW, J. (1937) – Ein Beitrag zur Frage des Vertriebenwerdens der erwachsenen Jungvögel durch die Alten. *Beitr. Fortpfl. Vögel*, **13**: 54-55.

VERBOOM, J., A. SCHOTMAN, P. OPDAM & J.A.J. METZ (1991) – European Nuthatch metapopulations in a fragmented agricultural landscape. *Oikos*, **61**: 149-156.

WAUTERS, L.A., Y. HUTCHINSON, T.D. PARKIN & A.A. DHONDT (1994) – The effects of habitat fragmentation on demography and the loss of genetic variation in the red squirrel. *Proc. Roy. Soc. Lond. B*, **255**: 107-111.

Belg. J. Zool. - Volume 128 (1998) - issue 1 - pages 55-92 - Brussels 1998

Received: 18 February 1998

NEW AND KNOWN NEMERTODERMATIDA (PLATYHELMINTHES-ACOELOMORPHA) – A REVISION –

WOLFGANG STERRER

Bermuda Natural History Museum, Flatts FLBX, Bermuda
e-mail wsterrer@bbsr.edu

Abstract. Described in 1930-31 by Steinböck who considered it the most primitive bilaterian, the turbellarian genus *Nemertoderma* is known for its role in platyhelminth phylogeny as much as for its muddled taxonomy. On the basis of material collected in the Mediterranean, Atlantic and Pacific Oceans since 1964 this paper re-diagnoses the known 4 genera and 7 species (*Nemertoderma bathycola* Steinböck, 1930-31; *N. westbladi* Steinböck, 1938; *N. psammicola* Sterrer, 1970 (syn. *N. rubra* Faubel, 1976); *Meara stichopi* Westblad, 1949; *Meara* sp. (see SMITH *et al.*, 1994); *Nemertinoides elongatus* Riser, 1987; and *Flagellophora apelti* Faubel & Dörjes, 1978), describes one new genus with 2 new species (*Ascoparia neglecta* n. g., n. sp. and *A. secunda* n. sp.), and provides observations from living material on morphological variability, body size *vs.* reproductive state, statocyst structure and statolith variability, and sperm morphology and dimorphism. The paper concludes with diagnoses for the known taxa of Nemertodermatida, including the new family Ascopariidae.

Key words: Platyhelminthes, free-living, marine; systematics, new species.

INTRODUCTION

In 1930-31 Otto STEINBÖCK described *Nemertoderma bathycola* from a single tiny worm which he and Erich Reisinger had dredged from a muddy bottom, at 300-400 m depth, off Greenland. *Nemertoderma* caused a small sensation, not only because Steinböck, a meticulous observer, was also an assertive character (who liked to express himself double-spaced, with exclamation marks added) but because *Nemertoderma* was indeed unusual. Steinböck insisted that he had hit upon the ur-acoel, the «mother of all turbellarians»: with a novel, two-stoned statocyst, an unusually thick and gland-rich epidermis, a peripheral nervous system, and a mixed, lacunar gonad without accessory organs. A heated argument ensued when, in 1937, Einar WESTBLAD described a similar worm from the Swedish west coast suggesting, not unreasonably, that STEINBÖCK's specimen had been immature, and adding that his «speculations... seemed to originate to an alarming degree from preconceived notions.» WESTBLAD left it open whether his and STEINBÖCK's were the same species, and the assignation of the name *N. westbladi* by

STEINBÖCK in his 1938 reply was not generally accepted. WESTBLAD kept the heat on by describing, in 1949, *Meara stichopi*, endocommensal of a holothurian, and clearly related to *Nemertoderma*. The initial nomenclatural muddle was exacerbated by subsequent authors who variously referred to «*Nemertoderma*, Westblad's form», «North Sea form», «Skagerrak form» and «Adriatic form» (RIEDL, 1960), «species I & II» (STERRER, 1966), and «species A, B, C & D» (TYLER & RIEGER, 1975, 1977). Additional taxa were described by STERRER (1970: *Nemertoderma psammicola*), FAUBEL (1976: *Nemertoderma rubra*), FAUBEL & DÖRJES (1978: *Flagellophora apelti*), and RISER (1987: *Nemertinoides elongatus*).

My observations of living material of Nemertodermatida, gathered since 1964 in the Mediterranean, Atlantic, and Pacific Ocean, include all described species as well as new ones (Table I). The purpose of this paper is to (1) clarify the species problem in *Nemertoderma*, largely on the basis of sparse but consistent finds over more than 30 years, (2) provide observations on morphological variability, body size *vs.* reproductive state, statocyst structure and statolith variability, and sperm morphology and dimorphism, (3) describe a new genus with two new species, and (4) present an analysis and diagnoses of the currently known taxa, including the erection of a new family. Throughout the paper I emphasize features observable in living specimens, and consider micro-anatomical and ultrastructural features only where necessary.

There are a number of reasons why species identification in Nemertodermatida is problematic. First, with the exception of *Meara stichopi* and *Nemertoderma westbladi*, most species are known by only few specimens, which are often only partially mature, or fragments (as especially in the filiform species *Nemertoderma psammicola* and *Nemertinoides elongatus*). In addition, characters seem to vary widely even within a population. This applies to size in relation to sexual maturity (*e.g.*, *N. westbladi*), statolith numbers (most species), colour (*e.g.*, *N. psammicola*), presence or absence of epidermal glands and rhabdoids (*e.g.*, *M. stichopi*), differences between auto- and allosperm, etc. On the other hand, there is a high degree of similarity, even in details such as sperm dimensions, between specimens from geographically distant locations, *e.g.*, *N. psammicola* from Sweden, Florida and New Zealand. Finally, several species may co-occur, as for instance *N. westbladi* and *Flagellophora apelti* (the latter quite unexpectedly) in a mud sample from Kristineberg, or *N. psammicola*, *N. elongatus* and *Ascoparia neglecta* in Florida. The result is that each specimen may present a slightly different aspect, which aggravates the diagnosing of species that lack hard, measurable characters in the first place.

In view of these difficulties I have chosen to define few but broadly distributed species, all the while emphasizing differences that may eventually lead to their splitting. At this time I consider *M. stichopi, N. westbladi* and *A. neglecta* to be reasonably well characterized whereas *N. bathycola* is still insufficiently contrasted against *N. westbladi*, and *N. psammicola.* The species *F. apelti, N. elongatus* and *Ascoparia secunda* may turn out to be clusters of sibling species.

TABLE 1

Date	*Sample number*	*Country/ ocean*	*Region*	*Locality detail*	*Depth*	*Substrate*	*Species (of specimens)*
10/13/1964	461-64 (E3)	Norway	Bergen	Raunefjord	250 m	mud	*M. stichopi* (20)
10/13/1964	462-64 (E4)	Norway	Bergen	Kors Fjord	690 m	mud	*N. bathycola* (1)
10/17/1964	467-64 (E6)	Norway	Bergen	Fana Fjord, NW of Korsnes	260 m	mud	*N. bathycola* (3)
10/17/1964	468-64 (E7)	Norway	Bergen	Raune Fjord, off Sletta	120 m	mud	*N. bathycola* (7)
10/29/1964	488-64 (E12)	Norway	Bergen	off Steinneset	620 m	mud	*N. westbladi (1)*
11/5/1964	K64-17	Sweden	Kristineberg	Essvik	30 m	mud	*N. westbladi* (115)
11/8/1964	K64-18	Sweden	Kristineberg	Klubban beach	0.5-2 m	fine sand	*N. psammicola* (11)
3/29/1965	R65-14	Adriatic	Rovinj	Punta Croce	6 m	clean coarse sand	*N. elongatus* (5), *F. apelti* (7)
4/2/1965	R65-19	Adriatic	Rovinj	Punta Croce	5 m	medium sand	*N. psammicola* (1)
5/25/1965	V (Rieger coll.)	Adriatic	Venice	Lido at Alberoni	1 m	fine sand	*N. psammicola* (5)
Jul-Oct 65	K65	Sweden	Kristineberg	Klubban beach	1-5 m	fine sand	*N. psammicola* (5)
Jul-Oct 65	K65	Sweden	Kristineberg	Essvik	30 m	mud	*N. westbladi* (2), *F. apelti* (1)
5/8/1966	R66 (Rieger coll.)	Adriatic	Rovinj	off train station	3-4 m	coarse sand with detritus	*N. psammicola* (1)
8/25/1966	P66-D	N. Ireland	Portaferry			sand	*N. psammicola* (1)
8/26/1967	R67-17	Adriatic	Rovinj	Val di Cuvi	3 m	coarse sand	*F. apelti* (1)
9/19/1967	F67-44	Mediterranean	Marina di Carrara	in harbour	3-4 m	sand	*N. psammicola* (1)
9/24/1967	F67-52	Mediterranean	Fiascherino	in harbour	1-2 m	sand	*N. westbladi* (2)
9/25/1967	F67-53	Mediterranean	Portovenere	in mid-bay	5-6 m	sand	*N. psammicola* (2)
11/25/1968	NC76 (E 48-68)	off US E coast	34°45.0'N, 75°45.0'W		41 m	clean sand	*F. apelti* (4), *A.secunda* (1)
11/27/1968	NC81 (E48-68)	off US E coast	34°28.6'N, 76°43.4'W		20 m	sand	*A. secunda* (1)
12/29/1968	FL15	Florida	Big Pine Key	patch reef	3 m	fine sand	*N. psammicola* (1), *N. elongatus*(2), *A. neglecta* (4)
3/18/1969	Eastward 4	off US E coast	off Beaufort, NC		130 m	sand	*N. westbladi* (1)
7/19/1969	D69-1	Adriatic	Dubrovnik	offshore	340 m	mud	*N. westbladi* (1)
7/25/1969	D69-8	Adriatic	Dubrovnik	offshore	400 m	mud	*N. westbladi* (1)
7/20/1969	D69-2	Adriatic	Dubrovnik	Lapad	5 m	sand between *Zostera*	*N. psammicola* (1)
9/1/1969	B66-23	W Mediterranean	Banyuls-sur-Mer	Cap Ouilleret	20 m	medium sand	*N. westbladi* (1)
2/13/1973		Bermuda	Castle Harbour	Castle Roads	8 m	clean coarse sand	*F. apelti* (2)
5/1/1974	Eastward 24303-3D	off US E coast	34°07.3N, 75°57.7W)	(cf. Coull et al. 1977)	400 m	fine sand	*F. apelti* (1)
7/2/1986	NZ 5	New Zealand	N Island	N of Leigh	upper intertidal	fine, clean sand	*N. psammicola* (1)
2/16/1996	Can 1	Gran Canaria	Las Palmas	Playa de Las Canteras	2-3 m	fine sand with *Cymodocea*	*N. psammicola* (1)
2/17/1996	Can 1	Gran Canaria	Puerto de San Nicolas	harbour	2 m	fine sand	*F. apelti* (1)
2/20/1996	Can 9	Gran Canaria	Las Palmas	Playa de Las Canteras	2-3 m	fine sand with *Cymodocea*	*N. psammicola* (1)
2/21/1996	Can 10	Gran Canaria	Arinaga	inside harbour	4-5 m	fine sand with *Cymodocea*	*N. psammicola* (1
8/13/1996	W7	Queensland	Stradbroke Island	sand flat by lab	intertidal	fine sand	*N. psammicola* (2)
8/27/1996	LZ9	Queensland	Lizard Island	Watson's Bay, S end	intertidal	fine sand with fine sea grass	*N. psammicola* (1)
9/25/1996	PNG6	Papua New Guinea	Madang	Wongat Island	8 m	*Halimeda* debris	*N. psammicola* (7), *Ascoparia sp.* (2)

MATERIAL AND METHODS

Sediment samples were taken in the intertidal by means of a spade, in the shallow subtidal by snorkeling or SCUBA, and by various dredges (Mortensen, Ockelmann) in deeper waters. Animals were extracted from sand samples with the magnesium chloride method (STERRER, 1971). Mud samples from deep bottoms, kept in tubs at 4° C in a dark room, were superficially stirred up every few days, and the suspension was filtered through a 100-200 µm plankton net mounted on a plexiglass ring (RIEGER & STERRER, 1968). The filter was then placed in a petri dish with chilled seawater from where specimens were pipetted out under a dissecting microscope. Individual specimens were photographed, and drawn and measured by means of a drawing tube. Wherever several specimens were measured, a range is given, in addition to the mean (in brackets). A relative scale is used for body length and positions; it takes the total body length of a specimen as 100 units (U), U 0 being at the anterior tip of the animal (RIEGER & STERRER, 1968). A «body index» (length divided by width) is used as an indicator of body shape (*i.e.*, the greater the body index, the more filiform the animal). Mean, relative scale (U) and body index are expressed with one or two decimals, not only to distinguish them from individual measurements but also to express the often surprisingly high constancy of morphometric characters in these soft-bodied worms. I use the term «autosperm» for «autochthonous sperm», *i.e.* sperm found in the specimen which has produced it, and «allosperm» for «allochthonous sperm», *i.e.*, sperm found in a specimen that has not produced it, *e.g.*, a functional female.

The long time period over which these observations were gathered (and exchanged with colleagues) is invoked as an excuse for the loss of many of the preserved specimens and some photo negatives, which means that in some cases (Figs. 10.1-3, 15, 16) original prints must substitute for type material.

I am indebted to the directors and staff of many marine stations for providing assistance with collecting and lab facilities, and to the following colleagues for material, unpublished observations and discussions: Michael Crezee, Kennet Lundin, Rupert J.M. Riedl, Reinhard M. Rieger, Julian Smith III, and Seth Tyler. I am grateful to my home institution for giving me time and support for this work.

TAXONOMIC PART

Meara stichopi (Bock) Westblad, 1949

(Figs 1.1-7, 2.1-5)

Meara stichopi (BOCK); WESTBLAD, 1949: 43-57, Figs 1-6, plates I-III
Meara stichopi; HENDELBERG, 1977: Figs 1-4
Meara stichopi; HENDELBERG, 1983: Fig. 2
Meara stichopi; SMITH *et al.* 1994: (rhabdoids)
Meara stichopi; LUNDIN & HENDELBERG, 1995: Figs 1-6
Meara stichopi; LUNDIN & HENDELBERG, 1996: Figs 1A-D

Meara stichopi; LUNDIN (in press a): Figs 1A-C'
Meara stichopi; LUNDIN & HENDELBERG (in press): spermiogenesis.

New material. About 20 specimens in squeeze preparation.

Locality. Espegrend, near Bergen, Norway. In the holothurian *Parastichopus tremulus* (Gunnerus) collected 13 Oct. 1964 in Raunefjord (250 m).

Organization and behaviour. The three adult specimens (Figs 1.1, 2.1) ranged from 1700 to 2100 µm (mean 1916.6 µm) in length and 450 to 640 µm (570.0 µm) in width at U 27.6 (body index 3.40). The statocyst (Figs 1.2, 2.2) is located at U 2.7, only 55 µm behind the anterior tip of the animal. The statocyst (Figs 1.2, 2.2) is elliptical, somewhat more convex anteriorly than posteriorly, 20-21 (20.5) µm long and 32-35 (33.5) µm wide. It is bisected by a septum that projects at least halfway down from the dorsal wall, incompletely dividing the statocyst into a left and a right chamber. Each statolith is 12-13 (12.5) µm in diameter, near-spherical but dorsally slightly flattened, and capped by a 4 µm thick cell (the lithocyte) that appears as if composed of regular spherical blisters of about 1 µm diameter. The statoliths normally sit on the bottom of the statocyst, with the blister cap upward, as if buoyed by it. If the animal is slowly rotated around its longitudinal axes the statoliths do not follow suit but always come to rest at the deepest part of the statocyst, blister cap on top.

Of 20 specimens checked, only 5 had bundles of 3-6 needle-shaped rhabdoids of 7-10 µm length (Fig. 1.6) that were more or less evenly distributed throughout the epidermis; the remainder had no rhabdoids at all.

Of 12 specimens of *Parastichopus tremulus* investigated, 7 contained from 1 to 40 individuals of *M. stichopi,* most of them in the foregut immediately behind the mouth, only rarely in the body cavity. When not actively moving, animals are slightly bent, with an anterior dorsal hump (Fig. 1.7). Specimens could be kept alive in a refrigerator in *Parastichopus* body fluid or seawater for up to 6 days. Maintained this way, mature specimens regularly laid eggs; they first bend into a dorsally-convex shape, then protrude the circum-oral body surface (like puckered lips) to disgorge, via the mouth, an egg of about 120 µm diameter.

Digestive tract. One specimen was seen with a nematode in its gut.

Reproductive system. The follicular testes occupy most of the preoral part of the body (Figs 1.1, 2.1, 2.3, 2.4). The male copulatory organ (mco) opens terminally to slightly supraterminally. Autosperm (Figs 1.3-5, 2.5) is filiform, about 45-60 µm long; in phase contrast a division into head, middle piece and tail is not obvious. Some sperm were coiled, corkscrew-fashion, over half of their length; none showed any movement. Allosperm was not observed.

The ovary (Figs 1.1, 2.1, 2.4) occupies most of the postoral part of the body.

Discussion. The fact that statoliths, buoyed by their lithocyte cap, always orient themselves vertically explains WESTBLAD's puzzling observation that in histological sections of *N. westbladi* (WESTBLAD, 1937: 67, and fig. 7b) the two statoliths, like a pair of eyes, invariably pointed in the same direction. Bundled rhabdoids, which SMITH *et al.* (1994) suggest «are histochemically and ultrastructurally similar to true rhabdites», were also found by LUNDIN & HENDELBERG (1995); their spotty occurrence (in only 25% of specimens) may explain Westblad's failure to find «mucous cells with formed secretion, *e.g.*, rhabdite glands». I did not notice «pulsatile bodies» (LUNDIN & HENDELBERG, 1996). My

observation of oviposition via the mouth corroborates WESTBLAD's (1949) statement that the «matured eggs are lying in the innermost part of the ovary, near the intestine», and might be «emptying by the intestine». K. LUNDIN (pers. comm.) confirms WESTBLAD's finding that masses of spermatozoids, presumably from copulation, are «in all tissues of the postoral part of the body, in the epithelium as well as in the parenchyma, particularly abundant in the neighbourhood of the ovocytes». HENDELBERG (1977) gives a sketch and TEM micrographs of what is presumably autosperm, confirming its single, 9+2 axoneme which is partly surrounded by helically coiled mitochondrial derivatives; his measurements (Fig. 1) are about 20 µm for the head, 35 µm for the middle piece, and 5-10 µm for the tail. We do not know yet whether allosperm differs from autosperm in this species (see LUNDIN & HENDELBERG, in press).

Meara sp.

Meara sp.; SMITH *et al.* 1994 (rhabdoids)

Meara sp.; LUNDIN 1997: Fig. 1D

Meara sp.; LUNDIN (in press a): Fig. 1D' (in the «foregut» of *Holothuria lentigenosa enodis* Pawson & Miller, 1979, collected near Cockburn Town, San Salvador, Bahamas, at approximately 330 m depth).

SMITH *et al.* (1994) mention *Meara* sp. in addition to *M. stichopi*. HENDELBERG (pers. comm.) says that it is not clear yet if they both belong to the same species. Their occurrence in different hosts and the symbiosis with bacteria of similar ultrastructure but of quite different size (LUNDIN, in press a) makes it seem probable that we are dealing with two species. Three specimens of *M.* sp. from the Bahamas all had rhabdoids though much more densely occurring than in *M. stichopi* (LUNDIN, 1997).

Nemertinoides elongatus Riser, 1987
(Figs 1.8-17, 3.1-4, 4.1-26)

N. sp.; SMITH & TYLER, 1985: Figs 8.7, 8.8

Nemertinoides elongatus; RISER, 1987 (NW Atlantic)

Nemertinoides elongatus; SMITH *et al.* 1994 (serous glands).

New material: 7 specimens in squeeze preparation.

Localities: Adriatic and NW Atlantic.

Organization and behaviour. Colorless to yellowish or reddish. At 4200 µm length and 300 µm width at U 42.85 (body index 14), my only intact adult specimen is below the range of 6-10 mm given by RISER (1987); all other specimens were anterior fragments. A head region (Figs 1.8, 3.1, 3.4) is often set off against the rest of the body by a shallow restriction in the region of the statocyst. An abundance of bottle-shaped mucous glands make the animal rather opaque in transmitted light (Fig. 3.4). Bottle glands are rounded and 10-12 µm in diameter in the head (Fig. 1.11), but more elongated and larger (17 µm) in the posterior body region (Fig. 1.12). A different, rather hooklet-shaped type of epidermal gland of up to 23 µm length may also be present, especially in the posterior body

region (Fig. 1.14). A pair of ropey glandular strands filled with tiny rhammoids, 1.5-3 µm in length (Fig. 1.9), originate in front of the pharynx and open close to the anterior tip of the animal. Located about 100-200 µm behind the anterior tip of the body, the statocyst (Figs 1.10, 3.2, 3.3) is 15-25 (20.14) µm long and 17-30 (25.00) µm wide; it contains two statoliths of 10-12 (10.5) µm diameter, which have a lithocyte cap in blisters. Intact animals glide and writhe animatedly, seemingly tying themselves into knots.

Digestive tract. The mouth is located at about U 25. The post-oral part of the gut contains numerous granular club cells of 10-15 µm diameter (Fig. 1.13).

Reproductive system. The only intact adult had paired testes and vasa deferentia, and a dorsal, ciliated male pore at about U 42 (Fig. 1.8). Autosperm (or possibly a late spermatid, Fig. 1.16) consisted of 46 µm long head with a spiral tip and oblique striation, a 48 µm long opaque middle piece, and a 45 µm long tail. Figs 1.15, 4.1 and 4.2 show a late spermiogenesis stage consisting of a tubular part whose posterior portion is surrounded by spirally-arranged granular precipitates, and a tail. Allosperm (Figs 1.17, 4.3, 4.4) is thinner but has a pronounced head spiral of 40 µm length, and a middle piece of 29 µm. Single allosperm are found throughout the body, even in the head and between testes follicles.

Discussion. My only intact adult (from the Adriatic) agrees well enough with RISER's (1987) original description to be assigned to this species, which cannot be said for several other specimens (*e.g.*, Fig. 3.4), many of which were fragments. *N. elongatus* differs from *N. psammicola* primarily in its larger, plumper body and head constriction; much smaller rhammoids (2 µm vs. 11.5 µm), and the reproductive system, particularly the far forward, dorsal position of the male copulatory organ, the position of the ovary posterior to the male reproductive system, and the much larger sperm.

Nemertoderma psammicola Sterrer, 1970
(Figs 5.1-26, 6.1-3, 7.1-4)

Nemertoderma sp. I; STERRER, 1966: Fig. 1, 3 (North Sea and Adriatic)
Nemertoderma psammicola; STERRER, 1970 (in Riedl 1970: 197, diagnosis; and plate 58) (Adriatic)
?*Nemertoderma* sp.; TYLER, 1976: 57, Fig. 30A (Eilat, Red Sea)
Nemertoderma rubra; FAUBEL, 1976: 2730, Fig. 5 (Sylt, NE Atlantic)
Nemertoderma sp. B; TYLER & RIEGER, 1977: Fig. 1A, 4A, 5A, C-E (N. Carolina, NW Atlantic)
Nemertoderma rubra; BUSH, 1981: 88, Fig. 184 (key)
?*Nemertoderma* sp.; RISER, 1984: 242, 246 (Christchurch, New Zealand)
Nemertoderma sp. B; EHLERS, 1992: Figs 1-4 (N. Carolina, NW Atlantic).

New material: About 35 specimens in squeeze preparation.

Localities: Mediterranean (Rovinj, Venice, Dubrovnik, Marina di Carrara, Porto Venere), Atlantic (Canary Islands, N. Ireland, Sweden, Florida), Pacific (New Zealand, Australia and Papua New Guinea).

Organization and behaviour. Adults in male and female maturity (Figs 5.1, 6.2) to 3100 µm long and 160 µm wide at U 47 (body index 19.37); adults with male gonads only

(Figs 1.3-4, 2.1) are usually smaller. The mean values for 6 adults from Kristineberg and Leigh (New Zealand) were 1838.33 µm by 141.66 µm at U 63.07 (body index 12.83). The anterior part of the body, to somewhat behind the statocyst, is medio-dorsally flattened (Fig. 5.2). Most Swedish, and many Adriatic specimens were distinguished by a salmon- or crimson-coloured stripe that extends from behind the statocyst to about the pharynx, where it turns to brownish and blends into outline of the intestine. Canaries specimens were faintly red, whereas most others were greyish or brownish. In many but not all specimens the skin contains bottle-shaped mucous glands, to 12 µm in diameter (Fig. 5.7); in the New Zealand specimen I also noted additional glands with a rod-shaped content in the caudal region (Fig. 5.8). A pair of rhammoid glands open frontally and extend posteriorly to about U 23, i.e., the region anterior to the mouth. Individual rhammoids are needle-shaped, 8-22 (11.5) µm long and 1 µm wide (Figs 5.5-6). The animal moves serpent-like. Many individuals encountered were anterior fragments.

Located at U 8.31, the statocyst is oval, 14.25 µm long and 20.75 µm wide (Figs 5.23-26, 6.3). Each of the two statoliths is spherical, 6-9 (7.28) µm in diameter, and covered dorsally with a lithocyte in blisters. The statocyst is not bisected by a septum but constricted by one or two shallow ridges. Statoliths normally rest at the bottom of the statocyst, and are held in upright position by the blister cap. A statocyst with only one statolith (Fig. 5.24) was found in 2 of 9 specimens from Kristineberg 1964 (22.2%), one of 12 from Kristineberg 1965 (8.3%), one of 7 specimens (14.3%) from Stradbroke, and one of 3 specimens (33%) from Lizard Island.

Digestive tract. Mouth ventrally, at U 37.8, opening into a shallow, ciliated pharynx bordered anteriorly by a horseshoe-shaped group of glands (Figs 5.1, 5.4).

Reproductive system. Testes paired, extending posteriorly from about U 45, not far behind the mouth, to about U 60 where they continue as a pair of vasa deferentia that merge just anterior to the male pore (Figs 5.1, 5.4). Located dorsally at U 93.4 the male pore (Fig. 5.20) is ciliated, and receives rod-shaped rhabdoids (Figs 5.21-22). Autosperm was recorded from Kristineberg (Figs 5.12, 7.3), New Zealand (Fig. 5.13) and Lizard Island specimens; it is composed of a 18-22 (17.6) µm long, apically slightly spiralized head, a 9-10 (9.7) µm long middle piece, and 16-22 (19.3) µm long tail. It appears that the axoneme runs through the entire length of the middle piece. In addition to this type of sperm, several Kristineberg specimens had also thinner, less regionalized and somewhat lumpy sperm (Fig. 5.14). Spermiogenesis stages, as observed in Kristineberg and Portaferry specimens, are shown in Figs 5.9-5.11 and 7.4.

Paired ovaries from about U 50 to U 70, with oocytes maturing posteriorly, reaching more than 100 µm in diameter (Fig. 5.1). There is no vagina or bursa. Numerous specimens, even juveniles, contained allosperm in the epidermis in all body regions, including the head (Fig. 7.1). Presumably resulting from hypodermal impregnation, such allosperm was found to be remarkably similar in specimens from all localities (Figs 5.16-19, 7.2). It is about 30 µm long, consisting of a strongly spiralized head 15-22 (18.9) µm long, with a central filament (Fig. 5.15), and a middle piece 10-16 (12.8) µm long; a tail is reduced or lacking. In living animals individual allosperm could be seen rotating around their axis and moving back and forth in what appeared to be short, preformed canals in or immediately underneath the epidermis.

Discussion. A diagnosis for this species, which I first mentioned (STERRER, 1966) as *Nemertoderma* sp. I, was given by me in RIEDL (1970) under the name *N. psammicola*. It is clearly identical, in all its features including the unusual red colouration, with *N. rubra* Faubel, 1976, which thus becomes a junior synonym. FAUBEL's (1976) description was based on specimens in male maturity only. Although my material contains but a few intact and fully mature specimens, it appears that this species is remarkably homogeneous throughout its global distribution range, particularly with regard to gross anatomy, and the size and proportions of body, statocyst, and auto- and allosperm. Variability between specimens, and possibly between geographically distant populations, is found in the colouration, and the presence and structure of glands. Male maturity seems to precede female maturity. The presence of allosperm throughout the body even of juveniles suggests that the latter may serve as the passive recipients in copulation, and then possibly store allosperm until they mature themselves. The different aspect of allosperm, and their movement in the host individual have yet to be explained.

Nemertoderma westbladi (Westblad) Steinböck, 1938

(Figs 8.1-5, 9.1-9)

Nemertoderma sp.; WESTBLAD, 1937: Drøbak (Norway), Sweden
N. westbladi; STEINBÖCK, 1938: p. 21 (species name)
N. «Nordseeform» (Norway and Sweden), *N.* «Skagerrakform» (Sweden), *N.* «Adriaform» (Adriatic); RIEDL, 1960: Fig. 1-7
N. sp.; KARLING, 1967: Figs 3-6
N. spec.; DÖRJES, 1968: 217-218, Figs 58-59 (Helgoland)
N. cf. *bathycola*; EHLERS, 1991: Figs 2, 3B (no locality given)
N. sp. D; TYLER & RIEGER, 1977: Figs 2, 7A-F (N. Carolina)
N. westbladi; LUNDIN, 1997: Fig. 3A (Kristineberg)
N. westbladi; LUNDIN (in press a): Fig. 3 (Kristineberg).

New material: About 125 specimens in squeeze preparation.

Localities: Atlantic (Sweden, N. Carolina), Mediterranean (Dubrovnik, Fiascherino, Banyuls).

Organization and behaviour. Very opaque in transmitted light, especially intestine and ovary, less so the epidermis, which creates a «halo» impression (Fig. 8.1-5). Epidermis with numerous mucous bottle glands (Fig. 9.8-9). Free-swimming animals are plump-sausage shaped (Fig. 8.1) or bottle-shaped (Fig. 8.4). The following phases can be distinguished in squeeze preparation: juvenile (without any reproductive structures), male (with copulatory organ and/or testes), female (with eggs), and hermaphrodite (both male and female organs present). My measurements of 107 specimens from the same sample (Kristineberg, Essvik, 30 m) confirm Westblad's observation that body size does not increase with maturity; on the contrary: 96 juveniles measured 240-950 (mean 539.12) µm in length and 140-550 (314.20) µm in width (body index 1.75) whereas 11 adults (Table 1) measured 360-750 (500.45) µm in length and 200-520 (311.80) µm in width (body index 1.65). The smallest male, also from a Kristineberg sample, was 245 µm long and 140 µm wide; the smallest female was 315 µm long and 200 µm wide. Similarly,

Westblad (1937: 48) recorded 12 immature specimens with an average length of 600 µm and width of 400 µm, but 9 mature specimens with an average length of 400 µm and width of 250 µm. Beyond the fact, however, that sexually mature individuals are smaller on average than immature ones there is no correlation between male, female or hermaphrodite stages and body size, and thus no clear evidence for protandry or protogyny (Table 2).

TABLE 2

Nemertoderma westbladi. *Body length (in µm) of 11 mature specimens in relation to the presence of male copulatory organ, testes and eggs.*

specimen	length	male organ	testes	eggs
1	360	+		
2	410	+		+
3	410	+	+	
4	425	+	+	
5	430			+
6	440	+		+
7	450	+		
8	500	+	+	
9	590	+		+
10	740			+
11	750	+		+

The statocyst (Fig. 9.1-5) is located at U 14.25 and measures 18-24 (21.11) µm in length and 22-36 (27.22) µm in width. It normally contains two spherical statoliths of 8-14 (11.09) µm diameter, each covered with a «blister cap» (Fig. 9.1-2). The statocyst lumen is not bisected by a septum but slightly constricted by one or two shallow ridges along the sagittal plane of the animal. Of 112 specimens taken from the same sample (Kristineberg, Essvik, 30 m), 3 had only one stone (2.7%; Fig. 9.4), and one specimen had 3 (0.9%; Fig. 9.3). A specimen taken from the same locality but on a separate occasion contained 4 statoliths (Figs 8.5, 9.5). Statocysts with aberrant statolith numbers tend to be more spherical than the normal two-stoned ones, and statoliths may be smaller.

Reproductive system. A pair of globular testes is located in mid-body region, from which sperm bundles lead to a massive vesicula seminalis in front of the male copulatory organ, which opens at or above the posterior end of the animal (Fig. 8.5). Autosperm is filiform, 83-105 µm long, slightly spiralized only at the very tip, and not conspicuously divided into a head-middle piece of 67-75 µm length, and a tail of 55-62 µm length.

Specimens in male and female maturity (Fig. 8.4-5) and those in female maturity often contain more than one large egg in the posterior body region, sometimes up to 10. Allosperm (Fig. 9.6-7) was seen in only 2 specimens, both juveniles from Fiascherino. Located throughout the body, in or immediately under the epidermis and slowly rotating, allosperm is 40 µm long, of which the head takes up 30 µm, the middle piece (plus tail?) 10 µm. Strongly spiralized around a central filament, the head is very thin for the anterior 10 µm, then swells to a diameter of 2 µm posteriorly.

Discussion. My data support WESTBLAD's (1937: 69) conclusion that, because sexually mature individuals are not only smaller than immature ones but also seem to lack a functional mouth opening, animals stop feeding at a certain age and then draw on body substance for reproduction. The presence of allosperm in juveniles also confirms WESTBLAD's (1937: 82) supposition that animals may be inseminated, by subdermal impregnation, before they reach maturity. My statocyst data suggest that aberrant statolith numbers, as encountered by RIEDL (1960), are to be expected in large enough samples and cannot be used as a diagnostic character. Thanks to WESTBLAD's careful original description, *N. westbladi* is one of the best defined species in the order.

Nemertoderma bathycola Steinböck, 1930

(Fig. 10.1-3)

Nemertoderma bathycola; STEINBÖCK, 1930 (Greenland)

N. bathycola; STEINBÖCK, 1938 (discussion)

N. cf. *bathycola*; HENDELBERG, 1977 (Norway)

N. cf. *bathycola*; LUNDIN, 1997: Fig. 3B (Kristineberg, Sweden)

N. cf. *bathycola*; LUNDIN (in press a) (Kristineberg).

New material: 12 specimens in squeeze preparation.

Locality: Norway.

Organization and behaviour. Body length of adults (Fig. 10.1-3)is 260-400 (mean 330.0) µm, body width 35-130 (87.5) µm at U 55.0 (body index 4.88). Freeswimming animals may be even more slender than that (to a body index of 9). Specimens range widely in transparency, mostly due to abundance or absence of bottle-shaped mucous glands. Located at U 12.16, the statocyst is 11 µm long and 17 µm wide. Statoliths are 8 µm in diameter, with the lithocyte cap in blisters. Of 12 specimens, one (8%) had only a single statolith in an otherwise normal statocyst.

Digestive tract. One specimen contained a large prey turbellarian.

Reproductive system. Of 7 adults, 5 contained both a male organ and a mature egg, whereas two were in male phase only. The conical male copulatory organ, often with sperm bundles on its anterior end, is located at U 89.3 (Fig. 10.3), and opens slightly supraterminally. A single mature egg was found in five individuals; it may reach 120 µm in length, or 40% of the total body length (Fig. 10.1).

Discussion. The 12 Espegrend specimens differed from *N. westbladi* in the following: adults were considerably smaller on average (X=330.00 µm vs. X=500.45 µm), more fusiform and slender (body index 4.88 vs. 1.65), and more transparent. Significantly, adults had only one mature egg, all of which agrees with the description given by Steinböck (1930: 48, Fig. 1) for *N. bathycola.* This species also seems to prefer deeper bottoms, although it is not alone at such sites, since specimens with the characteristics of *N. westbladi* were also occasionally encountered at depths below 100 m. While I am reasonably sure that the Espegrend specimens do not belong to *N. westbladi*, and that at least some of them are conspecific with *N. bathycola*, they may well represent more than one species.

Flagellophora apelti Faubel & Dörjes, 1978
(Figs 11.1-14, 12.1-5, 13.1-4)

Nemertoderma sp. II; STERRER, 1966 (Adriatic)
Flagellophora apelti; FAUBEL & DÖRJES, 1978 (North Sea off Helgoland and Scotland)
Nemertoderma sp. A; TYLER & RIEGER, 1975: fig. 1-4 (NW Atlantic)
Nemertoderma sp. A; TYLER & RIEGER, 1977 (NW Atlantic)
Flagellophora; TYLER, 1984: Fig. 13 (integument)
Flagellophora sp.; SMITH & TYLER, 1985: Fig. 8.9
F. sp.; SMITH *et al.*, 1986: Figs 5, 12
Flagellophora sp.; TYLER, 1986 (broom organ)
Flagellophora cf. *apelti*; SMITH & TYLER 1986.

New material: 21 specimens in squeeze preparation.

Localities: Kristineberg (NE Atlantic), Canary Islands, Bermuda.

Organization and behaviour. Adults (Figs 11.1-5, 12.1-5) are 210-735 µm long and 105-140 µm wide at U 72.31 (body index 3.80). Animals are rounded at both ends and often somewhat bottle-shaped, with a shallow constriction at about U 20. Located at U 20.46, the statocyst (Fig. 11.7-8, 13.1, 13.3) is 10-17 (mean 13.88) µm long and 14-22 (17.77) µm wide, with a blisterless statolith of 6-8 (7.4) µm diameter. Of 21 specimens, one (4.8%) had only one statolith. The glands of the frontal organ rarely extend caudally beyond the statocyst. The epidermis is sparsely set with hooklet-shaped glands (Fig. 11.6). All specimens had a proboscis (Figs 11.1, 11.9, 13.1-2), *i.e.*, a bundle of about 30 glands whose fiber-like necks are protrusible through a canal opening at the anterior tip of the body (Tyler 1986). Once extruded, the fibers may flare into a fan, with the distal ends appearing slightly swollen and adhesive (Fig. 11.9).

Digestive tract. Neither a mouth opening nor pharynx glands were seen.

Reproductive system. The male copulatory organ opens ventrally at U 91.37, in the center of a rosette of glands, and is usually surrounded by untidy sperm bundles (Figs 11.1, 12.4). According to FAUBEL & DÖRJES (1978) there is a single testis, which «occupies the central region of the posterior half of the body». Sperm consists of a pointed head, a segmented middle piece, and a tail, but there are differences between geographically distinct populations. In autosperm (from the testis) of a Bermuda specimen (Fig. 11.10), the head: middle piece:tail dimensions were 10:10:42 µm, with the middle piece showing a faint striation of about 15 narrow bands. For the remaining specimens I cannot say whether the measurements are for auto- or allosperm. In all of them, head and middle piece are bilaterally symmetric, with one contour slightly concave and smooth, the other slightly convex, and the middle piece composed of regularly spaced segments. Specimens from Rovinj (Figs 11.13-14, 13.4) had a 6:9:57 µm sperm, with 6 segments in the middle piece. One specimen from N. Carolina (Fig. 11.13) had a 16:9:45-62 µm sperm with a 9-segmented middle piece whereas another (Fig. 11.12), from a different sample, had a 5:4:35 µm sperm with a 4-segmented middle piece. TYLER & RIEGER (1975) give measurements of 6.5 µm each for head and middle piece of their N. Carolina specimen, stating that the middle piece contains 6-8 «crescent-shaped bodies» which «are presumably mitochondrial derivatives».

The female pore opens dorsally at U 60.53; it does not seem to be ciliated, and is often filled with sperm bundles, which may also be found in nearby vacuoles. The ovary lies behind it, with the eggs maturing caudally.

Discussion. TYLER (1986) showed that the peculiar «flagellar organ» (FAUBEL & DÖRJES, 1978) is in fact composed of gland necks and not flagella; RIEGER *et al.* (1991) therefore propose to call it «proboscis». We still ignore its function but guess that it may have to do with feeding, especially in the absence of a mouth opening. Such absence may be temporary, however, and related to the reproductive cycle as in *N. westbladi*. A female pore, with associated bundles of allosperm, is not found in other nemertodermatids (except the new *Ascoparia*). FAUBEL & DÖRJES (1978) state that «a vagina is not present», but there is an «immense cyst-like bursa seminalis» (p. 7). Since my observations on living animals always show a fairly deep, well defined invagination which could be interpreted as a vagina, I use the neutral term «female pore» pending further microanatomical clarification. The observed bilateral symmetry and middle piece segmentation of sperm agrees well with the ultrastructural data of TYLER & RIEGER (1975: fig. 4), but is different from the the striated sperm figured by FAUBEL & DÖRJES (1978: fig. 4C). The remarkable range in substratum (coarse to fine sand to mud) and depth (3 m to 400 m), together with the observed geographic variation in sperm structure and proportions, may already presage the future need to split into several species what I am here calling *F. apelti* .

Ascoparia neglecta nov. gen., nov. spec.
(Figs 14.1-10, 15.1-4)

Type material: 4 adults in squeeze preparation.

Type locality: Florida, Big Pine Key, fine coral sand near a patch reef at 4 m depth, coll. 29 December 1968.

Etymology: Genus name from Lat. *scopa* (broom), and *a-* (without), in reference to the lack of a witch's broom-shaped proboscis. The species name refers to its long dormancy in my files.

Organization and behaviour. The slender, colourless worms (Fig. 14.1-4, 15.1) measure 645-750 (mean 686.25) µm in length and 90-120 (105.00) µm in width at U 47.11 (body index 6.63). The head is rounded, and set off against the body by a slight constriction at U 19; the posterior ends in a short tail. The statocyst, located at U 19.01, is 18 µm long and 22 µm wide (Figs 14.10, 15.3). It contains two statoliths of 8 µm diameter whose lithocyte is not in blisters. A bundle of frontal glands originate behind the statocyst, at U 30; like a twisted rope their long necks run under the statocyst to open subterminally, at the anterior tip of the head. There are no rhammoids, rhabdoids, nor bottle glands, but the epidermis contains sparsely arranged «hooklet» glands (Fig. 14.8).

Digestive tract. A pair of granular glands converge ventrally at U 33.83 (Figs 14.1, 14.3, 14.4), which suggests that this is where the mouth should be expected, although no actual opening was observed.

Reproductive system. A deeply invaginated, ciliated male pore is located ventrally at U 89.63 (Figs 14.1, 14.3, 14.4, 15. 4). It is surrounded by a dense rosette of glands with granular secretion. Bundles of sperm are usually found in front of the male copulatory organ,

with the sperm heads pointing towards it. Sperm bundles were seen as far forward as U 70, but it was not clear whether these were autosperm or allosperm, nor could the location and number of testes be ascertained. Autosperm (Figs 14.5, 15.2) is filiform, with a slightly curved, 15 µm long head, a 10 µm long middle piece made up of 11-12 segments, and a 71-88 µm long tail. A spermatid (Fig. 14.6) was more curved, with a shorter tail.

The single ovary lies between U 43 and U 80, with eggs maturing caudally (Figs 14.1-2, 15.1). The largest egg may be 130 µm long, and extend to behind the female pore which is located dorsally at U 70.71. The female pore is ciliated, and in 2 of 4 specimens a bundle of allosperm was found protruding from it. In at least one specimen, vacuoles containing sperm bundles were seen to the left and right of the vagina. Allosperm protruding from the vagina (Fig. 14.7) had an undifferentiated, 16 µm long head-middle piece and a 65 µm long tail.

Discussion. The absence of a proboscis as well as the probable presence of a mouth justify erecting a new genus separate from *Flagellophora*, with which *Ascoparia* otherwise shares habitus, absence of blisters on the lithocyte, hooklet glands, location of male copulatory organ and ciliated female pore, and autosperm structure. It appears that sperm is transferred from the male copulatory organ to the female pore in bundles which are then stored in vacuoles (bursae?) prior to fertilization.

Ascoparia secunda nov. spec.
(Figs 14.11-16, 16.1-4)

Type material: One adult in squeeze preparation.

Type locality: NW Atlantic, off North Carolina (34°28-6'N, 76°43-4'W), clean coarse sand with shell at 20 m.

Additional material: One adult in squeeze preparation, NW Atlantic, off North Carolina (34°45-0'N, 75°45-0'W), clean coarse sand with shell at 41 m depth.

Etymology: The second species in the new genus.

Organization and behaviour. The type specimen («specimen A», Figs 14.15, 16.1) was 500 µm long and 110 µm wide at U 36.58 (body index 5.12). It was pointed both anteriorly and posteriorly. The statocyst, located at U 18, was 16 µm long and 19 µm wide (Figs 14.11, 16.3); statoliths are 8 µ in diameter, with the lithocyte cap not in blisters. A short bundle of frontal glands converge on the anterior tip of the head.

A second specimen («specimen B», Fig. 14.16, 16.4), which I tentatively list with this species, measured 410 µm in length and 80 µm in width at U 36.6 (body index 5.12). It was rounded both anteriorly and posteriorly, and had a narrow bundle of ropey frontal glands that extended posteriorly to the statocyst. The epidermis contained sparse, somewhat hooklet-shaped glands which reach a higher density only in the tail region (Fig. 16.2). Located at U 24.4, the statocyst (Fig. 14.12) was 16 µm long and 20 µm wide, with a blisterless lithocyte of 8 µm diameter.

Digestive tract. No mouth was seen in either specimen.

Reproductive system. Surrounded by a gland rosette, the male copulatory organ (Figs 14.15, 16.2) is situated ventrally at U 87 (U 93 in specimen B), with bundles of

sperm found dorsally from it. Sperm of the type specimen – I cannot say whether it was auto- or allosperm – consisted of an 8 µm long head with a slightly spiralized tip; a 6 µm long middle piece with 13 striations, and a 45-70 µm long tail. What may be an early spermatogenesis stage is shown in Fig. 14.13.

Specimen A (Fig. 14.15) had a female pore dorsally at U 54, which contained a bundle of allosperm that ended in a vacuole adjacent to an immature egg. The ovary extends between the female and the male pore; eggs mature caudally so that the largest egg is found immediately in front of the male copulatory organ.

Discussion. The habitus, absence of a proboscis, absence of blisters on the statoliths, location of male copulatory organ and female pore, and autosperm structure identify this species as belonging to *Ascoparia.* It differs from *A. neglecta* primarily in the location of the ovary behind the vagina. The second specimen may belong to a species yet to be described.

Ascoparia sp.

Material: Two adult specimens in squeeze preparation.

Locality: Papua New Guinea; Wongat Island off Madang, *Halimeda* debris from reef slope at 8 m.

The larger of the two adults was 450 µm long and 140 µm wide at U 50. Although these specimens could not be identified to species they are listed here as the only record of the genus in the Pacific Ocean.

THE TAXA OF NEMERTODERMATIDA

First defined as a family within the Acoela (STEINBÖCK, 1930), nemertodermatids were subsequently given the status of a suborder (and later an order, AX, 1961) within the Archoophora (KARLING, 1940). They are now considered an order within Acoelomorpha (EHLERS, 1984). The order is defined by the possession of a statocyst with two statoliths of which each is produced by a single lithocyte, and uniflagellate sperm with a 9+2 axoneme (HENDELBERG, 1983b). The nemertodermatid statocyst is symmetric, in contrast to the polylithophorous statocysts of Catenulida-Retronectidae (STERRER & RIEGER, 1974) and Rhabdocoela-Luridae (STERRER & RIEGER, 1990) which often contain an irregular number of irregularly shaped statoliths, and also differ in other aspects of their ultrastructure (EHLERS, 1991). Two statoliths must be considered the norm for the order, despite the occurrence, in four species, of aberrant statocysts with one, three, or four statoliths. Nemertodermatida share with Acoela the possession of a true frontal organ (SMITH & TYLER, 1986; but see EHLERS, 1992), and an interconnected system of epidermal ciliary rootlets (SMITH, 1990, LUNDIN & HENDELBERG, 1995, LUNDIN, 1997). But while Acoela lack rhabdites – rod-shaped, eosinophilic, epidermal secretions with a characteristic ultrastructure, histochemistry, and mode of formation (SMITH *et al.*, 1994) – the rhabdoids of *Meara* «are histochemically and ultrastructurally similar to true rhabdites» of Rhabditophora, and the glands of *Nemertinoides elongatus* appear to be making the same kind of mucus as is found in true rhabdites (SMITH *et al.*, 1994). Furthermore,

Nemertodermatida are the only Platyhelminthes with primitive, uniflagellate sperm with a 9+2 axoneme (TYLER & RIEGER, 1975, HENDELBERG, 1983b, LUNDIN & HENDELBERG, in press). The taxon Acoelomorpha, therefore, which has also been linked to the peculiar vermiform *Xenoturbella* (SMITH, 1990; but see EHLERS, 1991, NORÉN & JONDELIUS, 1997, ISRAELSSON, 1997, LUNDIN, in press b), needs to be reassessed, especially in light of new evidence from 18S rDNA analyses (CARRANZA *et al.*, 1997, JONDELIUS, 1998).

The following features might be used for establishing relationships between the genera: epithelial *vs.* insunk brain; presence and structure of mouth and pharynx; presence of proboscis, frontal organ and other glands; paired *vs.* unpaired testes and ovaries, and their orientation to the body axes; epithelial *vs.* lacunar nature of reproductive organs; and sperm structure. Most of these, however, are still too inconsistently known to be useful, especially for field identification. This leaves mainly the auxiliary reproductive structures. The male copulatory organ, present in all genera, opens either supraterminally (as in *M. stichopi, N. bathycola, N. westbladi*, and *N. psammicola*), or subterminally (*F. apelti, A. neglecta* and *A. secunda*). Only in *Nemertinoides elongatus*, the male copulatory organ is located far forward dorsally, possibly in connection with the extreme elongation of the body and the need to protect this organ in the event of body fragmentation. There are two features, however, that separate the order into two groups of genera. The first is the presence of a female pore (vagina?) and auxiliary organ (bursa?) for receiving and storing sperm in *Flagellophora* and *Ascoparia*, *vs.* the absence of such organs in all other genera. I would expect differences in sperm structure, such as the bilateral symmetry in the sperm of *Flagellophora* and *Ascoparia*, and the differentiation between autosperm and allosperm in *Nemertoderma* and *Nemertinoides,* to be linked to this character. The second feature is inconspicuous yet nevertheless as consistent; it is the observation that in all species of *Meara, Nemertoderma* and *Nemertinoides*, the lithocyte which caps the statolith is organized in the form of blisters (Fig. 1.2) whereas it is smooth in *Flagellophora* and *Ascoparia*. On the basis of these differences I propose to amend the diagnosis for the family Nemertodermatidae, and erect a second family, Ascopariidae.

DIAGNOSES

(*see also Table 3*)

Order Nemertodermatida Karling, 1940

(*emended diagnosis*)

With or without a ventral mouth and pharynx simplex; gut with lumen and granular club glands. Statocyst symmetric, with two statoliths. Without protonephridia. Sperm uniflagellate, with 9+2 microtubular pattern. Free-living or symbiotic, marine.

Fam. Nemertodermatidae Steinböck, 1930

(*emended diagnosis*)

Nemertodermatida without a female pore; sperm radially symmetric. Male pore located supraterminally or dorsally. Lithocyte in blisters. Usually with epidermal bottle glands. Three genera:

Genus *Nemertoderma* Steinböck, 1930: Nemertodermatidae with male pore located supraterminally. Testes and ovaries in posterior two thirds of body. Three species:

– *N. bathycola* Steinböck, 1930: Mean length 0.3 mm, body index 4.9. Female phase usually with only one mature egg.

Northeastern Atlantic; on mud, from 120 m to 690 m depth.

– *N. westbladi* (Westblad) Steinböck, 1938: Mean length 0.5 mm, body index 1.6. Statocyst 14 µm long, 21 µm wide, statolith 7 µm in diameter. Female phase usually with more than one mature egg. Autosperm with 18 µm long head, 9.5 µm long middle piece, and 18 µm long tail.

Northern Atlantic and Mediterranean; on mud or muddy sand, from the subtidal to 620 m depth.

– *N. psammicola* Sterrer, 1970: Mean length 1.8 mm, body index 12.8. Statocyst 18 µm long, 22 µm wide, statolith 8 µm in diameter. Autosperm with 15 µm long head, 10 µm long middle piece, and 71-88 µm long tail.

Northern Atlantic, Mediterranean and Western Pacific; in fine sand, from the intertidal to 8 m depth.

Genus *Meara* Westblad, 1949: Nemertodermatidae with male pore located supraterminally. Testes in preoral, ovaries in postoral region. Commensals in holothurians. Two species:

– *M. stichopi* Westblad, 1949: Mean length 2.2 mm, body index 3.3. Often with bundled rhabdoids of 10 µm length; bottle glands lacking. Statocyst 20 µm long, 33 µm wide, statolith 12 µm in diameter. Autosperm with 20 µm long head, 35 µm long middle piece, and 5-10 µm long tail.

Northeastern Atlantic; in *Parastichopus tremulus*.

– *M.* sp. Smith *et al.*, 1994.

Bahamas (in the foregut of *Holothuria lentigenosa enodis*).

Genus *Nemertinoides* Riser, 1987: Nemertodermatidae with male pore located dorsally. Testes in postoral, ovaries in caudal region. One species:

– *N. elongatus* Riser, 1987: Mean length 6 mm, body index 14. With bottle glands and 2 µm long rhammoids. Statocyst 20 µm long, 25 µm wide, statolith 10 µm in diameter. Male copulatory organ at about U 40. Autosperm with 46 µm long head, 48 µm long middle piece, and 45 µm long tail.

Northwestern Atlantic and Mediterranean; in coarse sand, from the intertidal to 6 m depth.

Fam. Ascopariidae nov. fam.

Nemertodermatida with a dorsal female pore; sperm bilaterally symmetric. Male pore located suberminally. Lithocyte not in blisters. Usually without epidermal bottle glands. Two genera:

Genus *Flagellophora* Faubel & Dörjes, 1978: Ascopariidae with an eversible proboscis. One species:

– *F. apelti* Faubel & Dörjes, 1978: Mean length 0.5 mm, body index 3.8. Statocyst 14 µm long, 18 µm wide, statolith 7 µm in diameter. Male copulatory organ at about U 91,

TABLE 3

Taxa of Nemertodermatida, and diagnostic characters

	av. length in mm	*body index*	*frontal organ*	*pro-boscis*	*rhabdoid length*	*bottle glands*	*hooklet glands*	*statocyst length*	*statocyst width*	*statolith diameter*	*blisters on statolith*	*mouth*	*male pore location*	*male pore at U*	*female pore location*	*female pore at U*	*autosperm symmetry*	*autosperm length head*	*autosperm length middle piece*	*autosperm length tail*
Fam. Nemertodermatidae																				
Meara stichopi	2.2	3.3	-	-	10µm	?	-	20	33	12	+	+	supraterminal	96	-		radial	20 µm	35 µm	5-10 µm
Nemertinoides elongatus	6	14+	+	-	2 µm	+	23 µm	20	25	10	+	+	dorsal	42	-		radial	46 µm	48 µm	45 µm
Nemertoderma psammicola	1.8	12.8	+	-	11.5 µm	+	?	14	21	7	+	+	supraterminal	93	-		radial	18 µm	9.5 µm	18 µm
Nemertoderma westbladi	0.5	1.6	+	-	?	+	?	21	27	11	+	+	supraterminal	98	-		radial	(h + mp	67-75 µm)	55-62 µm
Nemertoderma bathycola	0.3	4.9	?	-	?	+	?	11	17	8	+	?	supraterminal	98	?		?	?	?	?
Fam. Ascopariidae																				
Flagellophora apelti	0.5	3.8	+	+	-	-	10 µm	14	18	7	-	-	ventral	91	dorsal	60	bilateral	5-16 µm	4-9 µm	35-62 µm
Ascoparia neglecta	0.6	6.6	+	-	-	-	10 µm	18	22	8	-	?	ventral	90	dorsal	71	bilateral	15 µm	10 µm	71-88 µm
Ascoparia secunda	0.5	4.8	+	-	-	-	15µm	16	19	8	-	?	ventral	80	dorsal	54	bilateral	8 µm	6 µm	45-70 µm

female pore at about U 60. Autosperm with 5-16 µm long head, 4-9 µm long middle piece, and 35-62 µm long tail.

Northern Atlantic and Mediterranean; in sand (occasionally sandy mud), from 2 m to 400 m depth.

Genus *Ascoparia* nov. gen.: Ascopariidae without an eversible proboscis. Two species:

– *A. neglecta* nov. spec.: Mean length 0.6 mm, body index 6.6. Statocyst 18 µm long, 22 µm wide, statolith 8 µm in diameter. Male copulatory organ at about U 90, female pore at about U 71. Autosperm with 15 µm long head, 10 µm long middle piece, and 71-88 µm long tail.

Northwestern Atlantic; in fine sand, at 3 m depth.

– *A. secunda* nov. spec.: Mean length 0.5 mm, body index 4.8. Statocyst 16 µm long, 19 µm wide, statolith 8 µm in diameter. Male copulatory organ at about U 80, female pore at about U 54. Autosperm with 8 µm long head, 6 µm long middle piece, and 45-70 µm long tail.

Northwestern Atlantic; in sand, from 20 m to 41 m.

–*A.* sp.

Papua New Guinea; in *Halimeda* debris, 8 m.

ABBREVIATIONS USED IN FIGURES

als	allosperm	mp	middle piece of sperm
aus	autosperm	ov	ovary
bg	buccal glands	pb	proboscis
fo	frontal orgn	rh	rhammoids
fp	female pore	sp	sperm
h	head of sperm	t	testis
m	mouth	tl	tail of sperm
mco	male copulatory organ	vd	vas deferens

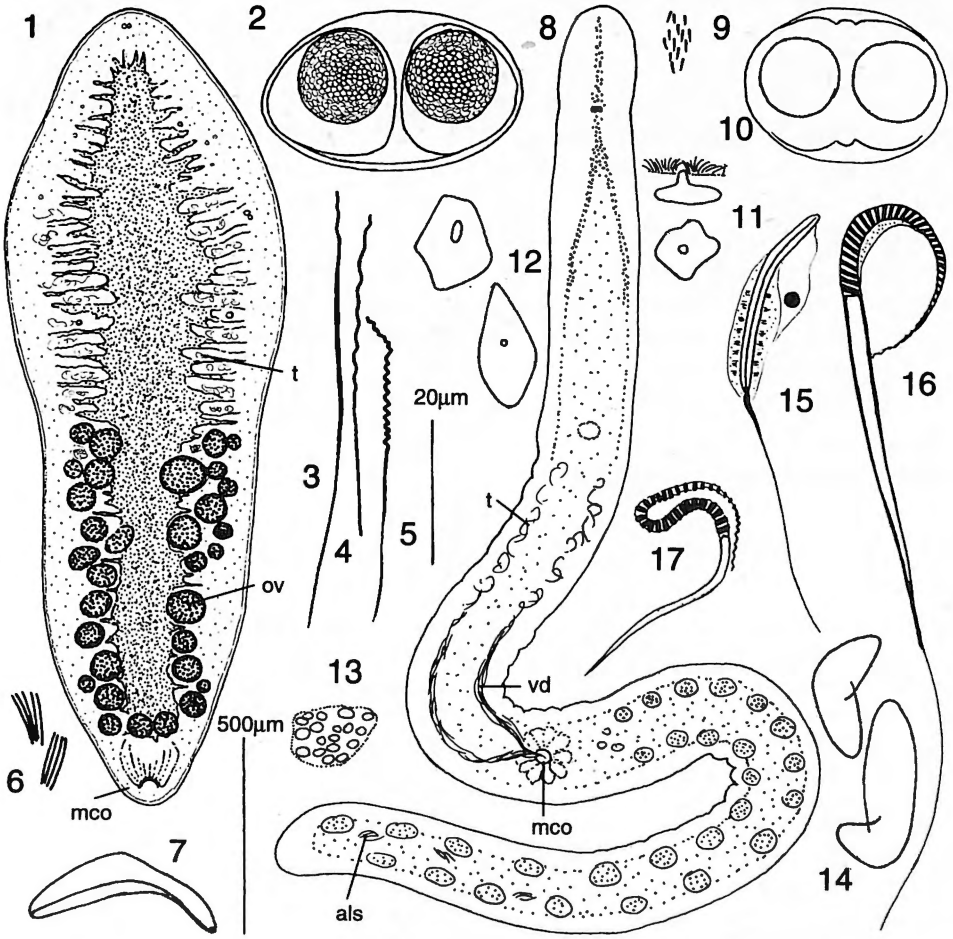


Fig. 1. – *Meara stichopi* (1-7) and *Nemertinoides elongatus* (8-17). – *Meara stichopi:* 1 mature specimen, dorsal view; 2 statocyst; 3-5 three autosperm; 6 rhabdoid bundles; 7 sketch of habitus, left ventral view. – *Nemertinoides elongatus:* 8 dorsal view of mature specimen from Rovinj (semischematic, with posterior body region completed after Riser, 1987); 9 rhabdoids; 10 statocyst («blister cap» omitted); 11 mucous gland of anterior body region, lateral (above) and dorsal (below) view; 12 mucous gland of posterior body region, dorsal view; 13 granular gland, and 14 «hooklet» gland of posterior body region; 15 spermatid; 16 autosperm or late spermatid; 17 allosperm. One scale applies to 1, a second to the remaining figures except 7 which is not to scale.

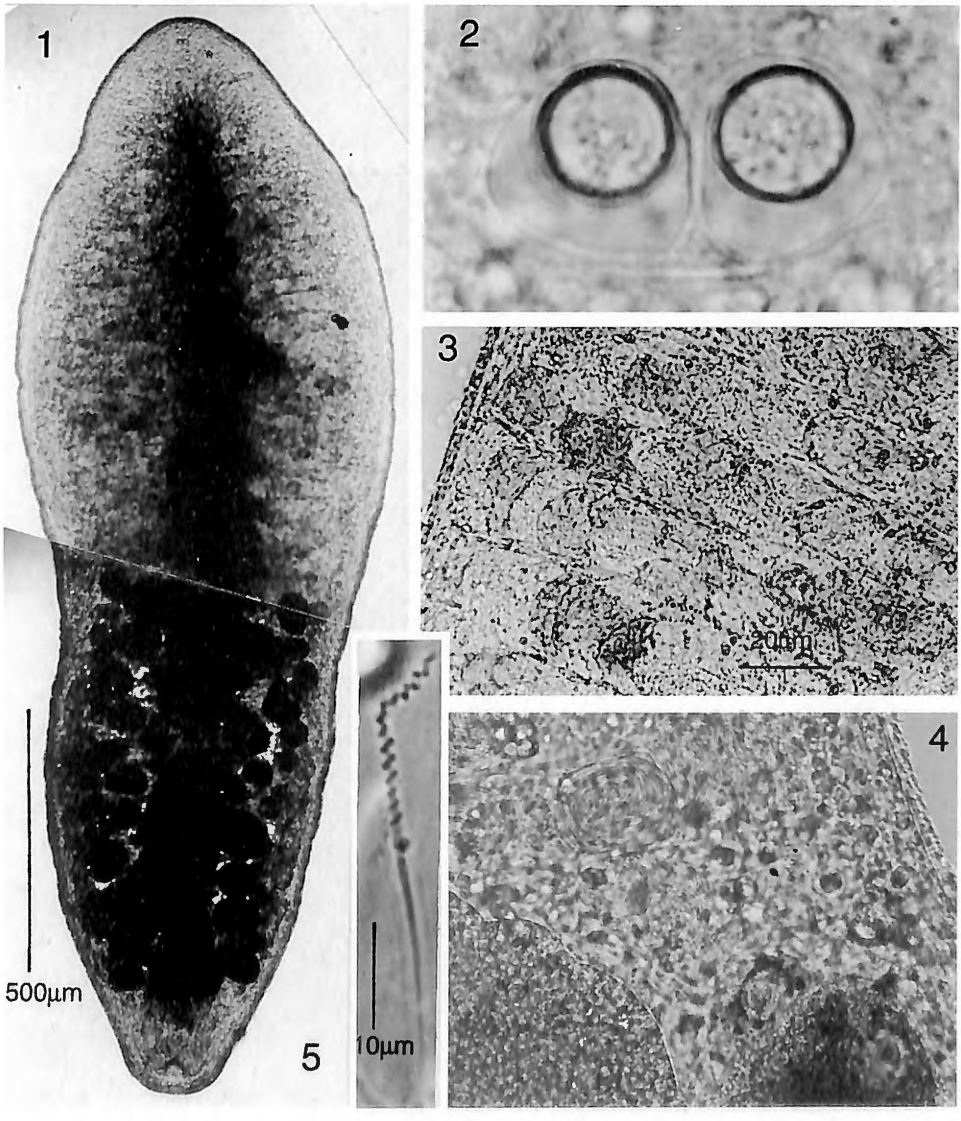
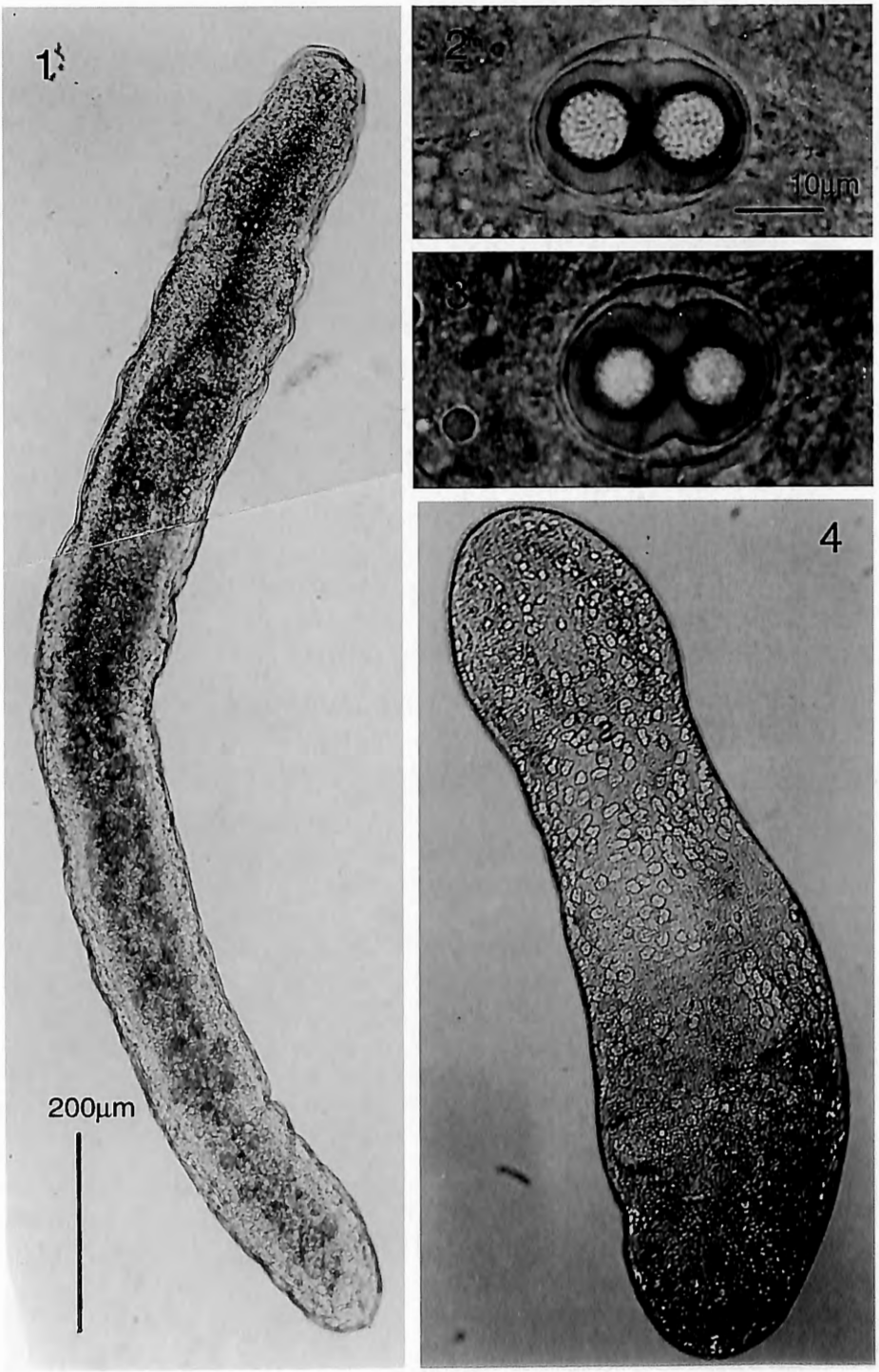


Fig. 2. – *Meara stichopi,* microphotographs of live specimens from Espegrend. – 1 habitus of adult, dorsal view; 2 statocyst, dorsal view; 3 testis follicles in left anterolateral body region; 4 sperm balls anterior to eggs in right mid-body region; 5 autosperm. One scale applies to 1; and a second to 2 and 5; and a third to 3 and 4.


1
200µm
2
10µm
3
4

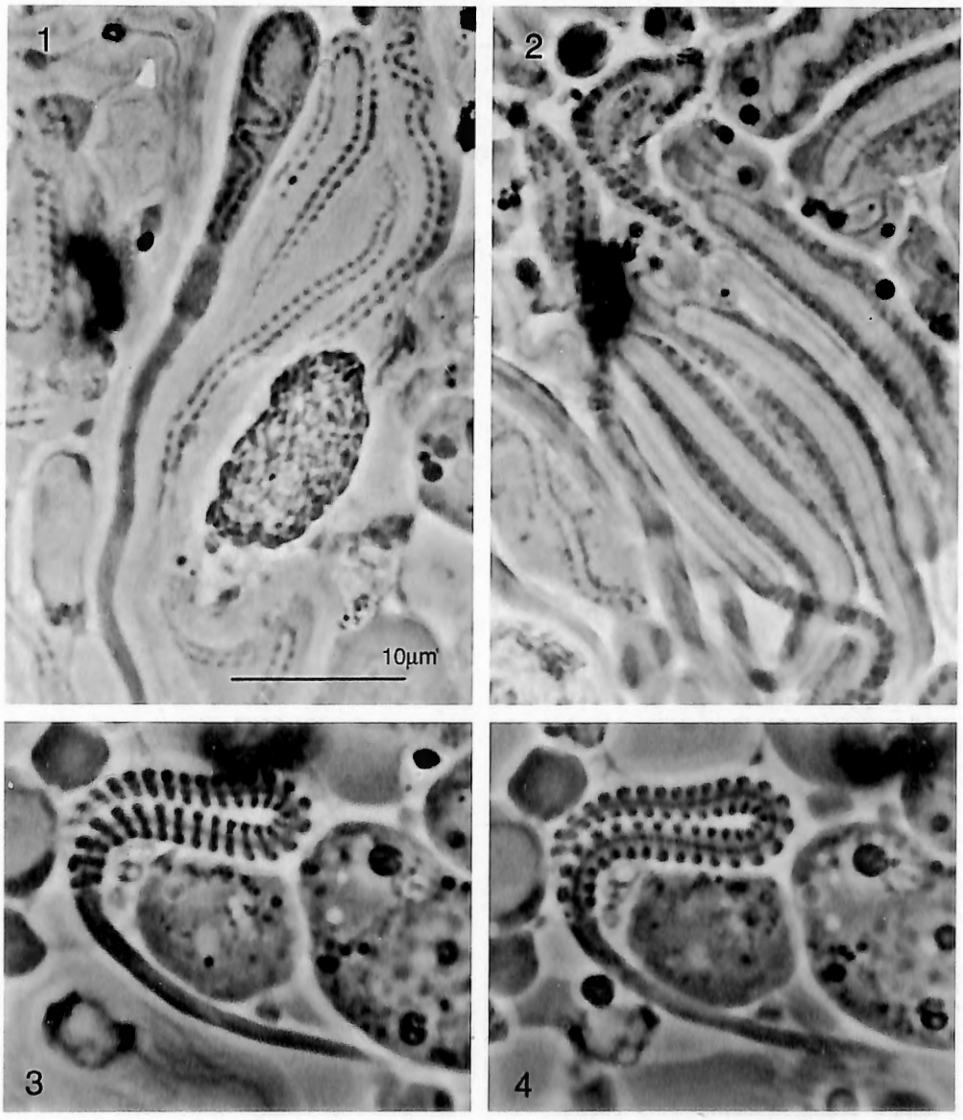


Fig. 4. – *Nemertinoides elongatus,* microphotographs of live specimens from Florida. – 1 & 2 spermiogenesis stages; 3 & 4 allosperm, at two different planes of focus. All to the same scale.

Fig. 3 (page 76). – *Nemertinoides elongatus,* microphotographs of live specimens from Rovinj. – 1 anterior fragment; 2 & 3 statocyst of two specimens: 4 anterior fragment with many bottle glands. One scale applies to 1 and 4; and a second to 2 and 3.

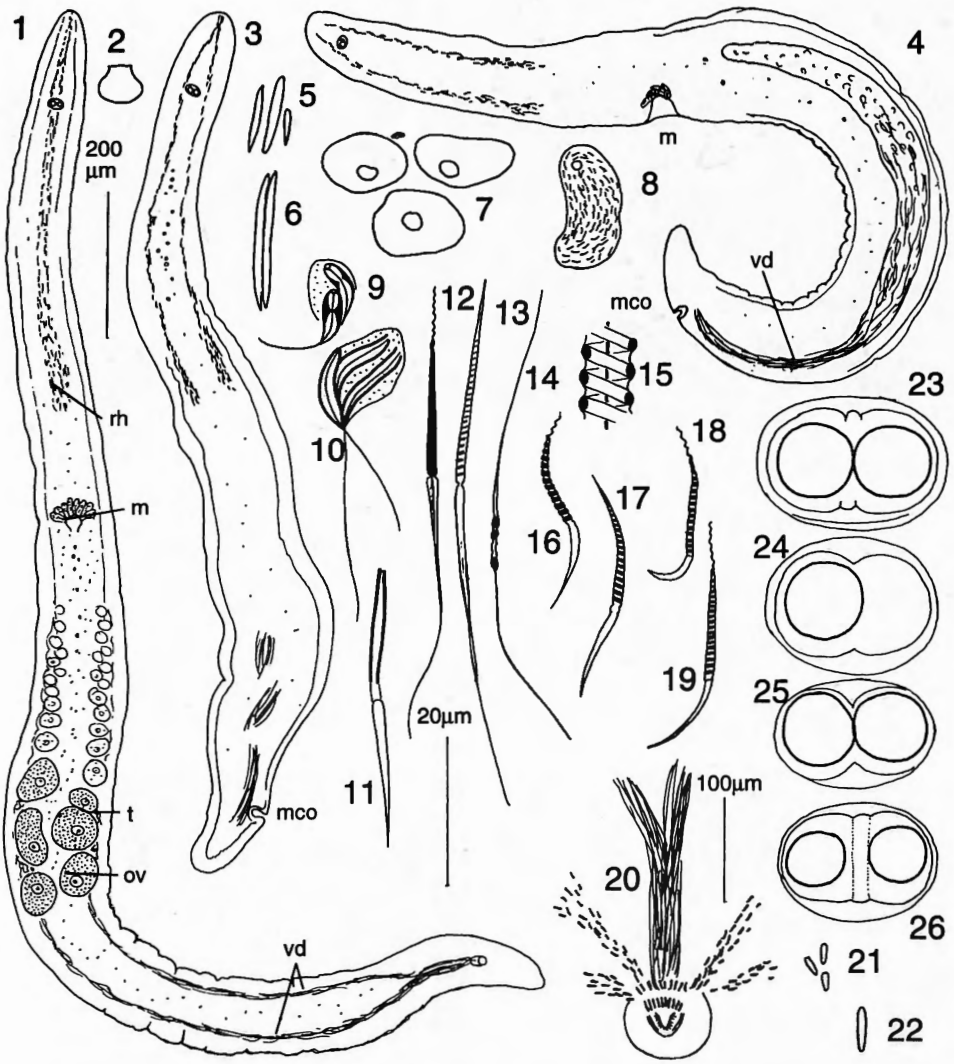


Fig. 5. – *Nemertoderma psammicola.* – 1 mature specimen from Kristineberg, dorsal view; 2 schematic cross section through the rostrum; 3 specimen in male phase from New Zealand, anterior in dorsal, posterior in right lateral view; 4 specimen in male phase from Kristineberg, left lateral view; 5 rhammoids of a New Zealand specimen; 6 rhammoids of a Florida specimen; 7 bottle glands of New Zealand specimen; 8 rod gland of New Zealand specimen; 9-11 successive spermatogenesis stages of a Kristineberg specimen; 12-13 autosperm of a Kristineberg (12) and New Zealand (13) specimen; 14 aberrant autosperm of a Kristineberg specimen; 15 schematic view of head of allosperm; 16-19 allosperm of specimens from Florida (16), New Zealand (17), Gran Canaria (18) and Kristineberg (19); 20 vasa deferentia and male pore of New Zealand specimen; 21-22 rhabdoids associated with male pore; 23-26 statocysts («blister cap» omitted) of specimens from Kristineberg (23, 24), New Zealand (25), and Gran Canaria (26). One scale applies to 1, 3 and 4; a second to 20, and a third to the remaining figures.

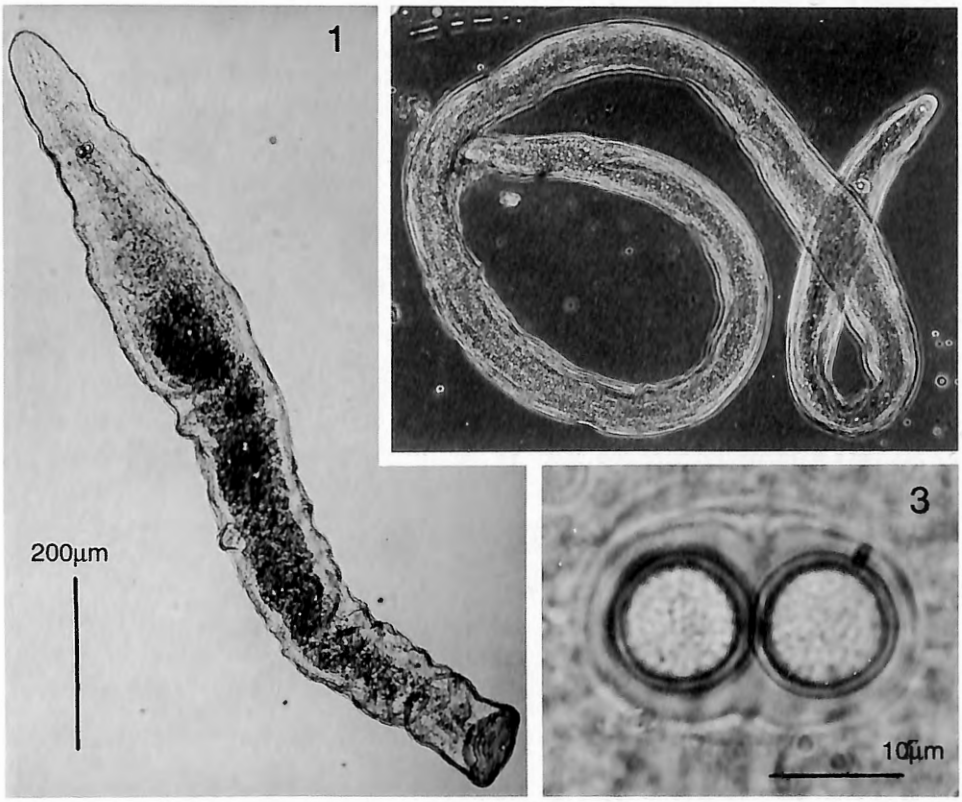


Fig. 6. – *Nemertoderma psammicola,* microphotographs of live specimens. – 1 adult from Fiascherino; 2 adult from Portaferry; 3 statocyst of specimen from Kristineberg. The same scale applies to 1 and 2.

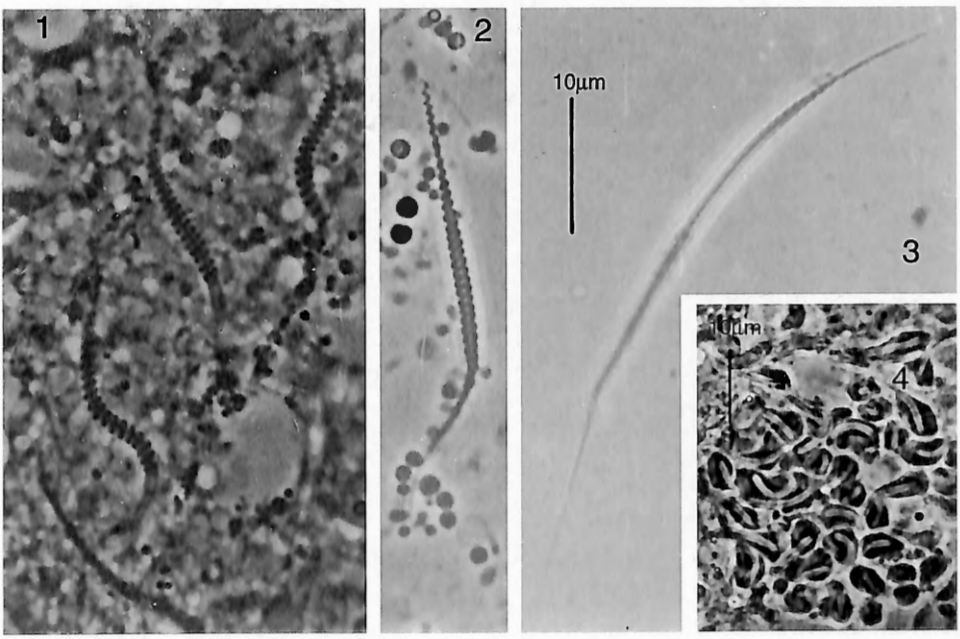


Fig. 7. – *Nemertoderma psammicola,* microphotographs of live specimens. – 1-3 sperm of the same specimen from Kristineberg: 1 allosperm in situ; 2 allosperm squeezed out of body; 3 autosperm; 4 spermiogenesis stages of a Portaferry specimen. The same scale applies to 1-3.

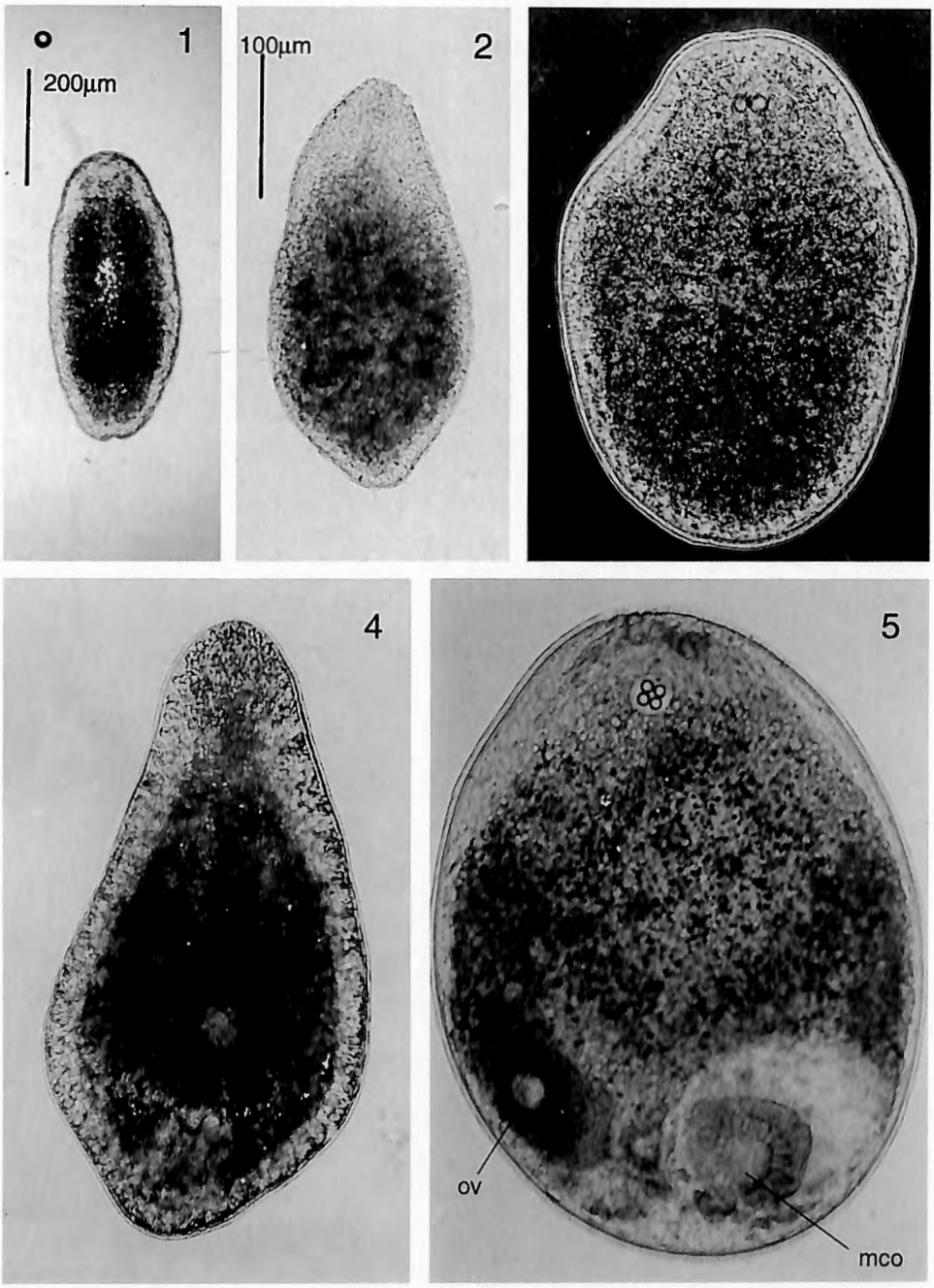
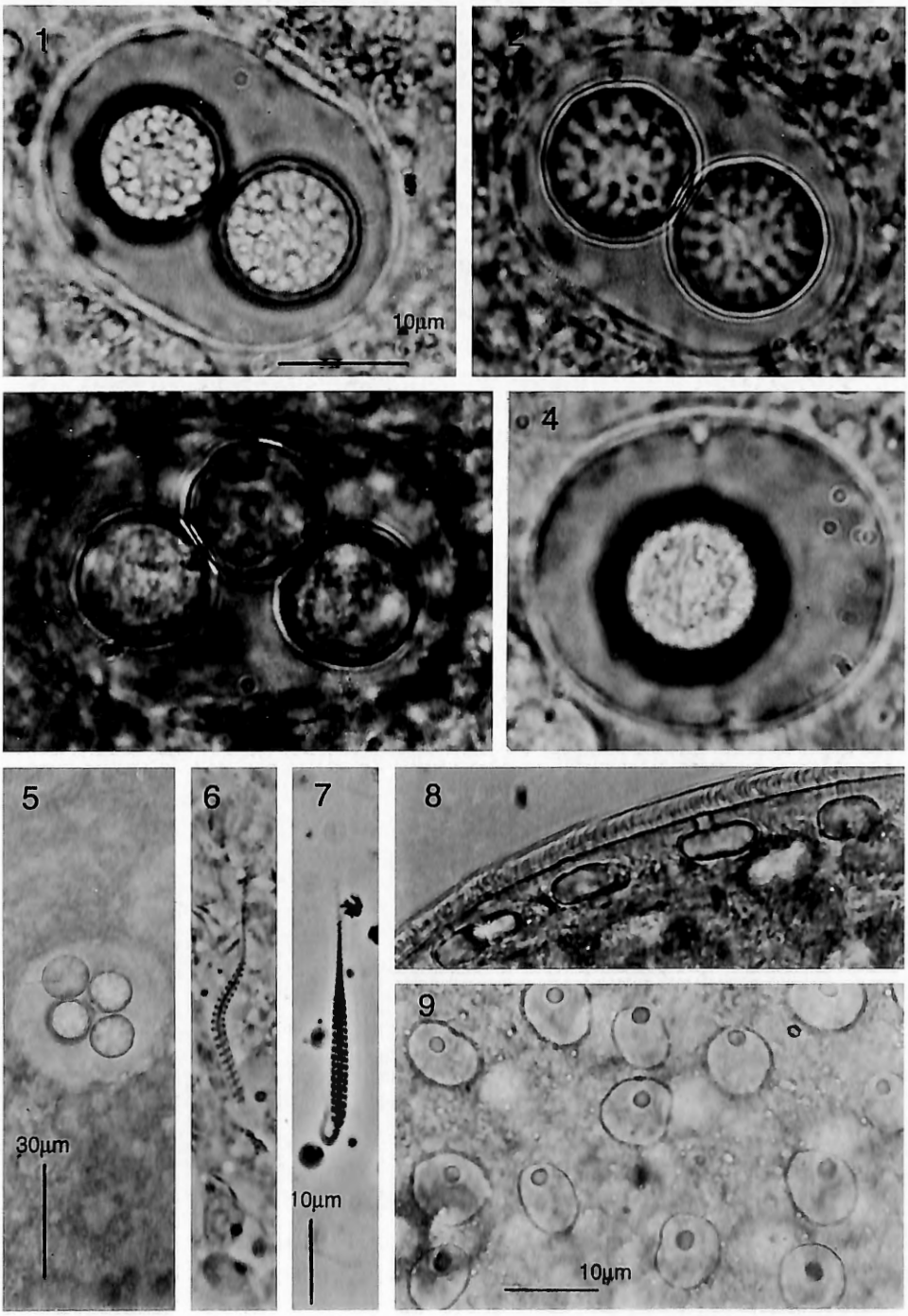


Fig. 8. – *Nemertoderma westbladi*, microphotographs of live specimens. – 1 juvenile from Kristineberg; 2 juvenile from Fiascherino; 3 juvenile from Banyuls; adult with mature egg from Dubrovnik; 5 adult with 4 statoliths, with egg and male organ, from Kristineberg. 3 and 5 are more strongly squeezed than the rest. The same scale applies to 2-5.


1
10µm
2
3
4
5
30µm
6
7
10µm
8
9
10µm

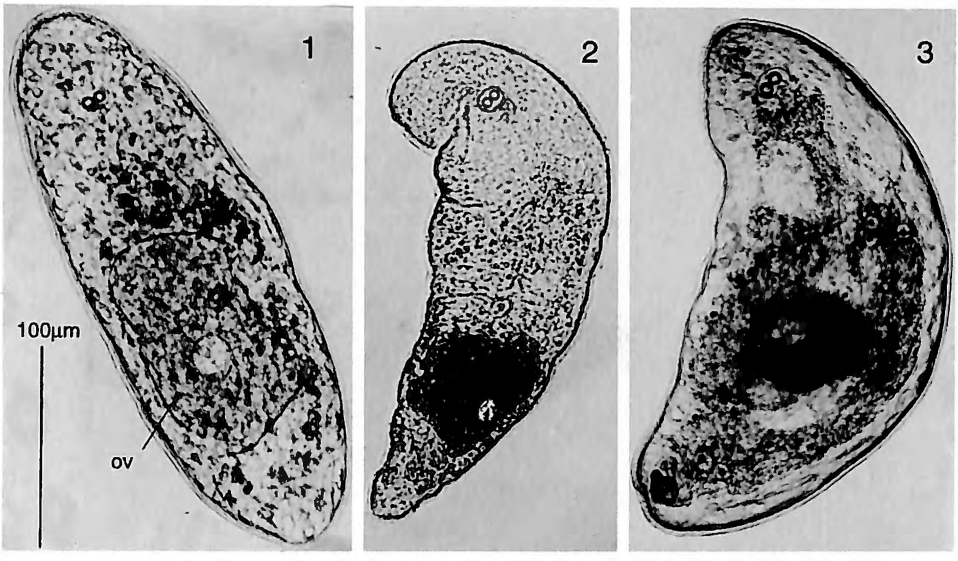


Fig. 10. – *Nemertoderma bathycola,* microphotographs of live specimens from Espegrend. – 1 specimen from 260 m depth; 2 specimen from 120 m depth; 3 specimen from 260 m depth. All to the same scale

Fig. 9 (page 82). – *Nemertoderma westbladi,* microphotographs of live specimens. – 1-2 statocyst of a Kristineberg specimen, at different planes of focus; 3-5 statocysts with aberrant statolith numbers (Kristineberg specimens); 6-7 allosperm of a Fiascherino specimen, in situ, (6) and squeezed out of specimen (7); 8-9 mucous bottle glands, lateral (8) and dorsal view (9). One scale applies to 1-4; a second to 5, a third to 6-7, and a fourth to 8-9.

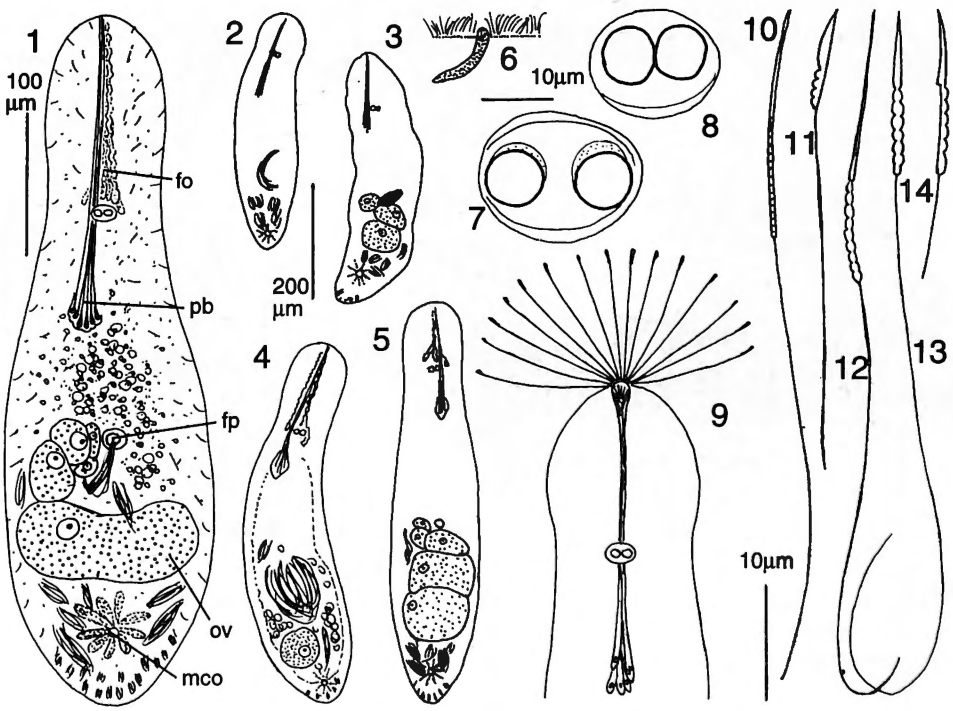


Fig. 11. – *Flagellophora apelti.* – 1 mature specimen from Bermuda, dorsal view; 2-5 habitus of specimens from Kristineberg (2) and Rovinj (3-5); 6 «hooklet gland» of specimen from North Carolina, lateral view; 7-8 statocyst of specimen from North Carolina (7) and Bermuda (8); 9 protruded proboscis; 10-14 sperm of specimens from Bermuda (10), North Carolina (11, 12) and Rovinj (13 in top view, and 14 in side view). One scale applies to 1, a second to 2-5, a third to 6-8, and a fourth to 10-14. Fig. 9 redrawn from RIEGER *et al.* 1991, fig. 8J.

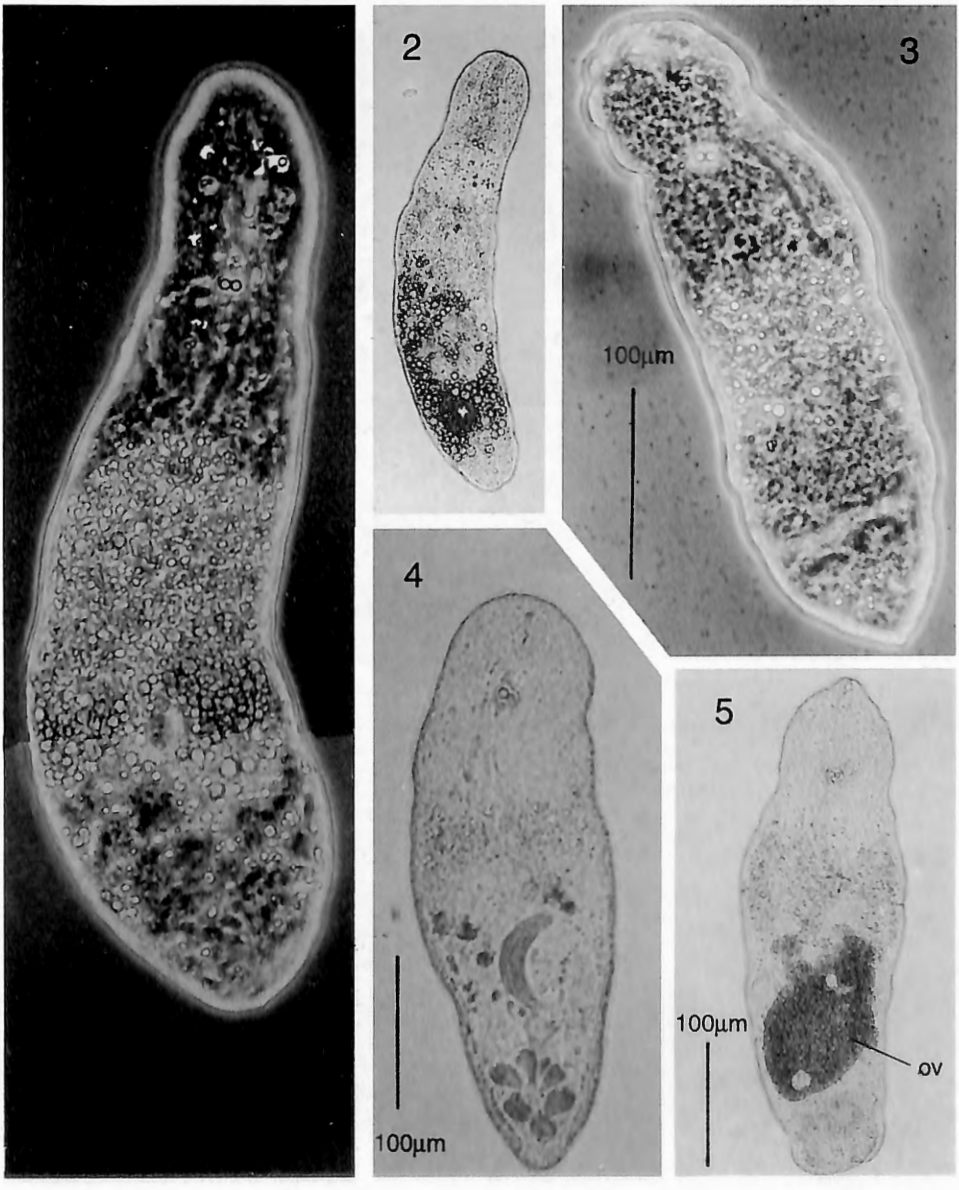


Fig. 12. – *Flagellophora apelti,* microphotographs of live, adult specimens. – 1 from Bermuda; 2 from Rovinj; 3 from North Carolina; 4 from Kristineberg; 5 from Rovinj. One scale applies to 1 and 3, a second to 2 and 5, and a third to 4.

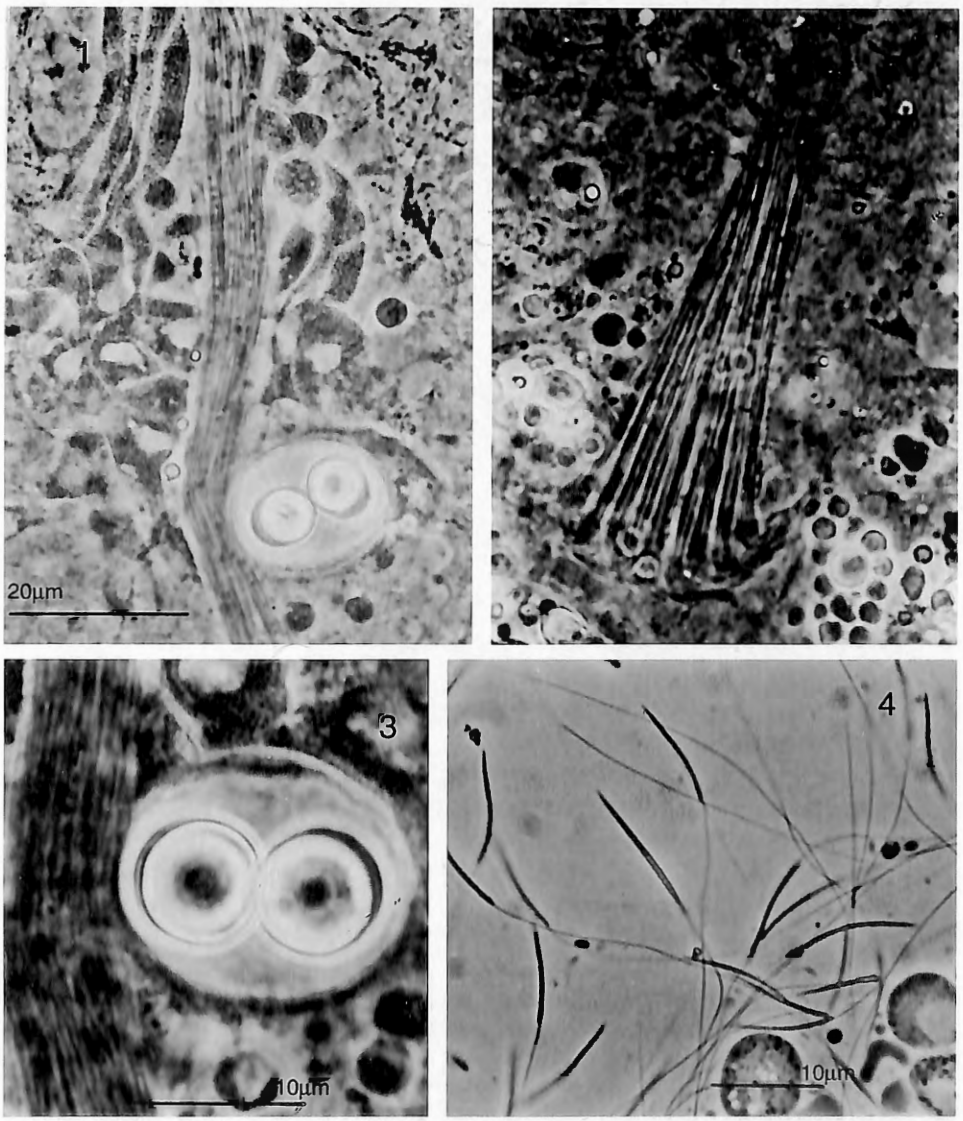


Fig. 13. – *Flagellophora apelti,* microphotographs of live specimens. – 1 statocyst, proboscis and frontal organ (Bermuda specimen); 2 posterior end of proboscis (Bermuda specimen); 3 statocyst (Bermuda specimen); 4 sperm (Rovinj specimen). One scale applies to 1-2, a second to 3, and a third to 4.

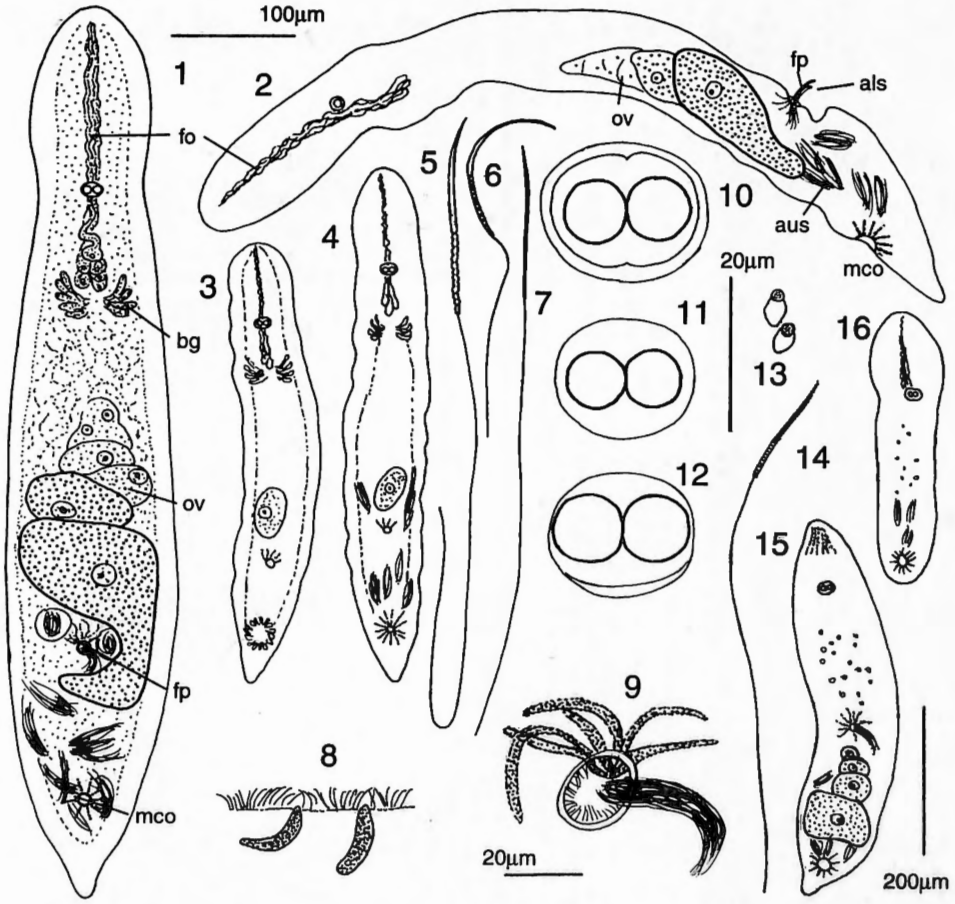


Fig. 14. – *Ascoparia neglecta* (1-10) and *A. secunda* (11-16). – *A. neglecta:* 1 mature specimen, dorsal view; 2 the same in left lateral view; 3-4 two other specimens, dorsal view; 5 autosperm; 6 spermatid; 7 allosperm; 8 epidermal 'hooklet' glands, lateral view; 9 vagina, dorsal view; 10 statocyst. – *A. secunda:* 11 statocyst of specimen A; 12 statocyst of specimen B; 13 spermatogenesis stage; 14 sperm; 15 specimen A, dorsal view; 16 specimen B, dorsal view. One scale applies to 1-2; a second to 3, 4, 15 and 16; a third to 9; and a fourth to the remaining Figs.

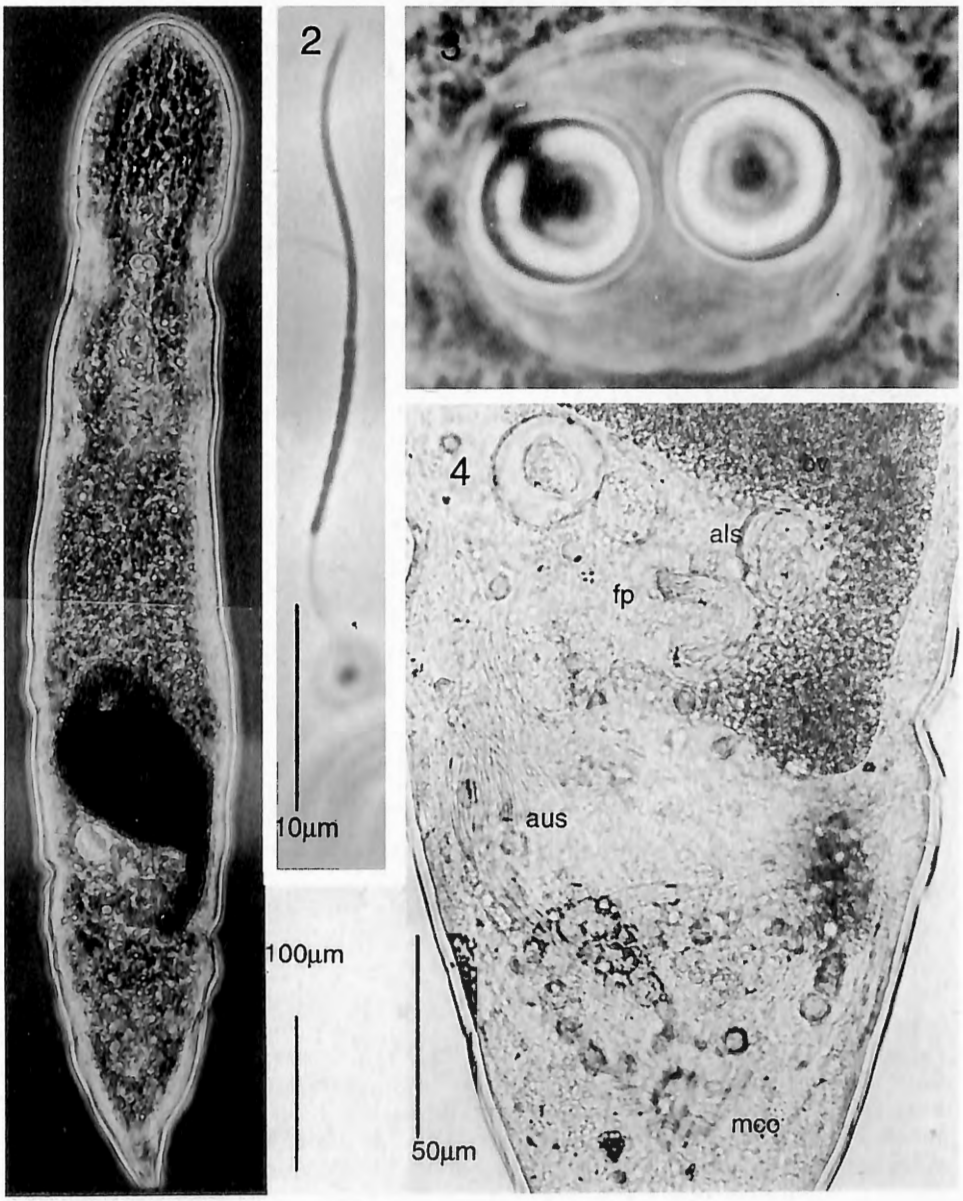


Fig. 15. – *Ascoparia neglecta*, microphotographs of live specimens. – 1 dorsal view of adult; 2 autosperm; 3 statocyst; 4 posterior body region, dorsal view. One scale applies to 1, a second to 2-3, and a third to 4.

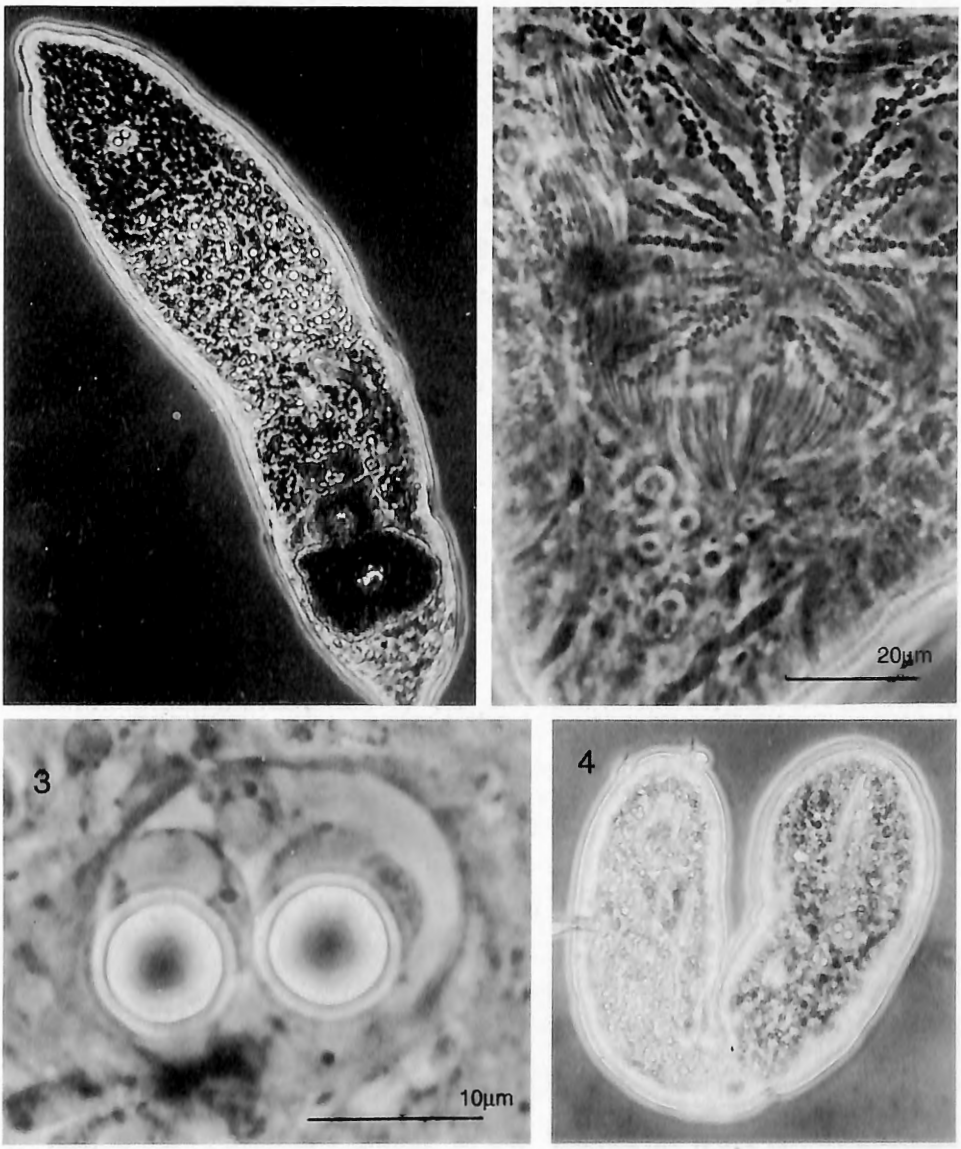


Fig. 16. – *Ascoparia secunda,* microphotographs of live specimens. – 1 specimen A, dorsal view; 2 specimen A, male pore; 3 specimen A, statocyst; 4 specimen, B dorsal view. The same scale applies to 1 and 4.

REFERENCES

AX, P. (1961) – Verwandtschaftsbeziehungen und Phylogenie der Turbellarien. *Ergebnisse der Biologie,* **24**: 1-68.

BUSH, L.F. (1981) – Marine flora and fauna of the northeastern United States. Turbellaria: Acoela and Nemertodermatida. *NOAA Techn. Report NMFS Circular 440*, 70 pp.

CARRANZA, S., J. BAGUÑA and M. RIUTORT (1997) – Are the Platyhelminthes a monophyletic primitive group? An assessment using 18S rDNA sequences. *Mol. Biol. Evol.*, **14**: 485-497.

DÖRJES, J. (1968) – Die Acoela (Turbellaria) der Deutschen Nordseeküste und ein neues System der Ordnung. *Z. zool. Syst. Evolutionsforsch*, **6**: 56-452.

EHLERS, U. (1984) – Phylogenetisches System der Plathelminthes. *Verh. naturwiss. Ver. Hamburg* (NF), **27**: 291-294.

EHLERS, U. (1985) – *Das phylogenetische System der Plathelminthes*. G. Fischer, Stuttgart-New York, 317 pp.

EHLERS, U. (1991) – Comparative morphology of statocysts in the Plathelminthes and the Xenoturbellida. *Hydrobiologia,* **227**: 263-271.

EHLERS, U. (1992) – Frontal glandular and sensory structures in *Nemertoderma* (Nemertodermatida) and *Paratomella* (Acoela): ultrastructure and phylogenetic implications for the monophyly of the Euplathelminthes (Plathelm.). *Zoomorphology,* **112**: 227-236.

FAUBEL, A. (1976) – Interstitielle Acoela (Turbellaria) aus dem Litoral der nordfriesischen Inseln Sylt und Amrum (Nordsee). *Mitt. Hamburg. Zool. Mus. Inst.,* **73**: 17-56.

FAUBEL, A. & J. DÖRJES (1978) – *Flagellophora apelti* gen. n. sp. n.: A remarkable representative of the order Nemertodermatida (Turbellaria: Archoophora). *Senckenbergiana marit.*, **10**: 1-13.

HENDELBERG, J. (1977) – Comparative morphology of turbellarian spermatozoa studied by electron microscopy. *Acta Zool. Fenn.*, **154**: 149-162.

HENDELBERG, J. (1983a) – Platyhelminthes-Turbellaria. In: Eds. ADIYODI K.G. & ADIYODI, R.G., *Reproductive Biology of Invertebrates*, Vol. 2, New York, John Wiley & Sons: 75-104.

HENDELBERG, J. (1983b) – Trends in the evolution of flatworm spermatozoa. In: *The Sperm Cell* (ed. J. André), Martinus Nijhoff Publishers, The Hague: 450-453.

ISRAELSSON, O. (1997) – *Xenoturbella's* molluscan embryogenesis. *Nature*, **390**: 32.

JONDELIUS, U. (in press) – Flatworm phylogeny from partial 18S rDNA sequences. *Hydrobiologia.*

KARLING, T.G. (1940) – Zur Morphologie und Systematik der Alloeocoela Cumulata und Rhabdocoela Lecithophora (Turbellaria). *Acta Zool. Fenn.*, **26**: 1-260.

KARLING, T.G. (1967) – Zur Frage von dem systematischen Wert der Kategorien Archoophora und Neoophora (Turbellaria). *Comm. Biol. Soc. Scient. Fenn.*, **30**: 1-11.

LUNDIN, K. (1997) – Comparative ultrastructure of the ciliary rootlets and associated structures in species of the Nemertodermatida and Acoela (Plathelminthes). *Zoomorphology,* **117**: 81-92.

LUNDIN, K. (in press a) – Symbiotic bacteria on species of the Nemertodermatida (Platyhelminthes). *Acta Zoologica.*

LUNDIN, K. (in press b) – The ciliary rootlets of *Xenoturbella bocki* revisited: new support for a possible kinship with the Acoelomorpha (Platyhelminthes). *Zoologica Scripta.*

LUNDIN, K. & J. HENDELBERG (1995) – Ultrastructure of the epidermis of *Meara stichopi* (Platyhelminthes, Nemertodermatida) and associated extra-epidermal bacteria. *Hydrobiologia,* **305**: 161-165.

LUNDIN, K. & J. HENDELBERG (1996) – Degenerating epidermal bodies («pulsatile bodies») in *Meara stichopi* (Plathelminthes, Nemertodermatida). *Zoomorphology,* **116**: 1-6.

LUNDIN, K. & J. HENDELBERG (in press) – Is the sperm type of the Nemertodermatida close to that of the ancestral Platyhelminthes? *Hydrobiologia.*

NORÉN, M. & U. JONDELIUS (1997) – *Xenoturbella's* molluscan relatives. *Nature*, **390**: 31-32.

RIEDL, R. (1960) – Über einige nordatlantische und mediterrane *Nemertoderma*-Funde. *Zool. Anz.*, **165**: 222-248.

RIEGER, R. & W. STERRER (1968) – *Megamorion brevicauda* gen. nov., spec. nov., ein Vertreter der Turbellarienordnung Macrostomida aus dem Tiefenschlamm eines norwegischen Fjords. *Sarsia,* **31**: 75-100.

RIEGER, R.M., S. TYLER, J.P.S. SMITH III & G.E. RIEGER (1991) – Platyhelminthes: Turbellaria. In: Eds. Harrison, F. W. & Bogitsh, B. J., *Microscopic Anatomy of Invertebrates*, Vol. 3, New York, John Wiley & Sons: 1-140.

RISER, N.W. (1984) – General observations on the intertidal interstitial fauna of New Zealand. *Tane*, **30**: 239-250.

RISER, N.W. (1987) – *Nemertinoides elongatus*, gen. n., sp. n. (Turbellaria: Nemertodermatida) from coarse sand beaches of the Western North Atlantic. *Proc. Helminthol. Soc. Washington,* **54**: 60-67.

SMITH, J.P.S. (1981) – Fine-structural anatomy of the parenchyma in the Acoela and Nemertodermatida (Turbellaria). Ph.D. Dissertation, Univ. North Carolina, Chapel Hill.

SMITH, J.P.S. (1990) – Ultrastructure of the ciliary rootlet system in Acoelomorpha: Phylogenetic significance. *Am. Zool.* **30**(4): 46A (257) (Abstract).

SMITH, J.P.S., M.B. THOMAS, R. CHANDLER & S.F. ZANE (1988) – Granular inclusions in the oocytes of *Convoluta* sp., *Nemertoderma* sp., and *Nemertinoides elongatus* (Turbellaria, Acoelomorpha). *Fortschritte Zool.,* **36**: 263-269.

SMITH, J.P.S. & S. TYLER (1985) – The acoel turbellarians: kingpins of metazoan evolution or a specialized offshoot? In: Conway Morris C, George JD, Gibson R, Platt HM (eds) *The origins and relationships of lower invertebrates*. Oxford University Press, Oxford: 123-142.

SMITH, J.P.S. & S. TYLER (1986) – Frontal organs in the Acoelomorpha (Turbellaria): ultrastructure and phylogenetic significance. *Hydrobiologia,* **132**: 71-78.

SMITH, J. P. S. & S. TYLER (1988) – Frontal organs in the Nemertodermatida (Turbellaria). *Am. Zool.,* **28**: 140A (Abstract).

SMITH, J.P.S., S. TYLER, D. BOATWRIGHT & K. LUNDIN (1994) – Rhabdite-like secretions in Acoelomorpha: evidence for monophyly? *Trans. Amer. Microsc. Soc.,* **113**: 97 (Abstract).

SMITH, J.P.S., S. TYLER & R.M. RIEGER (1986) – Is the Turbellaria polyphyletic? *Hydrobiologia,* **132**: 71-78.

SMITH, J.P.S., S. TYLER, M.B. THOMAS & R.M. RIEGER (1982) – The morphology of turbellarian rhabdites: phylogenetic implications. *Trans. Am. Microsc. Soc.*, **101**(3): 209-228.

STEINBÖCK, O. (1930-31) – Ergebnisse einer von E. Reisinger & O. Steinböck mit Hilfe des Rask-Ørsted Fonds durchgeführten Reise in Grönland 1926. 2. *Nemertoderma bathycola* nov. gen. nov. spec., eine eigenartige Turbellarie aus der Tiefe der Diskobay; nebst einem Beitrag zur Kenntnis des Nemertinenepithels. *Vidensk. Medd. Dansk Naturhist. Foren.*, 90: 47-84.

STEINBÖCK, O. (1938) – Über die Stellung der Gattung *Nemertoderma* Steinböck im System der Turbellarien. *Acta Soc. Fauna Flora Fennica,* **62**: 1-28.

STERRER, W. (1966) – New polylithophorous marine Turbellaria. *Nature,* **210**: 436.

STERRER, W. (1970) – Turbellaria. In: RIEDL, R.(ed.), *Fauna und Flora der Adria,* Hamburg: Parey: 196-201 (2nd ed.).

STERRER, W. (1971) – Gnathostomulida: problems and procedures. *Smithsonian Contributions to Zool.,* **76**: 9-15.

STERRER, W. & R.M. RIEGER (1974) – Retronectidae – a new cosmopolitan marine family of Catenulida (Turbellaria). In: *Biology of the Turbellaria*, N.W. RIESER & M.P. MORSE (eds.), McGraw-Hill. New York: 63-92.

STERRER, W. & R.M. RIEGER (1990) – New species of the statocyst-bearing marine dalyellioid genus *Lurus* Marcus (Luridae nov. fam., Turbellaria-Rhabdocoela). *Cahiers de Biologie Marine*, **31**: 485-500.

TYLER, S. (1976) – Comparative ultrastructure of adhesive systems in the Turbellaria. *Zoomorphologie*, **84**: 1-76.

TYLER, S. (1984) – Turbellarian platyhelminths. in: BEREITER-HAHN, J., A.G. MATOLTSY & K.S. RICHARDS (eds.), *Biology of the Integument*, vol. 1 Invertebrates. Springer Verlag, Berlin: 112-131.

TYLER, S. (1986) – Ultrastructure of a remarkable food-gathering organ in *Flagellophora* sp. (Turbellaria, Nemertodermatida). *Trans. Am. Microsc. Soc.*, **105**: 90 (Abstract)

TYLER, S. & R.M. RIEGER (1975) – Uniflagellate spermatozo a in *Nemertoderma* (Turbellaria) and their phylogenetic significance. *Science*, **188**: 730-732.

TYLER, S. & R.M. RIEGER (1977) – Ultrastructural evidence for the systematic position of the Nemertodermatida (Turbellaria). *Acta zool. Fenn.*, **154**: 193-207.

WESTBLAD, E. (1937) – Die Turbellariengattung *Nemertoderma* Steinböck. *Acta Soc. Fauna Flora Fenn.*, **60**: 45-89.

WESTBLAD, E. (1949) – On *Meara stichopi* (Bock) Westblad, a new representative of Turbellaria Archoophora. *Ark. Zool.*, **1**: 43-57.

Belg. J. Zool. - Volume 128 (1998) - issue 1 - pages 93-95 - Brussels 1998

SHORT NOTE

A CRITICAL ASSESSMENT OF THE ALCIAN BLUE/ALIZARINE DOUBLE STAINING IN FISH LARVAE AND FRY

PIERRE VANDEWALLE, ISABELLE GLUCKMANN AND FRANCE WAGEMANS

Université de Liège, Institut de Zoologie, Laboratoire de Morphologie fonctionnelle et évolutive, 22 quai Van Beneden, B-4020 Liège, Belgique.
e-mail: P.Vandewalle@ulg.ac.be

Key words: fry skeleton, staining.

Methylene blue and/or toluidine blue were initially used for staining cartilage, in-combination with alizarin red S for staining of bone, while a KOH solution was used for clearing the tissues after the staining process (1, 2, 3, 4). In 1967, TAYLOR (5,6) developed a trypsin-based enzymatic technique for clearing tissues of small vertebrates prior to alizarin staining. SIMONS & VAN HORN (7) proposed the use of alcian blue instead of methylene blue or toluidine to stain the cartilaginous skeleton of chick embryos. DINGERKUS & UHLER (8) simultaneously used TAYLOR's (5,6) and SIMONS & VAN HORN's (7) methods on all groups of vertebrates successfully, except with some amphibians and fish. POTTHOFF (9) and TAYLOR & VAN DYKE (10) adapted the method to stain fish larvae and fry.

We have, however, become aware of, and wish to draw attention to major problems we have encountered with interpretation of results of the alcian blue/alizarine staining according to DINGERKUS & UHLER (8), POTHOFF (9) and TAYLOR & VAN DYKE (10) when applied on larvae (from hatching onward), fry and adults of *Barbus barbus* (L., 1758) (11), *Chrysichthys auratus* (Geoffroy Saint Hilaire, 1808) (12), *Heterobranchus longifilis* (Valenciennes, 1840) (13), *Scophthalmus maximus* (L., 1758) (14), and *Dicentrarchus labrax* (L., 1766).

With adults of each of these species, alcian blue/alizarin staining of cartilage, bone, or both simultaneously was perfectly successful. With larvae and fry, separate staining of cartilaginous and bony skeletal structures again yielded excellent results. Simultaneous staining, on the other hand, proved only fully satisfactory for cartilage: the bony structures were less intensely stained than with alizarin alone and fewer structures were revealed. Fig. 1 illustrates this effect in *Dicentrarchus labrax* (GLUCKMANN, unpubl. data). The results were the same regardless of the glacial acid concentration (20%, 30%, up to 40%) or neutralisation methods used as proposed by the different authors (8, 9, 10).

POTTHOFF (9) cautioned against erroneous interpretations of the staining data. He mentioned variable and sometimes very weak staining of cartilage and noted that some bones do not stain at all but appear transparent with a conspicuous outline. This was observed in the case of the dentaries and maxillaries of some stages of *Scopthalmus maximus*, but not *e.g.* in the case of the frontals or pterygoids (14). In this species more bones were revealed by alizarin staining than by double staining up to day 57 post-hatching (14). Not until day 61

did the staining profiles become practically identical. This means that until this point in the development, the bony structures are not sufficiently calcified to compensate for the loss of calcium during the decalcification with glacial acid. To make sure all skeletal structures are revealed, it is thus necessary to perform three types of staining: alcian blue and alizarin separately, and both combined. If double staining does not reveal the same bony structures as alizarin staining alone, one must conclude that some structures have been decalcified, leaving only highly calcified elements to adsorb the dye. A subsequent staining with alcian blue and alizarin separately should then be performed, even though this separate staining does not provide as much detail for a precise description of skeletal construction.

Our observations reveal the need for great caution in the presentation and interpretation

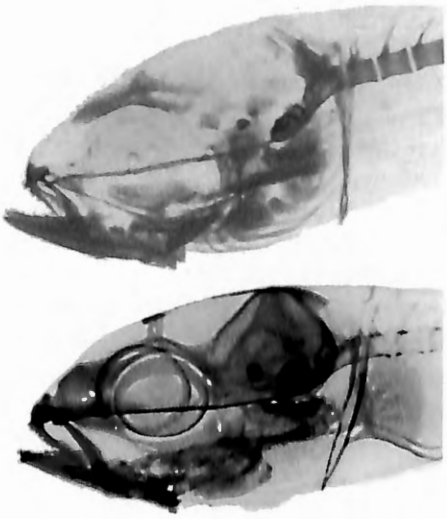


Fig. 1. – *Dicentrarchus labrax*. – 30-day fry. A, bony structures stained with alizarin alone; B, alcian-blue-stained cartilaginous structures and alizarin-stained bony structures. (GLUCKMANN, unpublished).

of data from simultaneous staining of the skeleton of developing fish larvae and fry. Perhaps earlier observations should be reconsidered or at least considered with great caution.

The authors thank K.Broman for linguistic assistance. This work was supported by a grant n° 2.4508.94 from the Fonds National de la Recherche Scientifique de Belgique. I.G. and F.W. are researchers at the Fonds pour la Recherche dans l'Industrie et l'Agriculture.

REFERENCES

1. LUNDVALL, H. (1927) – Färbung des Skelettes in durchsichtigen Weichteilen. *Anat. Anz.*, **62**: 353-373.

2. WILLIAMS, T.W. (1941) – Alizarin red S and toluidine blue for differentiating adult or embryonic bone and cartilage. *Stain Technology*, **16**: 23-25.

3. BECHTOL, C.O. (1948) – Differential *in toto* staining of bone, cartilage and soft tissues. *Stain Technology*, **23**: 3-9.

4. BURDI, A.R. & F. FLECKER (1968) – Differential staining of cartilage and bone in the intact embryonic skeleton *in vitro*. *Stain Technology*, **43**: 47-48.

5. TAYLOR, W.R. (1967a) – An enzyme method of clearing and staining small vertebrates. *Proc. U.S. Natl. Mus.*, **122** (3596): 1-17.

6. TAYLOR, W.R. (1967b) – Outline of a method of clearing tissues with pancreatic enzymes and staining bones of small vertebrates. *Turtox News*, **45**: 308-309.

7. SIMONS, E.V. & J.R. VAN HORN (1971) – A new procedure for whole-mount alcian blue staining of chicken embryos, adapted to the clearing procedure in potassium hydroxide. *Acta Morphol. Néer. Scand.*, **8**: 281-292.

8. DINGERKUS, G. & L.D. UHLER (1977) – Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. *Stain Technology*, **52**: 229-232.

9. POTTHOFF, T. (1984) – Clearing and staining techniques. *In*: Ontogeny and systematics of fishes. Special publication n° 1, *American Society of Ichthyologists and Herpetologists*: 35-37.

10. TAYLOR, W.R. & G.C. VAN DIJK (1985) – Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium*, **9**: 107-119.

11. VANDEWALLE, P., B. FOCANT, F. HURIAUX & M. CHARDON (1992) – Early development of the cephalic skeleton of *Barbus barbus* (Teleostei, Cyprinidae). *J. Fish Biol.*, **41**: 43-62.

12. VANDEWALLE, P., P. LALÈYÉ & B. FOCANT (1995) – Early develcpment of cephalic bony elements in *Chrysichthys auratus* (Pisces, Siluriformes, Bagridae). *Belg. J. Zool.*, **125**: 329-347.

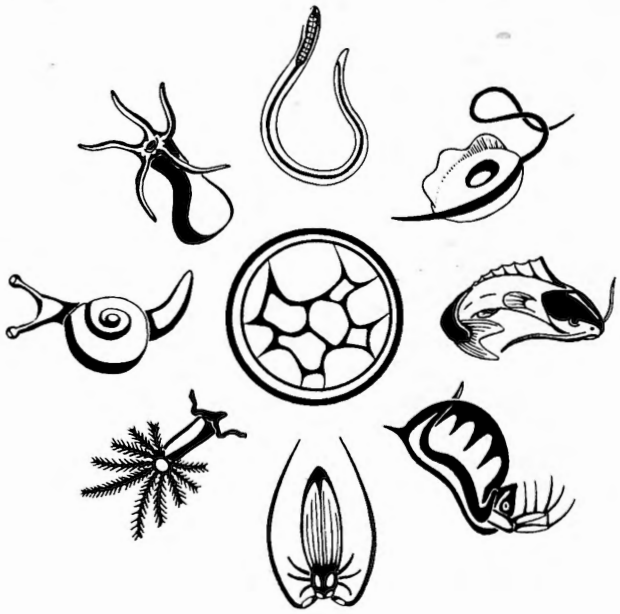
13. VANDEWALLE, P., I. GLUCKMANN, E. BARAS, F. HURIAUX & B. FOCANT, B. (1977) – Postembryonic development of the cephalic region in *Hetrobranchus longifilis*. *J. Fish Biol.*, **50**: 227-253.

14. WAGEMANS, F., B. FOCANT & P. VANDEWALLE (1998) – Early development of the cephalic skeleton in the turbot. *J. Fish Biol.*, **52**: 166-204.

(Received: 18 March 1998)

5th Benelux Congress of Zoology

Phylogeny and Biodiversity



November 6-7, 1998

University of Gent, Belgium

HIKW, Ledeganckstraat 35, 9000 Gent

Registration before **October 1** (with hotel reservation: **september 1**).

For more information and registration, contact

A. Huysseune – Vakgroep Biologie
Ledeganckstraat 35 – B-9000 Gent
Phone 09/264.52.29 – Fax 09/264.53.44
E-mail: Ann.Huysseune@rug.ac.be

or visit the website: http://kbvd-www.uia.ac.be/kbvd/

GUIDE FOR AUTHORS

1. The Belgian Journal of Zoology publishes, in English or French, original papers and notes in all fields of zoology. Reviews on topics of general interest or current importance may be considered after invitation by or agreement with the editors. Manuscripts must contain significant new findings and must not have been published elsewhere nor be simultaneously under consideration by any other journal. Single-species descriptions will not be accepted unless they have broader relevance such as implications for phylogeny or biogeography. Papers submitted in French must include English translations of the title and of the abstract.
2. Members of the Society, as well as non-members benefit from a waiver of page charges up to 15 printed pages. This waiver is subject to annual consideration of the Council of the Society. Additional pages are charged to authors at cost price (1300 BEF per page, incl. illustrations). Authors will receive 50 reprints free of charge. To order additional reprints, the completed order form must be returned together with the galley proofs.
3. Each manuscript is reviewed by at least two referees, usually one Belgian and one non-Belgian referee. After acceptance, authors remain fully responsible for their article.
4. Authors are requested to follow rigorously the lay-out as shown in the articles in this issue, paying particularly attention to the format of references. Papers should be written in clear, concise language and consist of an abstract (summarising the essential results and conclusions), introduction, material and methods, results and discussion. Sections and sub-sections should not be numbered but authors may suggest a printing format to provide structure to the article. Author names in text citations should be in SMALL CAPITALS and in the following format: PERRIN & KOKKINN (1985) for two authors (with ampersand (&) and not «and» as previously used) or WILSON *et al.* (1987) for more than two authors. Consecutive references must be separated by a semicolon (;). Figures in the text are referred to by «Fig. n» or «Figs n-m». Names of species (and names of higher taxa in axonomic papers) should be followed, on the first occasion of use, by the name of the author who described the species or introduced the taxon name (in lower case) and the year. Latin expressions (*species names*, *e.g.*, *et al.* etc.) should be in italics.
5. Short notes not exceeding 4 printed pages (incl. figures) with new findings of temporary importance, comments on papers published in the journal, new records for (*e.g.*) the Belgian fauna, curiosa, etc. will be published in the next issue whenever possible. They should be written as continuous text without the various divisions of regular papers. References for Notes must be indicated in the text by numbers and listed and numbered in the order of their appearance in the text. Short notes will be reviewed as normal papers. Reviews of books of interest to the members (max. 1 p.) are welcome.
6. All parts of the manuscript including text, references, tables and legends for illustrations should be type-written, double spaced (on one side of the page only) and all pages numbered. Figures, tables and legends should remain separated from the text. Scale bars should be drawn on figures where appropriate and size indicated in the legend. Three copies of text and three quality copies of figures (not exceeding the journal page size must be provided. In addition, authors are requested to provide a computer diskette with the manuscript (in WP, MSWord or RTF file, DOS or Macintosh) to allow electronic transmission with referees and authors. Please indicate on the manuscript the author e mail address for correspondence where available). Whenever possible, authors are asked to also include on the diskette(s) computer generated or scanned figures and photographs (as PICT or TIFF files with an indication of the program and system used). Indicate on the hard copy where figures and tables are to be inserted, and provide a short running title and keywords (separated by commas).
7. Following acceptance of the manuscript, authors must provide the originals of the figures together with the final version of the text. Photographs should be mounted and clearly labelled. Hand made drawing should be in black ink (clearly readable after reduction). All figures should be labelled in pencil on the reverse side with the number of the figure, the name of the author(s), title of the paper, number of the MS and intended degree of reduction. Photocopies of photographs or figures are not acceptable.

Consult the Journal home pages http://www.luc.ac.be/research/groups/dierkunde/BJZ.html for the latest instructions before sending in your manuscript. The content and abstracts of the latest issues can be found there, as well as those of the forthcoming issue, one month before it appears.

The royal Belgian zoological Society organizes meetings by invitation of the members.
The contribution for members is 800 BEF. annually. For members living outside Belgium, the contribution is 900 BEF. Belgian students benifit from a contribution of 300 BEF. (foreign students, 400 BEF.). Familial contributions, comprising one subscription to the journal, are 900 BEF. Members receive the journal (two issues per year, resp. in June and December and the occasional special issues) free of charge.

The price for one volume (two issues) is 1.500 BEF.

C.C.P. 000-0049113-31, Société Royale Zoologique de Belgique, Bruxelles.

P.R. 000-0049113-31, Koninklijke Belgische Vereniging voor Dierkunde, Brussel

Requests for exchange and other library matter: Prof. Dr. J. Delinge (library administrator), ULB-CP 160/11, Av. Roosevelt, 50, B-1050 Brussels (Belgium).

General information on the society can be found on http://kbvd-www.uia.ac.be/kbvd/.

CONTENTS

Short note

The **BELGIAN JOURNAL OF ZOOLOGY** is covered in **Current Contents** and in Science Citation index.

ISSN 0777-6276