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OBSERVATIONS ON MITES INHABITING NESTS OF *BUBO BUBO* (L.) (STRIGIFORMES, STRIGIDAE) IN BELGIUM

by

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SUMMARY

The acarofauna of six nests of *Bubo bubo* (L.) collected in Belgium has been studied. The number and quality of the mites collected varied markedly from nest to nest. Pyroglyphid mites (i.e. *Dermatophagoides farinae* and *Euroglyphus maynei*), which are known as important producers of bronchial asthma in man, were found in two distinct nests. Among the species collected in one nest, two are new (*Amblyseius namurensis* n. sp. and *Histiostoma trogicola* n. sp.) and six had not been recorded previously from Belgium. The hypopial stage of *Myianoetus travei* FAIN, 1976, (not the adults) is transferred into the genus *Comyianoetus* FAIN and PHILIPS, 1979, with the name *Comyianoetus puteanus* FAIN n. sp. A possible scenario depicting, in three phases, the mite-bird relationships evolving with time is proposed.
Key words : Belgium, mites (Acari), nests of *Bubo bubo*

INTRODUCTION

Relatively little is known about the associations between birds and mites, apart from ticks which have been known to plague birds (as well as people) for centuries. Mites are a very ancient and diverse group of chelicerates consisting of probably more than 2000 genera and 500000 species (KRIVOLUTSKY and DRUK, 1986; WOOLLEY, 1987; O'DOWD *et al.*, 1991). In Europe, the insects and mites associated with certain birds' nests have already been studied by NORDBERG(1936) in Finland and by WOODROFFE (1953) in England. Yet both papers, which initiated an ecological analysis of the nest arthropod community, dealt mainly with the insect fauna.

Nests of birds, and especially those built by birds of prey, usually show a mite fauna very rich in both species and individuals (FAIN and PHILIPS, 1977a and b, 1978a and b, 1979, 1981). In addition, some nests may be inhabited by mites injurious to stored food products (for instance, among Astigmata, species belonging to genera *Acarus, Glycyphagus, Lepidoglyphus* and *Tyrophagus*) or harmful to man

(among Astigmata, pyroglyphid mites ; and, among Mesostigmata, species of the genera *Ornithonyssus* and *Dermanyssus* ; see observations by WOODROFFE and SOUTHGATE, 1952 ; BAKER *et al.*, 1976 ; FAIN *et al.*, 1991).

In their recent synthesis of data gained on free-living mites in Belgium since the beginning of the century, LEBRUN *et al.* (1989) have emphasized the fact that our current knowledge of mites occurring in nests of birds is quite fragmentary while a lot of papers have documented both the ecology and the biology of soil mites (see e.g. WAUTHY *et al.*, 1989, and VERA ZIEGLER *et al.*, 1990).

In order to partially fill the hiatus, this study is a first approach to the mite fauna associated with nests of *Bubo bubo* and captured in six nests originating from South Belgium (provinces of Namur and Luxembourg).

MATERIAL AND METHODS

Nests

In Belgium, the Eagle owl begins to lay in the second half of February. The nest is a shallow depression (diameter : app. 30 cm) scraped away by the male usually upon a cliff ledge. Being a typical cliff nester, the Eagle owl sets its nest in marked natural or artificial inclines.

Usually, a pair has on average three or four nest sites located in their territory and used consecutively year after year. The clutch consists of 2-4 eggs incubated for around 35 days. Young are altricial and nidicolous. They are cared for and fed by both parents, and when small brooded more or less continously by the female. The fledging period is 60-70 days or more. Frequently, however, the young leave the nest before fully fledged, walking in the surroundings at least if the ledge area is spacious enough.

Our survey of the mite fauna associated with nests of *Bubo bubo* refers to populations found in the organic matter gathering in the nest depression. This organic matter consists of scraps (a mixture of owl pellets made up of bird feathers, mammal hair and bones), fragments of uneaten prey and excrement, to which earthy materials may be added in more or less conspicuous quantity.

Six nests were studied and some data relating to their localization as well as to their immediate, physical and vegetational environments are shown in Table 1. For reasons of conservation, we omitted the name of the sites where the nests were found.

Mite sampling

The organic matter gathering in the depression of each nest was sampled during a single survey (dates in Table 1), when ringing the young. In each nest, in order not to disturb young, only one sample of organic matter (app. 1 dm^3 in volume) was withdrawn by hand from the centre of the depression, i.e. from this part of the

TABLE 1

Designation, number of young, short description of cliff ledge and ground organic substrate, and date of sampling for the six nests of *Bubo bubo* from distinct localities in the provinces of Namur (N) and Luxembourg (L).

Estimation of water content of organic substrate : *m*, moist ; *vd*, very dry. Composition of organic matter : *s*, prey scraps (+, very few ; ++ numerous) with miscellaneous compounds (*f*, feathers ; *h*, mammal hair ; *b*, bones) possibly mixed with earthy materials (*e*) and stones (*r*).

Nest Number	Young Number	Age	Presence	Nest localization and environment	Organic substrate		Province	Date
					Composition	Moisture		
1	3	3	yes	Upon a small rocky ledge, the surface of which was completely occupied by the nest and weakly covered by earthy material ; not direct exposure to the sun owing to a bush overhanging the nest	s(+:f,b)	m	N	16.V.92
2	4	4	yes	Upon a wide earthy ledge, in the vicinity of a herbaceous area ; direct exposure to the sun ; site drought by a substantial draining of the rocky substrate	s(++:f,b)	vd	N	16.V.92
3	4	5	yes	Upon an elongate, earthy ledge ; direct exposure to the sun ; site covered by a cushion of short grasses	s(++:f,b,h),e	vd	N	28.V.92
4	2	5	yes	Upon a small, stony ledge, the surface of which was bare and almost occupied by the nest ; direct exposure to the sun	s(+:f,b),e	vd	L	28.V.92
5	3	6	yes	Upon an earthy, stony ledge ; reduced exposure to the sun owing a small shield of plants overhanging the edge ; site used for many years	s(++:f,b,h),e,r	m	N	6.VI.92
6	1	8	no	At the corner of a wide rocky slip covered by earthy material and invaded by vegetation ; clearly reduced exposure to the sun owing to both the wall of rock and plants ; site used for many years	s(+:f,b,h),e,r	m	N	30.VI.92

nest which usually is in contact with young birds. The mite fauna was extracted over one week in a Berlese funnel using 15 W light globes as a heat source.

RESULTS

Assemblage composition : richness and density

A total of 33 species and more than 700 mite specimens were found in the six nests surveyed. Table 2 illustrates both the specific attributes of mite assemblages established in each nest and the numerical size of mite populations at the time of sampling.

Among the six nests studied, the assemblage of nest no. 6 appeared to be the one with the highest species richness (25 species, Table 2) while the others consisted at best of three species. In addition, abundances registered in nest no. 6 were patently high since population size of five species was over the noticeable value of 100 individuals per 1 dm^3 of organic substrate. In the same way, the assemblage of nest no. 3 diverged from others by one population showing substantial establishment (*Tyrophagus palmarum*, Table 2).

Species composition : taxonomy, trophic behaviour, medical and economical importance

From a taxonomic point of view, Acari are triphyletic in origin GRANDJEAN, 1970) and are subdivided into seven orders. The mites collected in the nests of *Bubo bubo* belonged to 24 families and five orders : Metastigmata (1 species); Mesostigmata (4 species); Prostigmata (6 species); Astigmata (13 species); and, Oribatida (9 species).

Where feeding habits of mites are concerned, a distinction between free-living forms and parasitic ones is required. Free-living mites are predaceous, phytophagous, microbivorous (i.e. feeding on fungi, yeasts, bacteria or algae, or combining two or more of these habits), saprophagous, coprophagous or necrophagous organisms (KRANTZ, 1978). In addition, two blood-sucking parasites of man and animals were found in nests surveyed, i.e. *Ixodes ricinus* and *Ornithonyssus sylviarum*. Another parasitic form (*Leporacarus gibbus*) known as feeding on hair follicle secretions of its vertebrate host was likewise registered among mites occurring in the nest no. 6.

On the other hand, several species captured in the nests of *Bubo bubo* are well-known pests of stored food and have thereby a great economic importance (e.g. *Acarus* spp., *Tyrophagus* spp., and *Lepidoglyphus destructor*). Finally, two other taxa (i.e. *Dermatophagoides farinae* and *Euroglyphus maynei*), which usually live in house dust, are able to cause bronchial asthma in man.

TABLE 2

List of mite species found in six nests (no. 1, 2, 3, 4, 5 and 6) of *Bubo bubo* sampled in South Belgium. Registered densities at the time of sampling and usual feeding habits are indicated. Feeding habits : [*b*], blood suckers ; [*c*], organisms able to feed on corneous material of the skin of birds ; [*f*], phytophagous organisms ; [*m*], microbivorous ; [*p*], predators ; [*s*], saprophagous ; [*t*], ectoparasite feeding on secretions of its vertebrate host ; [*u*], unknown or unidentified. Ontogenetic stages : A^f, female ; A^m, male ; *n2*, deutonymph ; *n1*, protonymph ; *h*, hypopus ; *n*, nymphal stage when only one exists ; *i*, unidentified immature stage. Number of individuals : +, few (i.e. < 10) ; + +, numerous (i.e. > 50).

	Nest					
	1	2	3	4	5	6
Ixodes ricinus [b]						n:1 l:7
Ornithonyssus sylviarum [b]		A^f:6				
Macrocheles penicilliger [p]					A^f:+	A^f>100 n2:+ n1:+
Amblyseius namurensis [u]						A^f:2
Pelethiphis sp. [u]						n:2
Bdella sp. [u]				n:1		n:1
Linopodes motatorius [u]						A^f:1
Tarsonemus sp. [u]						A^f:4 A^m:1
Bryobia praetiosa [f]		A^f:1				
Cunaxoides croceus [p]						A^f:1
Neophyllobius saxatilis [u]			A^f:1			
Acarus siro [m+s]	A^f:+ A^m:+				A^f:+ A^m:+	
Acarus farris [m+s]				A^f:+ h:+ i:+		A^f:++ h:++ i:++
Tyrophagus similis [m+s]					A^f:++ i:++	A^f:++ i:++
Tyrophagus palmarum [m+s]			A^f>100 i:++			A^f>100 i:++
Lepidoglyphus destructor [m+s]	A^f:+ A^m:+ i:+			A^f:+ A^m:+ i:+		

TABLE 2

	Nest					
	1	2	3	4	5	6
Dermacarus sciurinus [m+s]						h:1
Dermatophagoides farinae [c]	A^f:8 A^m:4 n:2					
Euroglyphus maynei [c]		A^f:2				
Histiostoma trogicola [u]						h:24
Scheucheria mongolica [u]						h:1
Myianoetus turkorum [u]						h:25
Comyianoetus denticulatus [u]						h:13
Leporacarus gibbus [t]						A^m:1
Nothrus sp. [m+s]						n1:1
Trhypochthonius tectorum [m]						n2:1
Suctobelbella subtrigona [m]						A^f:1
Tectocepheus sarekensis [m+s]						A^f:1
Micreremus brevipes [u]						A^f:1
Trichoribates trimaculatus [u]						A^m:1
Galumna cf. lanceata [u]						A^f:1 n1:1
Dometorina pl. plantivaga [m]						A^f:1
Oribatula tibialis [m+s]						A^f:2

Study of the species

Data on the density and the number of nests where the following species of mites were registered are shown in Table 2.

METASTIGMATA

Ixodes ricinus (LINNAEUS, 1758)

This tick is wide-spread in Belgium. Larvae and nymphs occur frequently upon birds. *I. ricinus* is the main vector of Lyme disease in our country (FAIN, 1990).

MESOSTIGMATA

Macronyssidae

Ornithonyssus sylviarum (CANESTRINI and FANZAGO, 1877)

This haematophagous parasite is commonly found in birds' nests and on wild and domestic birds (e.g. pigeons, chickens, etc.) as well as on cage birds (canary, etc.). The manipulation of contaminated birds may induce an itching dermatitis in man.

Macrochelidae

Macrocheles penicilliger (BERLESE, 1904)

Mites of the family Macrochelidae are predators of nematodes or feed on eggs or larvae of numerous insects, especially Diptera. Although widely distributed in Europe and reported from the U.S.A. and New Zealand, *M. penicilliger* had so far never been collected in Belgium. Note the relatively high abundance of the two populations we recorded (clearly higher in nest no. 6 than in nest no. 5 ; see Table 2) and the lack of males in both populations.

Phytoseiidae

Phytoseids are efficient predators of phytophagous mites, viz. Tetranychidae and Eriophyidae, which are able to injure severely a great variety of cultivated plants. Therefore, phytoseids have been used with patent success for the biological control of these pests.

Amblyseius namurensis **nov. spec.**

Material : 2 females (holotype and 1 paratype), deposited in the Institut royal des Sciences naturelles de Belgique, Bruxelles.

Description of the holotype female (Figs 1-3).

Idiosoma : 360 µm long and 240 µm wide (maximum width).

Scutum : 330 µm long, and maximum width, 210 µm. The scutum carries 17 pairs of setae measuring as follows (in µm) : *j1* 18 ; *j3* 33 ; *j4* 19 ; *j5* 6 ; *j6* 8 ; *z4* 27 ; *z5* 9 ; *s4* 24 ; *s5* 42 ; *r3* 30 ; *J2* 8 ; *J5* 10 ; *Z1* 12 ; *Z4* 50 ; *Z5* 57 ; *S2* 33 ; *S4* 10 ; *S5* 12 ; *R1* 19. The scutum bears, anterolaterally and outside of setae *z4* and *s4*, two or three oblique striations while the rest of the scutum is devoid of any line or striation.

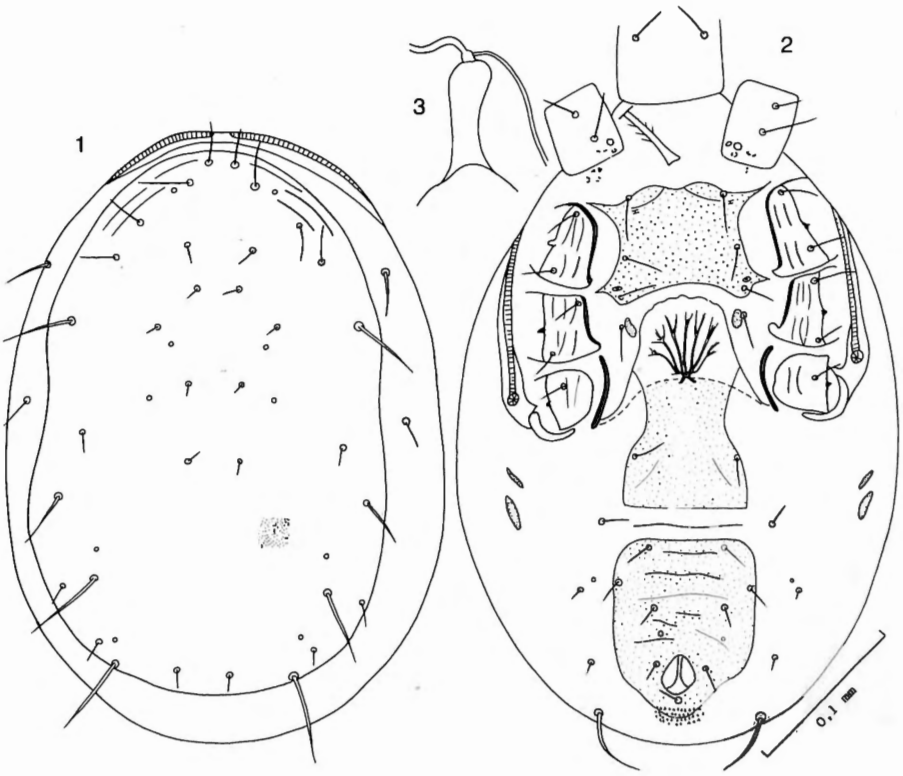
Venter. Sternal shield : 57 µm long (in midline), and 75 µm wide (at the level of setae *st2*). Genital shield with lateral margins slightly divergent behind the genital setae ; maximum width, 75 µm. Ventrianal shield : 105 µm long, and maximum width, 87 µm.

Chelicerae badly oriented, not observable. Inseminating organ as in *Amblyseius graminis* (CHANT, 1956) : the atrium and the calyx, together, are 18 µm long and 4.5 µm wide (in middle).

Type locality : Near Namur (nest no.6).

Remark

This species is rather close to *Amblyseius graminis*. It differs however from the latter by the following characters : (1) the shape of the genital shield with the lateral margins, behind the genital setae, almost parallel (these margins are strongly divergent in *A. graminis*) ; (2) the two pairs of metapodal shields are very unequal (they are subequal in *A. graminis*) ; (3) the long setae of the genu, tibia and tarsus IV are shorter (42, 30 and 56 µm in *A. namurensis* instead of 47, 33 and 70 µm in *A. graminis*) ; and, (4) the setae *Z5* are shorter (57 µm instead of 70-100 µm in *A. graminis*). See CHANT (1956) and KARG (1971).



Figs 1-3. *Amblyseius namurensis* n. sp., holotype female. — 1. in dorsal view. - 2. in ventral view. - 3. inseminating organ.

Eviphididae

***Pelethiphis* sp.**

The lack of adult individuals in the sample taken within the nest no. 6 did not allow a specific determination of both nymphs collected. Note, however, that all the known species of the genus *Pelethiphis* live in association with necrophagous Coleoptera.

PROSTIGMATA

Bdellidae

***Bdella* sp.**

Here also the lack of adults in our collection did not allow a specific identification of the two nymphs found in nests no.5 and 6. Some species of the genus *Bdella* prey on other mites.

Eupodidae

Linopodes motatorius (LINNAEUS, 1758)

As early as 1935, WILLMANN reported on the finding of this taxon in Belgium. It occurs in soil litter, in mosses and upon rocks.

Tarsonemidae

***Tarsonemus* sp.**

The biology of the species of the genus *Tarsonemus* is variable. Some taxa show clear predatory habits, feeding on eggs of tetranychid mites. Other species are commensal organisms associated with bark beetles. In Europe and North America, two species (*T. granarius* LINDQUIST, 1972, and *T. fusarii* COOREMAN, 1941) are commonly found in stored grains where they feed on fungi such as *Penicillium*, *Aspergillus*, etc.

Tetranychidae

Bryobia praetiosa C. L. KOCH, 1836

This world-wide distributed species is regarded as a major phytophagous pest on account of damage it inflicts on many cultivated plants, especially fruit-trees.

Cunaxidae

Cunaxoides croceus (C. L. KOCH, 1838)

Recently revised by SMILEY (1992), the species of the family Cunaxidae are predators of other microarthropods which inhabit mosses or soil organic horizons.

Although *C. croceus* shows a world-wide distribution, our finding is its first report from Belgium.

Camerobiidae

Neophyllobius saxatilis HALBERT, 1923

A single female specimen found in nest no.3 is assigned to *N. saxatilis*. This species, so far unrecorded from Belgium, was succinctly described by HALBERT (1923). The typical specimens were collected by HALBERT in the vicinity of Dublin (Ireland). VAN EYNDHOVEN (1938), from specimens collected in dunes near the city of Vogelzang (west coast of the Netherlands), redescribed this species. Note that the biology of camerobiid mites is still quite unclear.

ASTIGMATA

Acaridae

Acarus siro (LINNAEUS, 1758)

Being a major contaminant of stored products, this species may cause important depredations to flour, grain, cheese, etc. The heteromorphic deutonymphs (= hypopi) are motile organisms which attach to various species of insects.

Acarus farris (OUDEMANS, 1905)

Just as the previous species, *A. farris* occurs in stored food and show motile hypopi. In addition, it is usually captured in nests of birds (FAIN *et al.*, 1991).

Tyrophagus similis VOLGIN, 1949

Relatively ubiquitous, this species has been recorded from soil, grassland and diverse cultivated plants such as spinach, mushrooms, etc. (HUGHES, 1976).

Tyrophagus palmarum OUDEMANS, 1924

This taxon is an usual inhabitant of both stored food products and nests of birds (WASYLIK, 1963 ; FAIN *et al.*, 1991). Note that no male was found among the individuals we captured in this survey (Table 2).

Glycyphagidae

Lepidoglyphus destructor (SCHRANK, 1781)

This species is an important and very common pest of stored food, and is frequently found associated with *A. siro*. *L. destructor* is essentially mycophagous and is able to survive on different species of fungi. It produces hypopi of the « immobile » type.

Dermacarus sciurinus (C. L. KOCH, 1841)

As the adult stage of this taxon is reported to be a strict inhabitant of nests of the squirrel, it is very likely that the finding of one hypopus individual in nest no. 6 has to be inferred from the predatory activities of Eagle owl parents.

Pyroglyphidae

Dermatophagoides farinae HUGUES, 1961

This species is a common dweller in both mattresses and house dusts. In man, its pathogenic role is quite important since it may induce bronchial asthma.

Euroglyphus maynei (COOREMAN, 1950)

As the previous species, it is likewise a domicolous mite implicated in bronchial diseases of man.

Histiostomatidae

Histiostoma trogicola **nov. spec.**

Material : 24 heteromorphic deutonymphs (holotype and 22 paratypes deposited in the Institut royal des Sciences naturelles de Belgique, Bruxelles ; one paratype deposited in the British Museum, Natural History, London).

Description of the holotype (Figs 4-9).

Idiosoma : 156 µm long ; maximum width, 115 µm ; length and width in two paratypes : 167 × 114 and 153 × 108 µm, respectively. Posterior margin of body nearly straight in all our specimens.

Dorsum. Hysterosoma with numerous, small and rounded depressions especially well developed in the lateral parts of the body. Propodonotum with small depressions confined to the anterior part of the body. Length of setae (in µm) : *sce* and *sci*, 10-12 ; *d1* to *d3* thick, and 80, 66 and 65 long, respectively ; *d4*, *d5*, *l1* to *l4*, *h* and *sh* very small and thin (6-9 long). Setae *l5*, 18-20 µm long.

Venter. Palposoma, 16 µm long and 6 µm wide, bearing a pair of solenidions, 40 µm long. Suctorial plate, 70 µm wide ; anterior suckers, 7 µm wide ; posterior suckers, oval, 14 µm long and 11 µm wide. The setae *CxI*, *CxIII* and *gp* are conoids. Two pairs of conoids (lateral and paramedian ones) are present on the suctorial plate (see FAIN, 1973).

Legs. Length of tarsi : 42, 33, 27 and 30 µm, respectively (ambulacra not included). Tarsi I-III with an apical claw, and a long and wide foliate seta. Tarsus IV bearing a strong seta, 75 µm long. Length of solenidions *phi* I-IV : 55, 32, 31 and 9 µm long, respectively.

Habitat

Holotype and three paratypes, all hypopial deutonymphs, were found in nest no. 6 (near Namur). Twenty paratypes (hypopi) attached to a *Trox scaber* were found in the same nest.

Remark

This species is characterized by the aspect of the dorsal chaetotaxy showing only three pairs of very long and strong median setae while all the other dorsal setae are very thin and short. Other distinctive features are as follows : (1) the great size of conoids ; (2) the presence of large lanceolate setae on tarsi I-III ; and, (3) the pitted shape of the dorsum.

Scheucheria mongolica MAHUNKA, 1969

This species is represented in our collection by a single hypopus collected in nest no. 6. This species was described from Mongolia. Our specimen corresponds very closely to the original description of *S. mongolica.*

The genus *Scheucheria* MAHUNKA, 1969, includes another species, which is the type of the genus, *Wichmannia stammeri* SCHEUCHER, 1957, and whose hypopi were collected by SCHEUCHER (1957) from a staphylinid beetle in Erlangen, Germany. MAHUNKA (1969) did not examine the hypopus of this species when he described his *S. mongolica,* and it is possible that the differences he noted between the two species could be explained by an inadequate description of *S. stammeri.* In order to check this eventuality we asked the Director of the Institute of Zoology, University of Erlangen, Nürnberg, to send us the type specimen of *S. stammeri.* Unfortunately « there exists no prepared material of that species in that Institute » (in litt. Prof. H. W. Scheloske). *Scheucheria mongolica* had so far not been recorded from Belgium.

Myianoetus turkorum SCHEUCHER, 1957

About 20 specimens of this species, all heteromorphic deutonymphs, were collected in nest no. 6, and five specimens were collected from a *Trox scaber* found in the same nest. It is the first record of this species from Belgium.

Genus *Comyianoetus* FAIN and PHILIPS, 1979

So far, the genus *Comyianoetus* included only the type species *Comyianoetus denticulatus* FAIN and PHILIPS, 1979. These hypopi differ from those of the genus *Myianoetus* OUDEMANS, 1929, (see also MAHUNKA, 1972) by the following characters : (1) claws I and II not divided by a deep median cleft, but normally shaped, and with a small preapical tooth (Fig. 10) ; this tooth is anteroventral on both claws I and II, and posteroventral on claw III ; (2) in *Comyianoetus*, the anterior suckers of the suctorial plate are vestigial and replaced by small rings ; and, (3) the conoids located behind the posterior suckers are vestigial and replaced by very small setae.

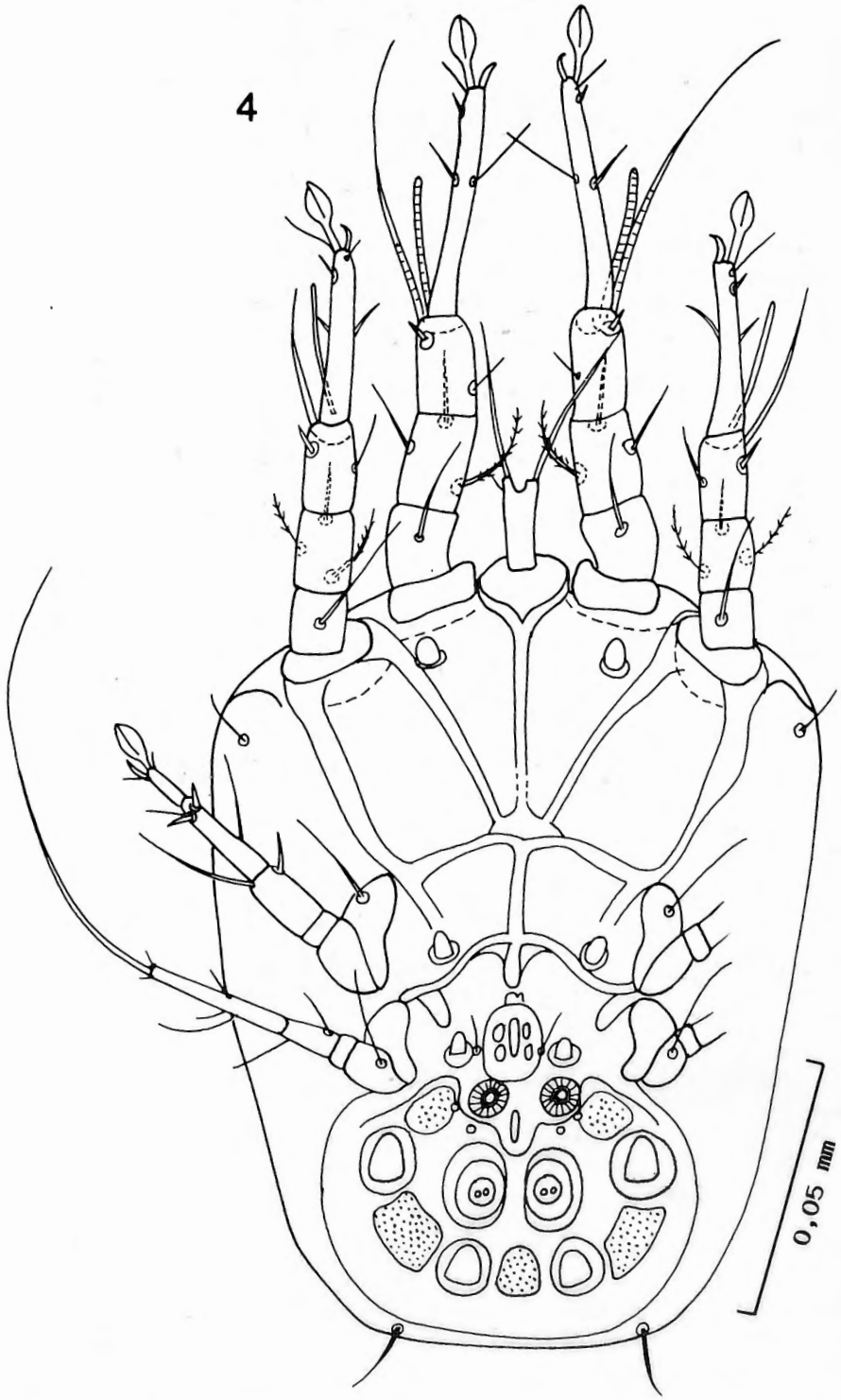
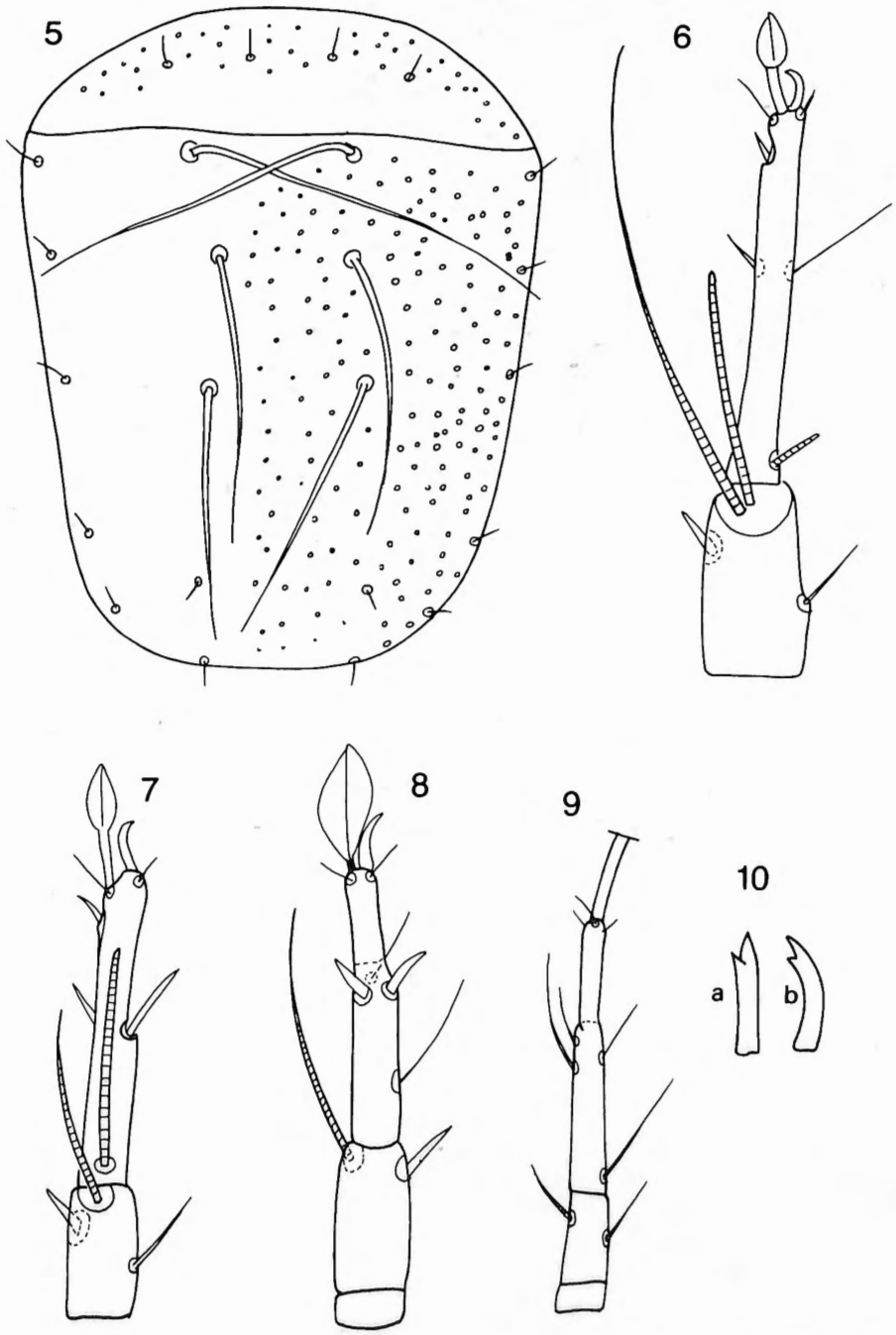


Fig. 4. — *Histiostoma trogicola* n. sp., heteromorphic deutonymph in ventral view.



Figs 5-9. *Histiostoma trogicola* n. sp. — 5. Heteromorphic deutonymph in dorsal view. - 6-9. Apical segments of legs I, II, III and IV.
Fig. 10. *Comyianoetus denticulatus* FAIN and PHILIPS, 1979. — Heteromorphic deutonymph : claw of tarsus I in ventral (a) and lateral (b) view.

Comyianoetus denticulatus FAIN and PHILIPS, 1979

We collected 12 hypopi of this taxon in nest no. 6. In addition, one hypopus was found attached to a *Trox scaber* collected in the same nest. It is the first record of this species in Belgium. In fact, the original description was based on individuals captured in nests of several birds of prey : in U.S.A., in nests of *Asio otus, Buteo virginianus* and *Buteo albonotatus*; and, in Norway, in nests of *Aegolius funereus.*

Comyianoetus puteanus **nov. spec.**
= *Myianoetus travei* FAIN, 1976 : 309, in part (hypopus)

Previously described under the name *Myianoetus travei*, the hypopus (not the adult) is now transferred into the genus *Comyianoetus* and becomes the type of a new species, *C. puteanus* n. sp. We think now that the deutonymphal stage (but not the adults) of *Myianoetus travei* FAIN, 1976, which was described from specimens collected in the Kerguelen Islands, also belongs to the genus *Comyianoetus*. In fact, the hypopi of this species were not found in either the same locality or biotope as the adults. Moreover, the holotype of the species was selected among the adults collected.

Listrophoridae

Leporacarus gibbus (PAGENSTECHER, 1862)

This taxon is a pilicolous parasite of the rabbit *Oryctolagus cuniculus*. The single male specimen found in nest no.6 was probably brought into this nest with prey captured by Eagle owl parents.

ORIBATIDA

All the oribatid mites were captured in nest no.6. The record of both several saxicolous and/or arboricolous taxa and one species unknown up to date in Belgium (*Trhypochthonius tectorum*) has to be emphasized. Data on saxicolous and arboricolous species originate from observations by VAN DER HAMMEN (1952), TRAVÉ (1963), ANDRÉ and LEBRUN (1979) and ANDRÉ (1984).

Nothridae

Nothrus **sp.**

The single deutonymphal specimen we found might be referred either to *N. anauniensis* CANESTRINI and FANZAGO, 1877, or to a species belonging to the *silvestris* group. Indeed, morphological traits allowing a definite identification to be achieved (e.g. interlamellar setae and posterior gastronotic phaneres) were unfortunately lacking or damaged.

Trhypochthoniidae

Trhypochthonius tectorum (BERLESE, 1896)

As often as not, this taxon occurs in mosses growing upon walls or rocks.

Suctobelbidae

Suctobelbella subtrigona (OUDEMANS, 1916)

In forest soils, this taxon is a common inhabitant of dead organic matter (litter and humus) (WAUTHY, 1982a ; LEBRUN *et al.*, 1989).

Tectocepheidae

Tectocepheus sarekensis TRÄGÅRDH, 1910

Mainly collected upon rocks, this species can also be found both upon trees and in leaf litter of forests where sometimes relatively high densities may be registered (TRAVÉ, 1963 ; WAUTHY, 1982a).

Micreremidae

Micreremus brevipes (MICHAEL, 1888)

Although exceptionally collected in soil organic matter, this species is one of the most common, arboricolous oribatid mites in Occidental Europe. It lives in lichens as well as upon leaves.

Ceratozetidae

Trichoribates trimaculatus (C .L. KOCH, 1836)

This taxon is patently a saxicolous organism. Yet, in foliose lichens growing upon trees, it can sometimes develop populations numerically important.

Galumnidae

Galumna cf. lanceata (OUDEMANS, 1900)

Galumnidae are a quite large group of oribatid species, the identification of which is still confused. Nonetheless, in Belgium, the species in question has been reported from dead organic matter of forest soils relating to distinct humus types. Its distribution displays however some preference for mull-moder soils (WAUTHY, 1982a ; LEBRUN *et al.*, 1989).

Scheloribatidae

Dometorina plantivaga plantivaga (BERLESE, 1895)

This lichenophagous organism lives upon trees much more than upon rocks.

Oribatulidae

Oribatula tibialis (NICOLET, 1855)

Although it can inhabit bark and lichens of trees, this taxon is mainly collected in litter of forests (TRAVÉ, 1963 ; LEBRUN *et al.*, 1989).

DISCUSSION

Abiotic conditions in the nests

The substrate filling up the nests from which acari were extracted appears to be a heterogeneous and constraining habitat for the mite fauna. Indeed, the substrate is usually an organic, more or less earthy material, and conceptually, this material might be regarded as a « hanging soil ». In fact, as in true soils, dead organic matter (food debris, excrement, fragments of feathers, etc.) settles continuously.

Although no attempt was made here to measure conspicuously the thermic and hydric conditions prevailing within nests, it could be fairly assumed that not only the orientation of the nest, its exposure to the sun and its neighbouring, physical environment (form of the ledge, vicinity of stones and shrubs, etc.) but also the presence of young and the « perturbating stimuli » (sensu REGIER and RAPPORT, 1978) they induce in connexion with both the intensity of their movements and the frequency of feeding and excreta are so much factors which contribute to make the nest an unpredictable habitat for mites.

Temperature and relative humidity are very important factors in the survival and the development of mites. Nothing is known about the conditions prevailing in the nests of birds but one may surmise that a temperature of 20-25°C and a RH of at least 80 % (probably more) are the optimal conditions for the species living in nests.

Richness and density

There are potentially a variety of micro-environmental variables which could influence the richness of species assemblages as well as the size of mite populations inhabiting the nests (e.g. physical structure and chemical composition of organic substrate), and these are unknown for all the sites studied. As in soils, it seems likewise that the presence of plants could have a mulching effect for thermic and hydric variations, and even a physical effect of both structuration and stabilisation of the nest habitat.

The great diversity and the high densities of mites registered within the nest no. 6 is probably explained by the fact that this nest was the most closely in conformity with the edaphic requirements. Indeed, this nest was located at the corner of an earthy ledge partially occupied by plants just along the rocky cliff. Moreover, it was shaded by various plants and rocks which most likely generated better conditions of temperature and RH than in other nests studied. It must also be added :

(1) that the site was used by the Eagle owl for many years as proved by the presence of many bones and feathers; (2) that the departure of the young, which had left the nest some days before sampling, is another factor that we cannot neglect. Finally, one may suppose that all these conditions were particularly favourable for the development of a rich mite fauna.

Surprisingly, in nest no.3, despite its localization in a widely open site and the presence of four young, one species (*T. palmarum*) showed a clearly high, population size that might square to a demographic explosion. Such dynamics are often registered in ephemeral or temporary biotopes (see examples in SOUTHWOOD, 1977) and relate to what is known in ecological terminology as « fugitive » (sensu HUTCHINSON, 1951) or « r-strategist » (sensu MACARTHUR and WILSON, 1967) species. Despite the physical disturbance due to young, there is no doubt that *T. palmarum* found in nest no.3 conditions favourable for growing.

Species composition

Regarding the mite assemblage evolving within the nest no. 6, what struck the most was the collection of mites belonging to two orders we did not find elsewhere, i.e. Metastigmata or ticks (1 species, Table 2) and Oribatida (9 species).

While ticks are parasites, Oribatida are primarily soil organisms. From an evolutionary standpoint, oribatid mites, which are among the most important secondary decomposers in the soil (see e.g. WAUTHY, 1982b, for a review), are dwellers of a very ancient, porous substrate (« porosphere » sensu VANNIER, 1983) where energetic, thermic and hydric conditions are substantially stable and predictable.

This leads to the assumption that the presence of oribatid mites within the organic substrate could be related to two causes improving their invasion of the milieu : (1) the cessation of physical perturbations due to the young, in addition to the coming back of thermic conditions more consistent with the ones tolerated by indigeneous species; (2) the presence of an organic substrate with both better climatic conditions and better physical structure due to plants growing upon the cliff edge. Yet, it must be noted that most populations established within the nest no. 6 belong to strictly saxicolous or arboricolous taxa. The few exceptions relate to species which up to now have been collected only in soils (i.e. *Suctobelbella subtrigona* and *Galumna cf. lanceata*) or to one species (*Micreremus brevipes*) which is a common tree dweller and originates probably from shrubs overhanging the nest.

Origins of the mites living in the nests of birds

The great heterogeneity observed in the mite fauna inhabiting birds' nests is probably directly related to the high diversity of their origins. In this connection, one may recognize three main categories of mites, as foilows (see also WOODROFFE, 1953).

1. *Species directly linked to birds.* In this category are the parasitic mites which live upon birds and may be occasionally collected within the nest.

2. *Species introduced into the nest :* (1) with food products (e.g. Acaridae, Glycyphagidae, etc.) ; (2) with prey (diverse ectoparasites of mammals and birds) ; or (3) with coprophagous insects (Diptera or Coleoptera) attracted by the droppings of the birds. These insects may carry hypopi (i.e. heteromorphic deutonymphs) of astigmatid mites or phoretic adults or deutonymphs of some Mesostigmata (e.g. Macrochelidae and Uropoda).

3. *Mites originating from the nest itself.* Some are present in the soil on which the nest is built (e.g. Oribatida) while others are introduced into the nest with the vegetal material used by the bird for making its nest. This is the case for the Dipper (*Cinclus cinclus aquaticus*) which lives in very wet habitats and whose nests are constructed with mosses which usually contain a rich fauna of Prostigmata. Some of these mites are able to survive for a long time in these nests (FAIN *et al.*, 1991).

Astigmatid mites and their evolution

Our current data on mites associated with *Bubo bubo* as well as data gained with other species of birds emphasize the fact that Astigmata clearly characterize and feature in all known mite assemblages found in nests of birds. In addition, as indicated above, several species of mites found in nests are clearly phoretic organisms : they belong either to the order of Astigmata, especially involved in phoresy, or to the order of Mesostigmata. Moreover, most hypopi use insects for moving, but a lot of others attach to hair of mammals with peculiar, very elaborate organs (FAIN, 1969).

Regarding the evolution of Astigmata, it seems very likely that parasitism has started in nests. It has been postulated that all the parasitic Psoroptidia of mammals and all the parasitic Analgoidea of birds could have been derived from the nidicolous Pyroglyphidae (FAIN, 1963). These mites occupy an intermediate position between free-living and parasitic forms. Ecologically, the Pyroglyphidae are still free-living mites. However, from a morphological standpoint, they show all the characteristics of the parasitic Psoroptidia. In this group of mites, it seems, firstly, that the regressive evolution towards parasitism has preceeded the invasion of the host as if there were a « pre-adaptation », and secondly, that this has probably been induced both by the repeated contacts between hosts and mites and by the fact that these mites feed mainly on the corneous material desquamating from the skin of their host (FAIN, 1979).

Structural and temporal organisation of mite assemblages

From an ecological standpoint, only the mite assemblage of nest no.6 appeared to be structurally organized. Indeed, this assemblage showed, in addition to haematophagous parasites feeding directly on birds, all the trophic levels known in

decaying systems (predators, phytophagous organisms, and sapro-microbirous ones ; see Table 2).

This observation and previous considerations lead us to propose a possible scenario depicting the mite-bird relationships evolving with time, as follows.

Phase 1. Although no observations on the mite fauna living in any nest site were conducted before the setting up of the nest by the male owl, it could be assumed that the activities of the male (choosing of the nest place, scraping of the substrate, etc.) generate a gap (i.e. a « catastrophe » sensu HOLLING, 1973) for mite populations which inhabit not only the exact place where the nest is burrowed but also its neighbourhood.

Phase 2. From the incubation period and mainly as soon as the young have hatched, the nest is refilled more or less rapidly with organic matter that may be regarded as a milieu chemically (e.g. toxins) and physically (compression, temperature, etc.) disturbed. Clearly, mites do not seem to create resident assemblages within nests during this phase. In addition, the number of species registered in nests as well as their population size does not seem to be linked with the number of young (Table 2). Yet, the too small number of nests studied does not lead us to reject the possibility that mites either develop specific assemblages subject to population change generated by perturbations (see SUTHERLAND, 1981, for examples, and CASWELL and COHEN, 1991, for a model) or might evolve according to successive stages just as it is known in insects inhabiting dung (see e.g. HANSKI, 1980).

Phase 3. Although in this survey only one nest already abandoned by young has been studied (nest no. 6), the observation in it of mites, which were not recorded in other nests before the departure of young, leads us to assume that from the time of departure of the young a population succession could occur in nests. If after the departure of young mites show such population successions, these clearly relate to successions following a perturbation. This means that the rate and pattern of populations occurring successively depend on diverse factors among which the most important, where mites are concerned, appear to be the invasive power and the reproductive traits of species living in the vicinity of the nests (see SOUSA, 1984, for theoretical considerations).

CONCLUSION

The previous scenario is today quite hypothetical, and it is obvious that the survey of a greater number of nests is necessary to confirm or not its relevance.

Yet, at this point of our study of mite-bird relationships, the results herein gained highlight the adequacy of the model « nest of *Bubo bubo* ». Indeed, all else being equal, this model appears to be relatively simpler than the one exemplified by nests of other species of birds. For instance, stronger, both biotic and abiotic perturbations due to the constant addition of organic matter to the nest occur in *Cinclus cinclus* during all the time for which the young grow up. However, due to

the localization of nests of *Bubo bubo*, the sampling of organic matter filling in nest depressions is conspicuously difficult (and even dangerous for the sampler!), and has to be carefully conducted in order to not disturb the young.

The model « nest of *Bubo bubo* » will certainly allow a better knowledge of non-parasitic relationships involving in Nature mites and other animals to be inferred, and this is certainly a quite wide programme. Finally, it should also be added that besides the mite fauna inhabiting nests of birds one finds very regularly a varied, sometimes very rich fauna of insects. Relationships prevailing in nests between mites and insects are another fascinating aspect of the study of these peculiar biotopes.

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A REDESCRIPTION OF *TRICASSA DESERTICOLA* SIMON, 1910, REPRESENTING THE TRICASSINAE, A NEW SUBFAMILY OF WOLF SPIDERS (ARANEAE, LYCOSIDAE)

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ABSTRACT

A detailed redescription is provided of *Tricassa deserticola* SIMON, 1910 after the discovery of male material. The species occurs along the south-western coast of Africa and has a distinctive genitalial structure. Its taxonomic position is discussed and a new subfamily, the Tricassinae, is proposed to accommodate this very peculiar spider species.

Key words : Araneae, Lycosidae, Tricassinae, *Tricassa,* Africa, taxonomy.

INTRODUCTION

Tricassa deserticola was discovered along the coast of Namibia, at Lüderitzbucht, during the 1903-1905 expeditions of the « Königliche Preussischen Akademie der Wissenschaften » and described by SIMON in 1910. Unfortunately, the very short Latin description of the two female type specimens (SIMON, 1910 : 213) is not accompanied by an illustration. The original description does not reveal the unusual habitus of this spider, which upon superficial examination does not ressemble a lycosid. The same applies to ROEWER's (1959-1960) redescription and illustration. His drawing (fig. 521, p. 942) of the epigyne is misleading and gives the impression of an « inverted T », so common in other lycosids.

Besides the type material, two females, only one report on other specimens is available : PENRITH and KENSLEY (1970) caught spiders on a supralittoral sandy beach during their study on rocky shores near Lüderitz ; the whereabouts of the specimens is unknown. The species is also listed by LAWRENCE (1965) and by GRIFFIN and DIPPENAAR-SCHOEMAN (1991).

Recently, several specimens of what appeared to be *Tricassa deserticola* were sent to us for identification. Two samples from separate localities in Namibia

included both males and females. Considering the lack of good illustrations of this peculiar spider and the fact that its position within the Lycosidae was unclear, a detailed redescription and an analysis of its taxonomic position seemed necessary.

Both SIMON (1910) and ROEWER (1959-1960) classified the species within the Lycosidae. This view is supported by the absence of the tibial apophysis on the male palp and by the typical eye arrangement found in *Tricassa,* both considered synapomorphies of the Lycosidae (DONDALE, 1986). Information on the behaviour of the species (carrying of egg cocoon and/or pulli ?) would corroborate this placement.

However, *Tricassa deserticola* cannot be placed in any of the existing subfamilies of Lycosidae. The species has several peculiarities which necessitate the erection of a new taxon at the subfamily level. In this paper, we describe the male for the first time, provide a detailed redescription of the female and discuss the taxonomic position of *Tricassa.*

ABBREVIATIONS

Material was provided by the following institutions :

MRAC = Musée Royal de l'Afrique Centrale, Tervuren, Belgium (R. Jocqué).
NCP = National Collection of Arachnida Pretoria, South Africa (A. S. Dippenaar-Schoeman).
NMSA = Natal Museum, Pietermaritzburg, South Africa (P. Croeser),
ZMB = Zoologisches Museum, Berlin, Germany (M. Moritz).

Other abbreviations used in the text are :

MNHN = Musée National d'Histoire naturelle, Paris, France
CW = Carapace width in mm.
CL = Carapace length in mm.
TL = Total length in mm.
AME = Outer diameter of one anterior median eye in mm
PME = Outer diameter of one posterior median eye in mm.
IV/CL = Ratio of total length of leg IV to carapace length.

DESCRIPTIONS

Tricassinae n. subfam.

Diagnosis : Representatives of the subfamily are recognized by the long anterior spinnerets, the large shaft-like, longitudinal median apophysis in the male palp, the simple epigyne with papillose surface of the scape and the long winding copulatory ducts in the female.

Type genus : Tricassa SIMON, 1910, with one species.

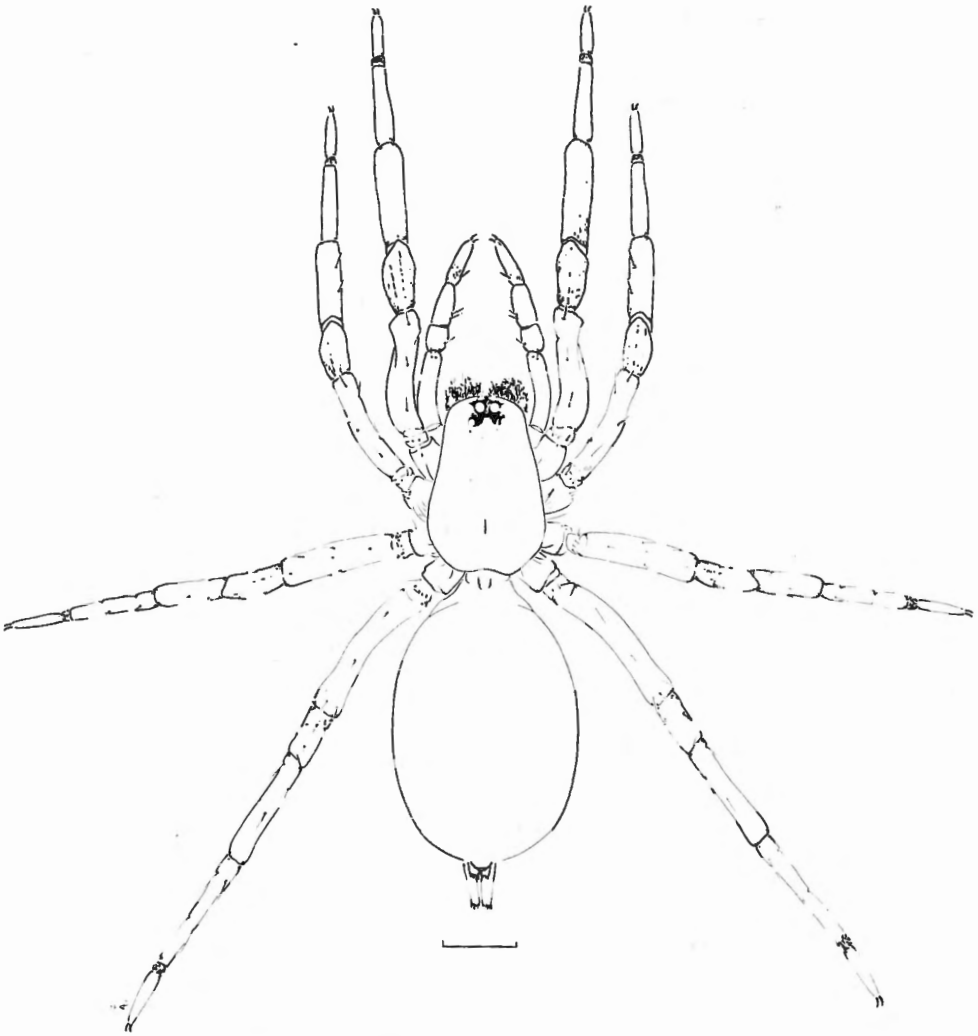
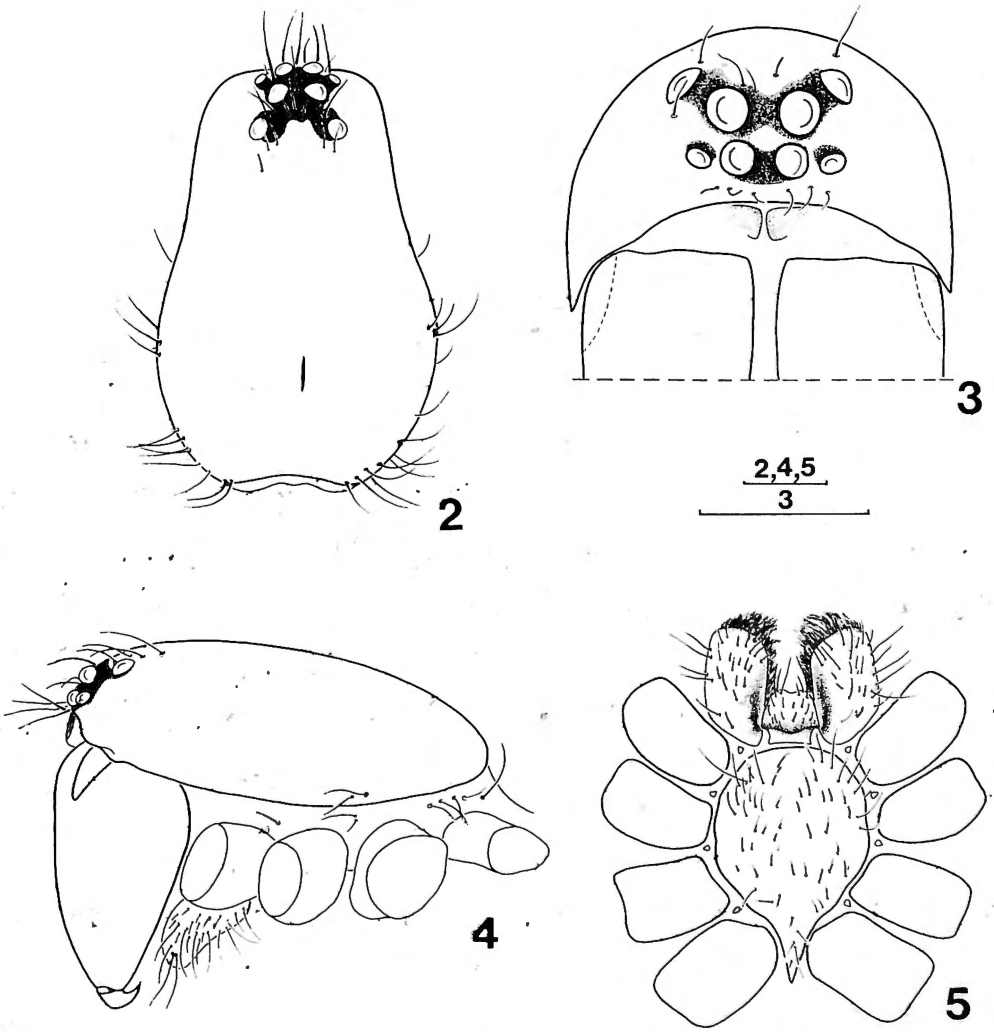


Fig. 1. — *Tricassa deserticola* — habitus of the female (scale = 1 mm).

Tricassa deserticola SIMON, 1910

Diagnosis :

Since *T. deserticola* is the only representative of the subfamily so far described, the species is diagnosed by the same characters.



Figs 2-5. *Tricassa deserticola* — 2-4. Female carapace, dorsal view (2), frontal view (3) and lateral view (4). - 5. sternum, labium and endites.
(Scale = 0.5 mm).

Type material :

Holotype ♀ : Namibia (South West Africa), Lüderitzbucht (ZMB 8566) (examined)

Paratype ♀ : same locality as Holotype (MNHN) (not examined)

Other material examined : South Africa : 1 ♂, 1 ♀ : Cape Province, Buffels Bay, Cape of Good Hope (34°19′ S — 18°26′ E), 25-29.X.1985, C. Griswold, J. Doyen and T.M. Griswold (NMSA). 1 ♂, 1 ♀ : Cape Province, Namaqualand, Port Nolloth, beach traps, VIII.1990,

A.M. McLachlan (MRAC 172.655). 3 ♂♂, 3♀♀ : Cape Province, Port Nolloth, beach traps, VIII.1990, A.M. McLachlan (NCP 91/77).

Distribution : Western coast of southern Africa, from Lüderitzbucht in the north to the Cape Peninsula in the south.

Description male (Figs 6-8, 12-13)

Measurements (mean, range, n = 3) : CW = 1.99 (1.80-2.16), CL = 2.72 (2.45-2.92), TL = 5.36 (4.60-5.88), AME = 0.14 (0.12-0.15), PME = 0.18 (0.17-0.19), IV/CL = 4.74 (4.66-4.86).

Carapace : uniformly pale yellowish white, without median or lateral coloured bands as present in many other lycosids. Profile domed, not falling sharply towards posterior margin as in female.

Sternum : uniform, yellowish white.

Clypeus : very narrow, much narrower than diameter of AME. Uniform pale, no colour difference with carapace. With some long hairs pointing forward. Chilum double, with two sclerites almost as wide as high, with faint lateral margin.

Chelicerae : relatively long and narrow. Pale yellow (compare with female) with two teeth on inner margin.

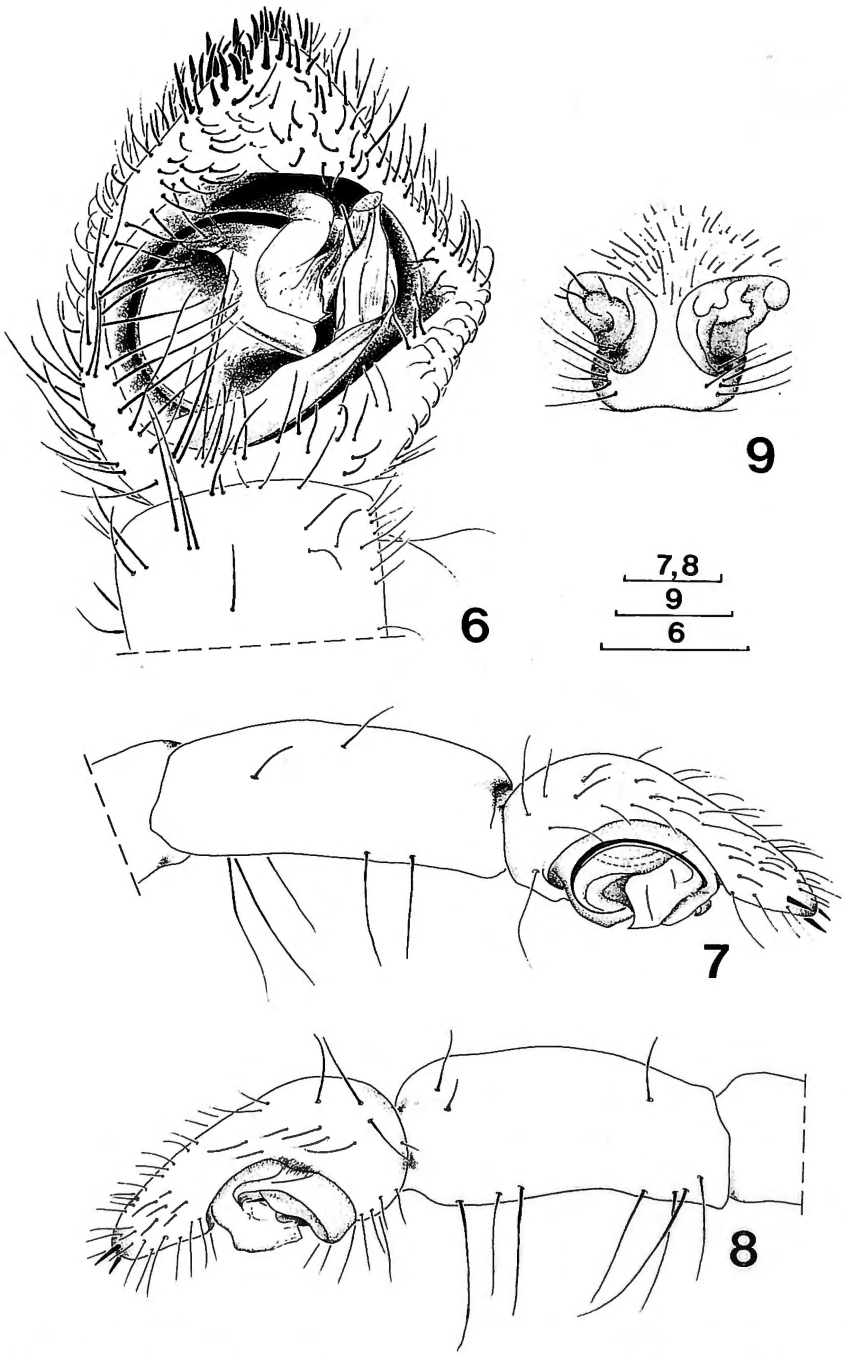
Eyes : anterior and posterior row clearly recurved. AME at least twice the diameter of ALE. PME enlarged but not strikingly so, only slightly larger than AME (see measurements). PLE only about one diameter from PME. Anterior row of eyes wider than second row of eyes. Ocular field with black patches and a few hairs.

Abdomen : almost completely uniformly pale yellow (no abdominal colour pattern), sometimes with a pink glimmer or somewhat transparent showing main blood vessel. With a group of stronger hairs on its dorso-basal edge. Venter uniformly pale yellow.

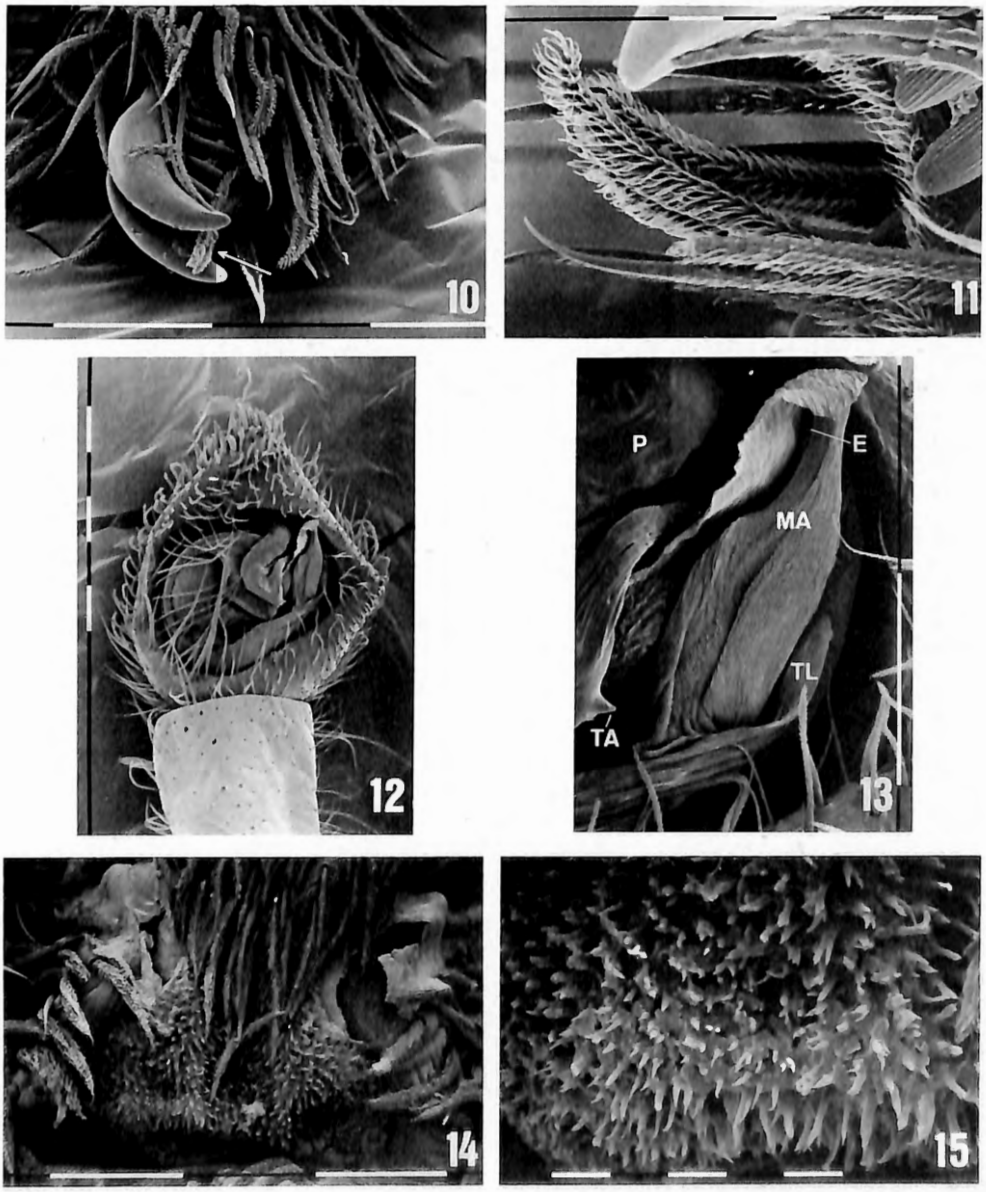
Spinnerets : uniformly pale. Anterior spinnerets cylindrical and strikingly elongated ; apical segment twice as long as basal one.

Legs : uniform, pale yellow, without darker patches or annulations. Legs long and slender, IV/CL relatively high (compare with female). Patella rather elongated. Tibia I with two pairs of long ventral spines and one additional, smaller apical pair (compare with female). Coxae clearly notched. Three tarsal claws, dorsal pair strong and relatively short with about six long ventral teeth. Tip of tarsi with curved modified hairs (Fig. 10, arrow ; Fig. 11).

Palp : elongated, with relatively long femoral and tibial segments. All segments pale yellow, same colour as legs and carapace, without any darker patches. Palpal tibia ventrally with widely spaced, long, slender hairs (length about one diameter of the tibia). Cymbium short, robust, retrolaterally angularly produced. Embolus very long with medio-apical insertion. Median apophysis and tegular lobe functioning as conductor.



Figs 6-9. *Tricassa deserticola* — 6-8. male palp, ventral view (6) mesal view (7) and lateral view (8). - 9. epigyne.
(Scale = 0.2 mm).



Figs 10-15. *Tricassa deserticola* — 10-11. tip of tarsus with modified hairs (10, arrow) and detail (11). - 12-13. male palp in ventral view, E = embolus, MA = median apophysis, P = palea, TA = terminal apophysis, TL = tegular lobe. - 14-15. epigyne, ventral view (14) and detail of scape (15).
(Scale = 0.1 mm in Figs 10-12, 14 ; scale = 0.01 mm in Figs 13, 15).

Description female (Figs 1-5, 9, 14-17)

Measurements (mean, range, n = 3) : CW = 2.32 (2.00-2.55), CL = 3.21 (2.86-3.50), TL = 7.84 (6.58-9.10 (abdomen swollen)), AME = 0.15 (0.13-0.18), PME = 0.20 (0.18-0.22), IV/CL = 3.26 (3.06-3.48).

Carapace : colour pale yellow, as in male. Shape somewhat more elongated than in male with widest point behind middle. Highest point of profile just behind ocular area, sharply falling towards posterior margin.

Sternum : pale yellow, as in male.

Clypeus : narrow and pale, as in male, but with more long hairs pointing forward. Chilum double, with two sclerites almost as wide as high and faint lateral margin.

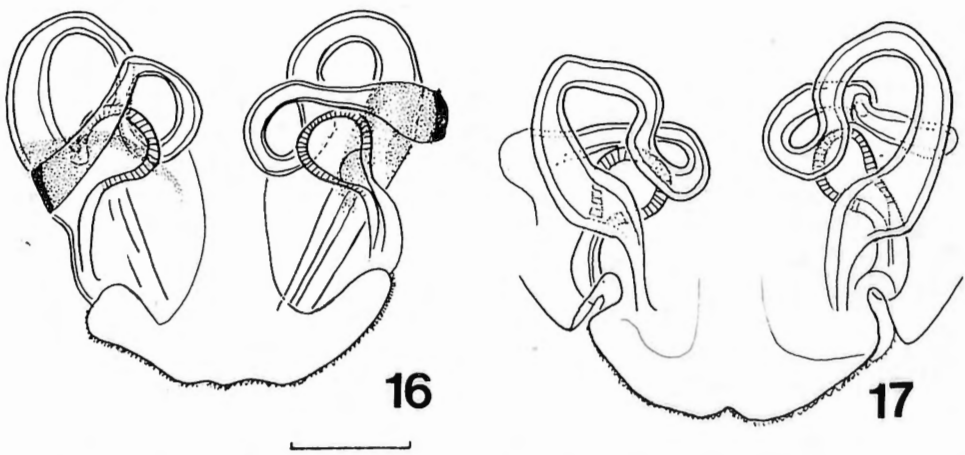
Chelicerae : relatively long, stronger than in male, chestnut brown (compare with male). Inner margin with two teeth.

Eyes : position and relative size exactly as in male ; ocular field also with black patches, but with more long hairs.

Abdomen : pale yellow to white.

Spinnerets : pale yellow to white. Anterior pair elongated and with apical segment twice as long as basal one.

Legs : Uniformly pale yellow, without darker patches or annulations. Much more robust than in male. Patellae and tibiae with many, short hairs ; to some extent organised in rows (on patellae). Patella, tibia, metatarsus and tarsus of leg I spineless, of leg II with very few spines (compare with male). Spines shorter and thicker than in male.



Figs 16-17. *Tricassa deserticola* : vulva, cleared in methyl salicylate — 16. dorsal view. - 17. ventral view.
(Scale = 0.1 mm).

Palp : uniformly pale yellow, same colour as carapace. Relatively long and strong. Tarsus somewhat darkened at tip, mainly due to occurrence of very dense, short spines on all sides. Tarsal claws strongly developed and relatively long (ca.0.24 mm), only slightly bent with some minute teeth at its very base (in contrast to leg claws).

Epigyne : consists of a simple median plate with a striking papillose surface (high magnification necessary).

Vulva : with entrance ducts long and winding. Copulatory openings near anterior lateral margin of epigyne. Large distal entrance ducts open ventrally into spermathecae but appear to be produced somewhat beyond that point. Fertilization ducts directed outwards and backwards, then curved inwards.

DISCUSSION

Habitat and behaviour of *Tricassa deserticola*

Little is known on the habitat and behaviour of this species. It seems to be restricted to coastal areas where it prefers sandy beaches. According to PENRITH and KENSLEY (1970) it does not occur in the sandy areas below high-water level of spring tides. It most probably makes a burrow in the sand although it is possible that adult males, in contrast to females, are no longer capable of making a new burrow (this can be deduced from their morphology, cf. ZYUZIN, 1990). Such a dimorphism has been recorded in other wolf spiders (ALDERWEIRELDT and JOCQUÉ, 1991), but requires confirmation from field or laboratory observations for this particular species.

Based on records of females with swollen abdomina, it is probable that egg cocoons are produced during October. This is the only element of the life cycle of *Tricassa* known at present.

Ethological data on this species would be of interest to establish if it behaves like a typical lycosid. It is not known if the female of *Tricassa* carries her egg cocoon attached to the spinnerets and the pulli on her abdomen as is the case in all other Lycosidae. Both characters are considered as synapomorphies of the Lycosidae (DONDALE, 1986).

Taxonomic position of *Tricassa*

As summarized by DONDALE (1986), the taxon Lycosidae is based on three autapomorphies. The first is the peculiar eye arrangement. Although the eye arrangement of *Tricassa* fits the general pattern known for typical Lycosidae, there are some small, but significant differences. The posterior median eyes are slightly larger than the anterior median eyes but the difference is much less pronounced than in most other lycosids. The distance between the posterior lateral and the posterior median eyes is small since the posterior lateral eyes are not shifted as far backwards as in typical Lycosidae. The eye arrangement thus resembles, to some

extent, that of other families, such as Pisauridae, Ctenidae, Zoridae or Zoropsidae. However, the genus does not belong in any of the latter three families because of the presence of three tarsal claws.

The second synapomorphy of the Lycosidae is the loss of a retrolateral apophysis on the male palpal tibia, as in *Tricassa deserticola*. Therefore it cannot be assigned to the Pisauridae.

Because of a lack of ethological information, it is not known if the third synapomorphy of the Lycosidae, *i.e.* active transport of the egg sac attached to the mother's spinnerets and of the young spiderlings on her abdomen, is present in *Tricassa*.

There are several peculiarities which make this species unusual within the Lycosidae. It has a very pale colour, with hardly any pattern, although this is also the case in some sand-dwelling *Arctosa* spp. (DONDALE, pers. comm.). Moreover, it is almost hairless, in contrast to the very hairy abdomen of most lycosids. Apart from the femur, the first leg of the female is spineless (cf. Fig. 1).

The structure of the genital organs is unusual for a lycosid. The embolus is very long, inserts medio-apically and is accomodated by a tegular process (tegular lobe) and a median apophysis (Figs 6, 12, 13), which probably act as a functional conductor. The palea is well developed and bears a very small, pointed terminal apophysis (Fig. 13). In the female, the papillose surface of the median plate of the epigyne is special (Figs 14, 15).

In discussing the systematic position of *Tricassa* we followed the classification proposed by DONDALE (1986). He divided the Lycosidae into five subfamilies : Sosippinae, Venoniinae, Allocosinae, Pardosinae and Lycosinae. ZYUZIN (1985), in his study on Palaearctic wolf spiders, added two new subfamilies : Evippinae and Wadicosinae. The representatives of the Sosippinae (*e.g.* the genus *Sosippus*, cf. BRADY, 1962) are characterized by the absence of a terminal apophysis and a palea. Moreover the embolus lies among a cluster of tegular processes and the tegular groove functions as conductor (DONDALE, 1986). In *Tricassa*, the terminal apophysis is, although small, clearly present and the median apophysis and tegular lobe act as conductor. The Venoniinae have a small and short embolus, situated distally (DONDALE, 1986 ; see also LEHTINEN and HIPPA, 1979) whereas the embolus of *Tricassa deserticola* is very long. *Tricassa* cannot be included in the Allocosinae either. It lacks the typical beaklike terminal apophysis and the median apophysis with two pointed processes (see DONDALE, 1986 ; DONDALE and REDNER, 1983). The Allocosinae lack a median septum in the epigyne, present in *Tricassa*.

The Pardosinae are characterized by the tooth-like terminal apophysis situated retrolaterally on the palea surface (DONDALE, 1986). Its tip is directed towards the tip of the embolus and conductor. As can be seen in Figs 6, 12, 13 and the description of the male, this structure is also found in *Tricassa deserticola*. The position and morphology of the toothlike terminal apophysis in *Tricassa* suggest that it might be homologous with that of the Pardosinae. On the other hand, *Pardosa* possesses a consistent synapomorphy absent in *Tricassa* : the shaftlike conductor along the basal margin of the palea extending to the retrolateral margin of the bulbus.

The large, exposed functional conductor of *Tricassa* illustrated in Fig. 13 excludes this species from *Pardosa* and the Pardosinae. Other differences between the typical representatives of the Pardosinae (genus *Pardosa*) and *Tricassa* exist but these might be interpreted as specific adaptations of *Tricassa deserticola* to its way of life.

ZYUZIN (1985) erected the subfamily Wadicosinae to accommodate the genus *Wadicosa,* characterized by the strongly developed, sclerotized tegular outgrowths which point outwards. These are absent in *Tricassa,* thus clearly excluding this species from the Wadicosinae. Finally, the Evippinae is characterized by the mesoapical insertion of the embolus in a wide and deep groove and by the vaulted tegulum. Females are characterised by the presence of pale epigynal atria (ZYUZIN, 1985). These characters are absent in *Tricassa.*

These comparisons show that *Tricassa deserticola* has a very unusual genital structure and that this justifies the establishment of a new subfamily.

ACKNOWLEDGEMENTS

We are very grateful to Dr. A.S. Dippenaar-Schoeman (NCP), Dr. P. Croeser (NMSA) and Dr. M. Moritz (ZMB) for providing material. We are very much indebted to Dr. A.S. Dippenaar-Schoeman, Dr. C. Dondale, Dr. T. Kronestedt and Dr. A. Russell-Smith for their valuable comments on an earlier version of the manuscript. We also thank Mr. A. Reygel for preparing the final line drawings.

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ON THE TAXONOMY AND ZOOGEOGRAPHY OF THE GENUS *GOMPHOCYTHERE* SARS, 1924 (CRUSTACEA, OSTRACODA), WITH THE DESCRIPTION OF A NEW SPECIES FROM THE NAHAL DAN (ISRAEL)

by

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SUMMARY

Gomphocythere ortali n.sp. is described from the Nahal Dan, one of the three main sources of the River Jordan. This area is well known as one of the southernmost Palaearctic enclaves in the Levant. The occurrence of an endemic species of an otherwise purely Ethiopian genus in such an area is therefore of interest. The present locality furthermore constitutes an important range extension for the genus, as the most northern locality of *Gomphocythere* thus far was Addis Ababa (Ethiopia).

Gomphocythere ortali n.sp. can easily be distinguished from all its congeners by the large, elongated valves in both sexes, by the relatively narrow female carapace in dorsal view and by the shape and anatomy of the hemipenis. New diagnoses for the tribe Cytheridellini and for the genus *Gomphocythere* are given; a number of morphological features and the validity of some species are reassessed. The origin and zoogeography of the genus and species are discussed.

Keywords : taxonomy, morphology, zoogeography, ecology, Ostracoda, Levant, *Gomphocythere*, Afro-Syrian Rift.

INTRODUCTION

MARTENS *et al.* (1992) published a preliminary checklist of non-marine ostracods reported from Israeli inland water and retained 25 species in 18 genera. Meanwhile, DIMENTMAN *et al.* (1992) and MARTENS (1993) added 6 genera and 8 species to this list. This total of 33 species still constitutes considerably less than half of the number of species present and/or expected in this country (MARTENS, unpublished records). Especially temporary pools, springs and subterranean habitats continuously prove to hold a very rich and diverse ostracod fauna. The present con-

tribution reports on a new species of an Ethiopian genus, *Gomphocythere* SARS, 1924.

Nahal (River) Dan is one of the three main headstreams of the River Jordan. The Dan spring emerges on the northern frontier at the foot of Mount Hermon from a powerful karstic exsurgence (POR *et al.*, 1986), where it forms a crystal-clear pond. Its waters, originating from melting mountain snow, are cold (15-16° C), even in mid-summer. The average flow of Nahal Dan is estimated at c. 252 million m^3 (ORNI and EFRAT, 1980) or at c. 8 $m^3.s^{-1}$ (ALLAN *et al.*, 1988). Both discharge and water temperature are unusually constant and there are no visible seasonal effects. Nahal Dan flows south in a series of small waterfalls, over a stretch of c. 6 km, very uniform from a faunal point of view, after which it unites with the Nahal Hermon and later on with the Nahal Senir to form the Upper Jordan River.

Our samples were collected near the actual spring of the Nahal Dan, i.e. near station 1 of ALLAN *et al.* (loc.cit.).

The new species, *G. ortali* n.sp., is here described and some problems with regard to taxonomy and morphology of the genus in general, as well as to origin and zoogeography of the new species in particular, are discussed.

ABBREVIATIONS USED IN TEXT AND FIGURES

With regard to the nomenclature of the chaetotaxy of the various limbs, we follow the model proposed by BROODBAKKER and DANIELOPOL (1982). Part of the terminology of the hemipenis is similar to the one developed in DANIELOPOL *et al.* (1990) and in MARTENS (1990a).

Soft parts

A1	antennula
A2	antenna
cop	copulatory processus of hemipenis
cs	caudal seta
dl	distal lobe of hemipenis
fs (1-2)	furcal setae 1-2
fl (1-3)	furcal lobes 1-3
fo	forked organ (« organe fourchu »)
fu	furca
GeO	genital orifice
hlo	hypostomeal lobes
lb	labium
Md	mandibula
Mx1	maxillula
P(1-3)	walking limbs
ro	rake-like organs
ur	upper ramus of clasping organ of hemipenis
Ya	aesthetasc on A1
Y	aesthetasc on A2

Valves

H	height of valves
L	lenght of valves
RV	right valve
LV	leftvalve

TAXONOMIC DESCRIPTIONS

Subfamily Limnocytherinae KLIE, 1938

Tribe Cytheridellini DANIELOPOL and MARTENS, 1990

Amended diagnosis. Carapace with important sexual dimorphism, i.e. ♀ caudally with widely dilated valves in dorsal view, forming brooding pouches for eggs and first (2 ?) instars. Valves with or without additional longitudinal ridges and crests on the external surface. Hinge adont to weakly lophodont. Mx1 with palp weakly sclerified, mostly undivided and with reduced number of apical and lateral setae. P(3) the largest walking limb (*Gomphocythere* and *Gomphodella*) or reduced to a short and curved cleaning limb (*Cytheridella*).

Genus *Gomphocythere* SARS, 1924

Type species : Limnicythere obtusata SARS, 1910

Amended diagnosis. Females with a brooding pouch. Hinge lophodont, with posterior cardinal tooth on LV (always ?) larger than anterior one. Ventral side in some species set with ridges.

A1 with penultimate segment set with 7 claw-like setae and weakly or not at all divided. P(3) the largest walking limb. Posterior part of female abdomen with one furca, bearing two furcal setae and three hirsute furcal lobes. Hemipenis with large, articulating distal lobe, without a lateral seta (present in *Cytheridella*).

Gomphocythere ortali sp.n.

Type locality. Nahal Dan, spring and river, northern Israel. Approximate coordinates: 35°37′33″ N, 33°15′29″ E (Israel Grid: 2110/2950). For description of the area: see introduction.

Type material. The following collections have been used for the present descriptions :

ISR/84/27 (no. IG.27401) : sample collected amongst littoral vegetation, at c. 200 m from actual spring, by MARTENS and ORTAL on 5/3/1984. Measurements at time of collection: Ph = 8.2, T° = 15.3° C. All illustrated specimens originate from this sample. Accompanying ostracod fauna : *Psychrodromus* n.sp., *« Stenocypris » subterranea, Ilyocypris* gr. *inermis.*

ISR/91/54 (no. IG.27757): sample collected amongst *Potamogeton* stands, c. 200 m from spring, by MARTENS and ORTAL on 13.6.1991. Accompanying ostracod fauna : *Psychrodromus* n.sp., *« Stenocypris » subterranea, Heterocypris rotundata.*

Holotype : a ♂, with soft parts dissected in glycerine in a sealed slide and with valves stored dry (no. OC.1698)).

Allotype : a ♀, dissected and stored as the holotype (no. OC.1699)).

Paratypes : 3 ♂ + 3 ♀ stored dry after use for SEM (nos. OC.1692-1697), 2 tubes which c. 50 specimens (♂, ♀, juv.) each (nos. OC.1700-1701).

Repository : all types, both dissected and in spirit, are stored in the Ostracod Collection of the R.B.I.N.Sc.

Derivation of name : this species is named after Dr Reuven ORTAL (The Hebrew University, Dept of Zoology and The Nature Reserves Authority, Jerusalem), in appreciation of his contribution to the research on and the conservation of aquatic environments in the Levant. This is also to thank him for the warm friendship that we have shared over the years.

Diagnosis. Large and elongated species, with straight dorsal margin sloping towards the caudal side, especially in the male. Posterior part of carapace in female relatively narrow and rounded in dorsal view. Mx1-palp weakly sclerified and undivided. Hemipenis with dl sub-triangular, with squarish base, blunt tip set with comb-like structure, medio-lateral indention without a protuberance; ur relatively short, cop short and curved in its distal half.

Additional description of ♂. Valves (Figs. 1E, F) strongly sclerified and externally reticulated, but without ridges or crests. Anterior margin broadly rounded, posterior margin more narrow, but still evenly rounded. Greatest height situated at about one fourth from the anterior, from there the straight dorsal margin sloping towards the caudal side. Ventral margin weakly sinuous. Hinge (Fig. 1L) lophodont, with prominent and elongated, but simple cardinal teeth on LV fitting in cardinal sockets on RV; intercardinal bar on RV smooth. Both valves (Figs. 1G, H) with prominent selvage; calcified inner lamellae narrow on both anterior and posterior sides. Ventro-caudal flanges irregularly serrated (Fig. 1M). Carapace in dorsal and ventral (Fig. 1K) views narrow, with nearly parallel sides.

A1 (Fig. 2D) with 4-segmented endopodite, penultimate segment undivided. First segment of endopodite c. twice as long as wide, with one long, medio-ventral seta. Second segment short and squarish, with one long apical seta. Third segment with one medio-ventral and two medio-dorsal setae and four apical (one ventral, three dorsal) setae. Terminal segment elongated, with two sub-apical setae and one apical aesthetasc, slightly fused at the base with a seta, this seta longer than the actual sensorial club.

A2 (Fig. 3E) with endopodite three-segmented. First segment short, bearing one long apical seta. Second segment long, with medio-ventrally two setae and one long aesthetasc (the latter reaching to tip of segment); medio-dorsally two unequal setae and apically one stout, claw-like seta. Terminal segment with three stout and short claws, one subapically and two apically inserted.

Md (Fig. 3D) without special features. Palp four-segmented. First segment with respiratory plate consisting of three rays only (two long, one short) and with two subequal ventral setae. Second segment short and squarish, bearing three apical setae. Third segment of similar shape, with 5 apical setae, three dorsally and two ventrally inserted. Terminal segment pyramidal, with two claws.

Mx1 (Fig. 2E) with three endites of normal shape and chaetotaxy; palp weakly sclerotized and unsegmented, set with three lateral and two apical setae.

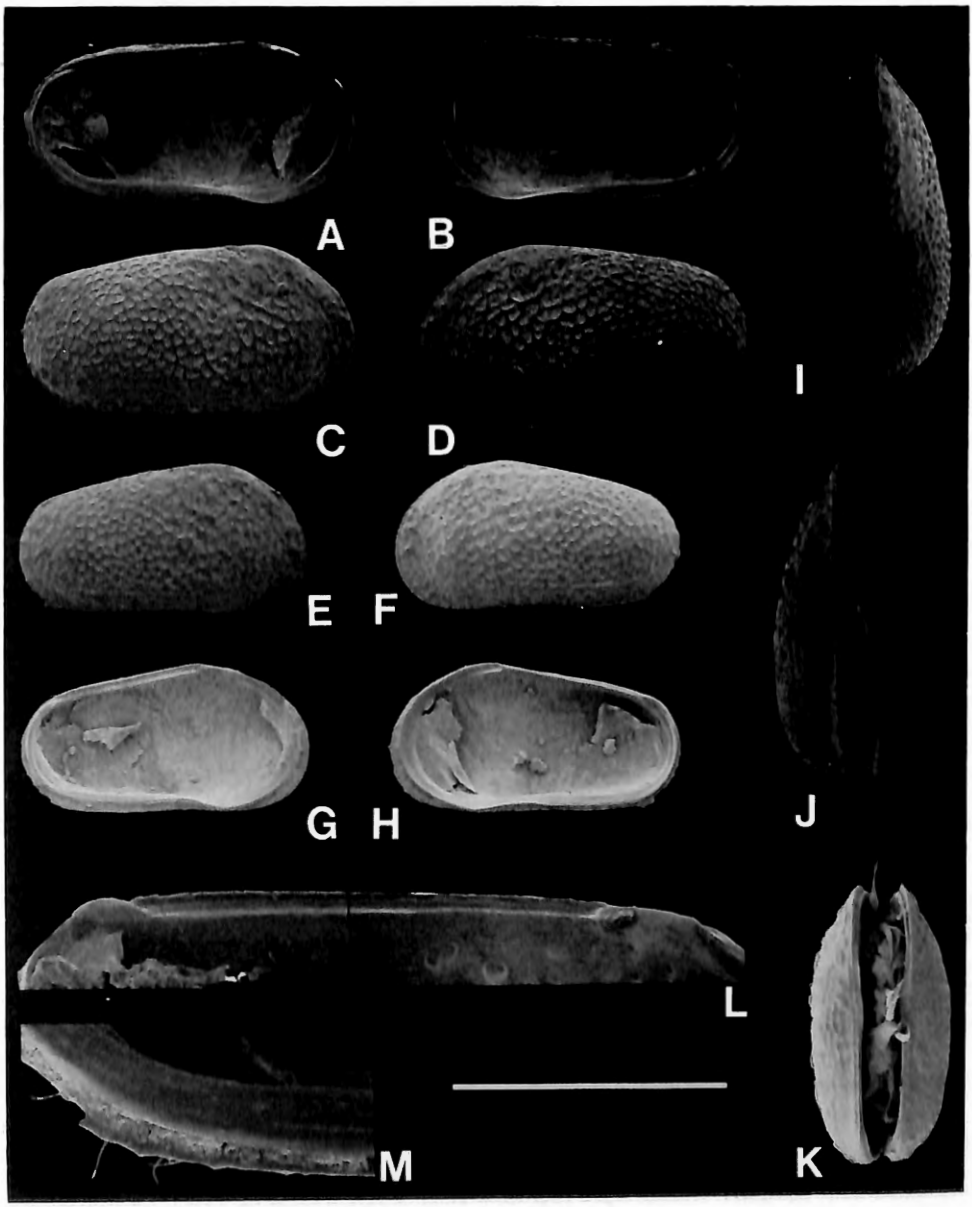

Fig. 1. *Gomphocythere ortali* n. sp. : SE micrographs of the valves. — A. ♀, LV, internal view (no. OC.1694). - B. ♀, RV, internal view (no. OC.1694). - C. ♀, RV, external view (no. OC.1693). - D. ♀, LV, external view (no. OC.1693). - E. ♂, RV, external view (no. OC.1697). - F. ♂, LV, external view (no. OC.1697). - G. ♂, LV, internal view (no. OC.1696). - H. ♂, RV, internal view (no. OC.1696). - I. ♀, Cp, dorsal view (specimen lost). - J. ♀, Cp, ventral view (no. OC.1692). - K. ♂, Cp, ventral view (no. OC.1695). - L. ♀, LV, internal view, detail of hinge (no. OC.1694). - M. ♀, LV, internal view, detail of posterior part (no. OC.1694).
(Scale = 556 µm for A-K ; 217 µm for L ; 139 µm for M.).

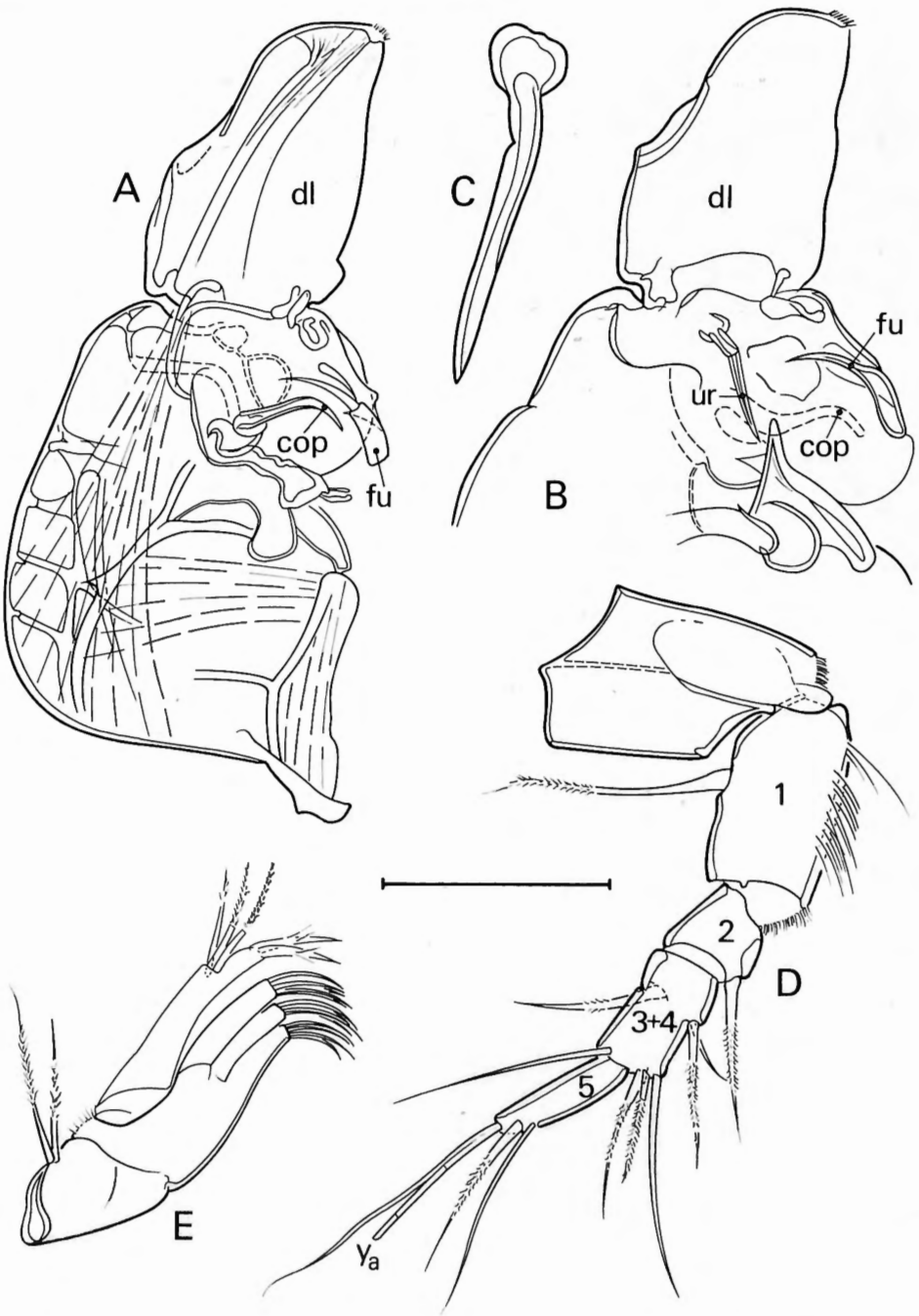

Fig. 2. *Gomphocythere ortali* n.sp. (♂, no. OC.1698). — A. Hemipenis, medial view. - B. Hemipenis, detail, medial view. - C. Hemipenis, detail or ur. - D. A1. - E. Mx1, respiratory plate not shown.
(Scale = 78 µm for A-E).

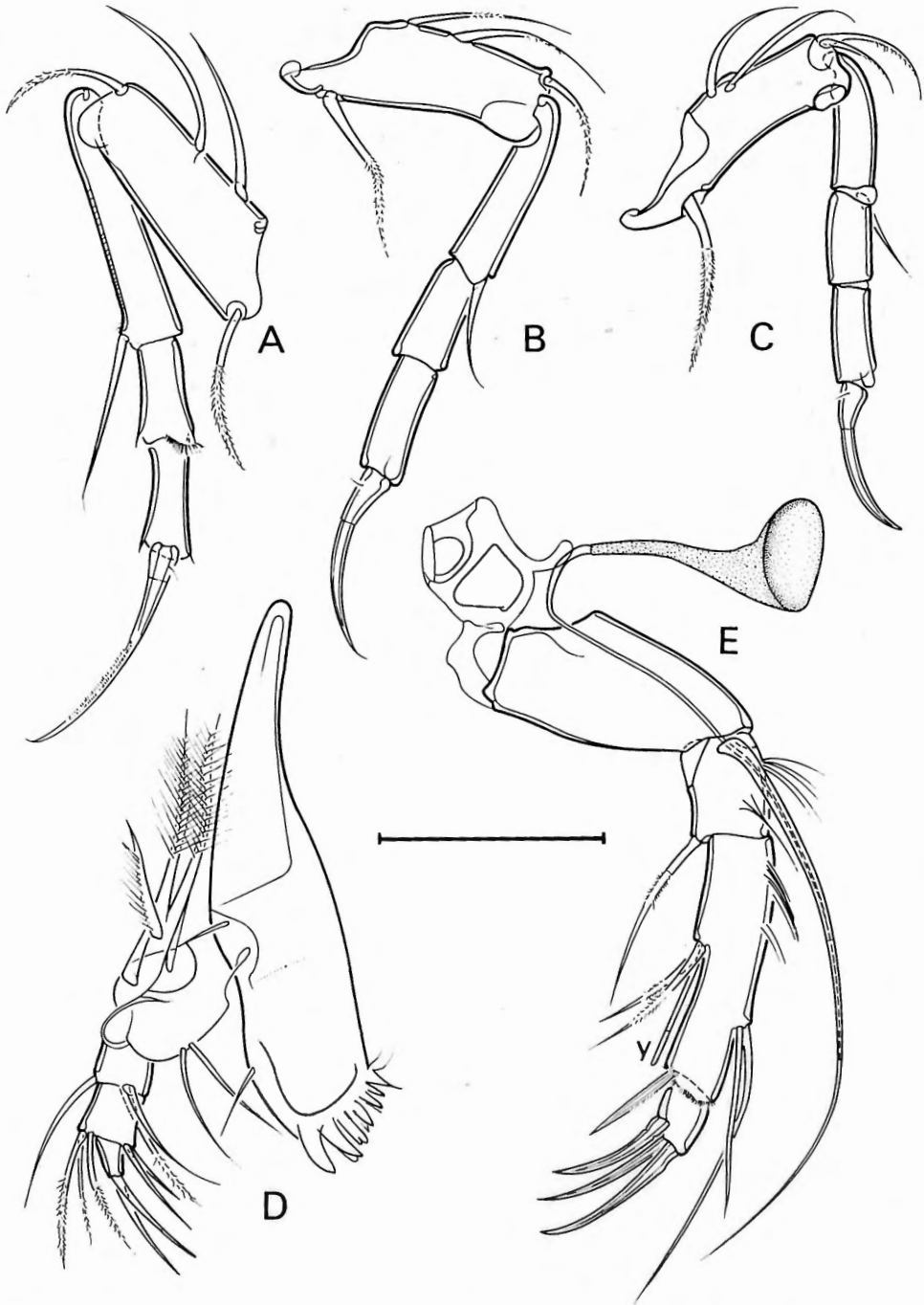

Fig. 3. *Gomphocythere ortali* n.sp. (♂, no. OC.1698). — A. P(3). - B. P(2). - C. P(1). - D. Md and mandibular palp. - E. A2.
(Scale = 78 µm for A-E).

P(1-3) (Figs. 3A-C) all walking limbs; P(1) the shortest and P(3) the longest and with the longest claw. First segment in these limbs with 2 long dorsal and one long and hirsute ventral seta (the latter proximally inserted) and with one (on P(2) and P(3)) or two (on P(1)) knee-setae as is typical of the subfamily.

Hemipenis (Figs. 2A-C) of the typical *Gomphocythere*-type, i.e. with a large, muscular body and a prominent, articulating distal lobe (dl), devoid of setae. Shape of the dl in this species broadly subtriangular, with a squarish base, a blunt tip set with a comb-like structure and with a sinuous dorsal margin devoid of protuberances. On main body, ur relatively short and straight, well sclerotized; cop short and distally curved.

Additional description of ♀. Valves (Figs. 1A-D) generally as in the ♂, but larger and with straight dorsal margin only slightly sloping towards the caudal side; anterior margin still more broadly rounded than posterior one. Structure of hinge and valve margin as in the ♂. Carapace in dorsal (Fig. 1I) and ventral (Fig. 1J) view with posterior part more swollen than in the ♂, but considerably less so than in most other species of this genus. Anterior tip in dorsal view bluntly pointed, posterior edge rounded.

All appendages (Figs. 4A-C, 5A-C) generally as in the ♂; aesthetasc Y on A2 (Fig. 4B) slightly shorter than the accompanying setae. Two pairs of « hypostomeal lobes » present posterior to labium (Fig. 5E), anterior lobes approximately twice as long as posterior ones. Sternum as illustrated in Fig. 4D. Posterior part of abdomen (Fig. 5D) with one furca bearing two furcal setae and three hirsute furcal lobes, one (caudal ?) seta and one forked organ (see discussion for homology). Genital orifice small and rounded.

Measurements

♀ : L = 667-578 (n = 6) ; H = 334-345 (n = 4), W = 311 (n = 1)
♂ : L = 578-589 (n = 5) ; H = 300 (n = 4).

Differential diagnosis. The new species can easily be distinguished from almost all of its congeners by the elongated valves and by the shape of the hemipenis.

DISCUSSION

Taxonomy of the genus Gomphocythere

Although most of the descriptions of the species in this genus are sufficient to allow identification, there are still a number of problematic taxa.

1. Sars (1910) described *Limnicythere* (sic) *obtusata* from Lake Victoria. Later on (1924), he designated this taxon as the type species of the genus *Gomphocythere*, offering a redescription of what he claimed to be *G. obtusata* from South Africa.

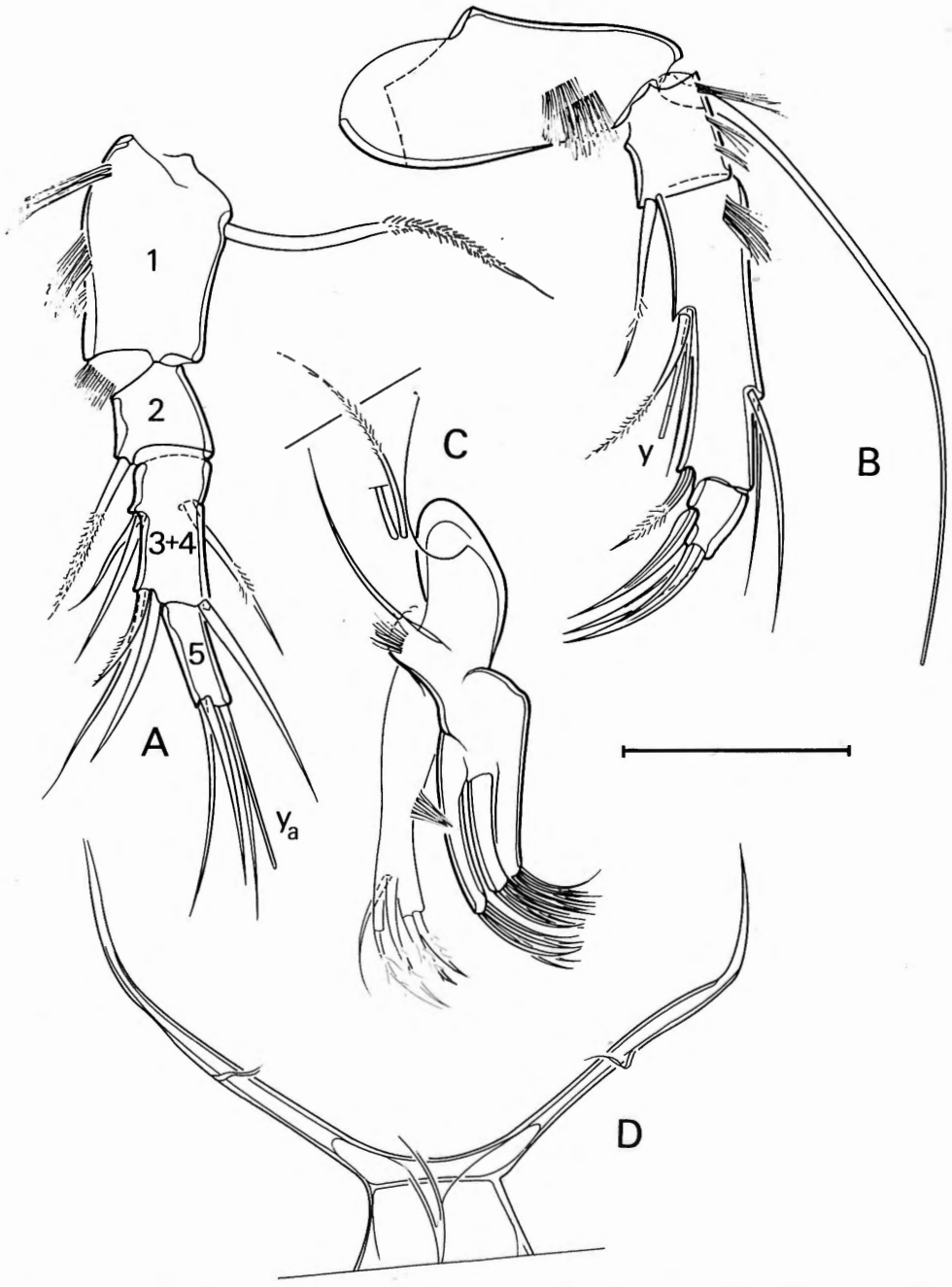

Fig. 4. *Gomphocythere ortali* n.sp. (♀, no. OC.1699). — A. A1. - B. A2. - C. Mx1, respiratory plate not shown. - D. Sternum.
(Scale = 78 µm for A-D).

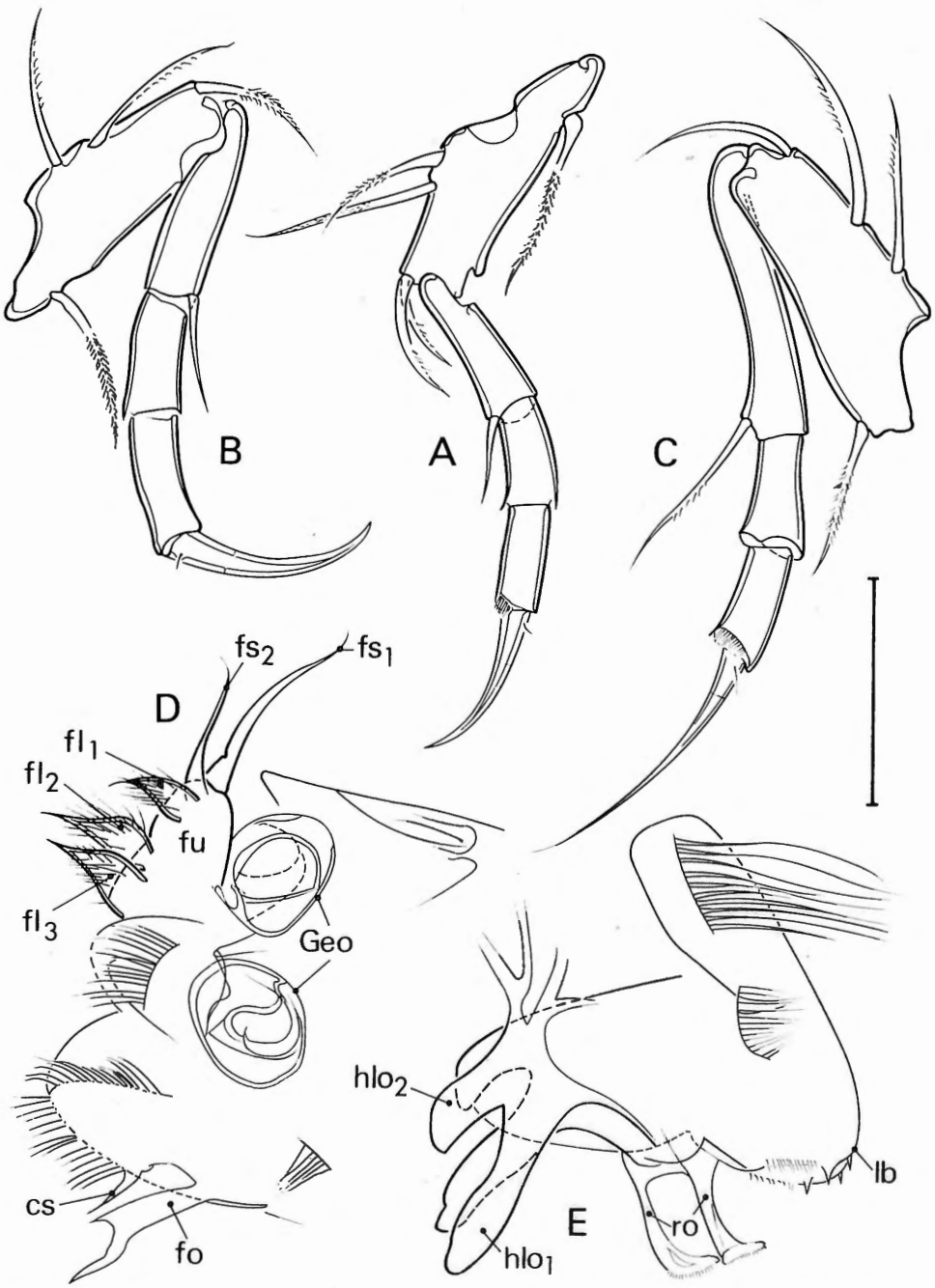

Fig. 5. *Gomphocythere ortali* n.sp. (♀, no. OC.1699). — A. P(1). - B. P(2). - C. P(3). - D. Furca and posterior part of abdomen. - E. Labium, rake-like organs and hypostomeal lobes. (Scale = 78 µm for A-E).

In the same paper he described *G. expansa*, which was later identified from East Africa by LOWNDES (1932) and LÖFFLER (1978). I have doubts with regard to the conspecificity of populations from East and South Africa, especially with regard to *G. obtusata* of which illustrations from both populations exist: the specimens from South Africa have a pronounced dorso-caudal corner on the valves, whereas this corner in Lake Victoria specimens is perfectly rounded. This is an important feature in this group. Unfortunately, no males are known from East Africa. Male specimens of both populations must be compared in order to solve this problem.

2. I have doubts with regard to the validity of *G. expansa* and *G. capensis*, the latter described as *Cytheridella capensis* by MÜLLER (1914) and characterized mainly by the complete absence of a brooding pouch in the ♀. From the same locality, MÜLLER identified females of *Cytheridella obtusata*, which actually belong to *Gomphocythere expansa* (fide KLIE 1939 and ROME and DE DECKKER 1977). The male valves and hemipenis of *G. capensis* are identical to those of *G. expansa*, whereas the ♀ supposedly belonging to *G. capensis* and illustrated by MÜLLER (*loc. cit.*) is similar to juvenile (A-1) ♀ of *G. expansa*. It would thus seem that *G. capensis* and *G. expansa* are synonyms, with the name *capensis* (described in 1914) then having priority over *expansa* (described in 1924). I have attempted to obtain the type material of *G. capensis* to check this assumption, but was thus far unsuccessful. Various new collections from the surroundings of Cape Town and the Cape Flats furthermore yielded several new populations of both *G. expansa* and *G. obtusata*, but never from *G. capensis*. Solutions to both of these problems will be described elsewhere (MARTENS, in prep.).

Morphology

There are a number of aspects of the morphology of this genus which need to be discussed here.

1. According to KLIE (1939), segments 3 + 4 of the A1 are fused in some species of this genus, separated in others. This is also described by ROME (1962, 1970) and ROME and DE DECKKER (1977). I have checked material of several species and it appears that a separation between these segments, if at all present, is always very superficial and never pronounced.

2. Ventral ridges and lateral crests were thought to be typical of *Gomphocythere*, until KLIE (1939) described *G. angusta*, which is completely devoid of both. This author also explicitely stated that these features should be lifted from the generic diagnosis, a fact which was ignored by DE DECKKER (1981) when he described the Australian genus *Gomphodella*. The ventral ridges were indeed cited by him as the main difference between *Gomphodella* and *Gomphocythere*. The other features from the diagnosis clearly separate *Gomphodella* from *Cytheridella*, but not from *Gomphocythere*. The Australian species should be re-investigated and new generic characteristics need to be defined.

3. The undivided and weakly sclerotized Mx1-palp with very reduced chaetotaxy is characteristic of the tribe Cytheridellini, as it occurs in all three genera belonging to this group. Nevertheless, for some species a two-segmented palp (e.g.

G. obtusata) or even a completely normal Mx1 (e.g. *G. expansa*) are described. At least for these two species I have been able to check new material from South Africa, and both species have the reduced Mx1-palp. The illustration of this appendage for *G. expansa* by Sars (1924) is completely erroneous. The degree to which the chaetotaxy is reduced (number of setae) does seem to show some interspecific variability. Some species (e.g. *G. obtusata* from South Africa) furthermore have a large lateral seta on the first endite of this limb. This seta was missing in all investigated specimens of *G. ortali* n.sp.

4. Some of Rome's species are described and illustrated with two endclaws on the A2, while all species examined by me clearly show 3 endclaws. ROME's material should be re-investigated in order to see if these observations are correct or not.

5. The forked organ was described by ROME and DE DECKKER (1977) as « organe fourchu », and occurs on the posterior part of the abdomen of females of both *Gomphocythere* and *Cytheridella*. In most species (including *G. ortali* n.sp.) it is indeed an undivided fork-like structure, but in *G. curta* ROME, 1962 it is illustrated as a solid base on which a small seta is inserted. Females of *Gomphocythere* and *Cytheridella* reportedly have only one furca (with furcal setae and furcal lobes), and the forked organ therefore in all probability represents the reduced second furca.

6. Fig. 5E shows part of the hypostomium (following nomenclature in SCHULTZ 1976), with lower lip (labium), rake-like organs and a pair of unsegmented lobes posterior to the labium. I have not found a homologue of these « hypostomeal lobes » in the literature.

Zoogeography

The extant (recent) species of the genus *Gomphocythere* are restricted to the Ethiopean Realm. A number of species outside Africa have been referred to this genus, but these are now lodged in other genera: the species from South America are in *Cytheridella*, while DE DECKKER (1981) created the genus *Gomphodella* for the Australian species. Including *Gomphocythere ortali* n.sp., there are 13 extant species (MARTENS, 1984). The distribution of this genus (Fig. 6) in Africa is very similar to that of the genus *Limnocythere* (see MARTENS, 1990a), with three exceptions : (1) *Limnocythere* is not exclusively Ethiopian, whereas *Gomphocythere* clearly is, (2) there are no *Limnocythere* species in the Cape Province (2-3 species of *Gomphocythere*) and (3) there are no *Gomphocythere* species in Namibia and Botswana (*Limnocythere tudoranceai* in both countries). The latter two observations, if at all valid and not due to an insufficiently dense sampling grid, are problematic, as they cannot be correlated with any physical or chemical environmental factor : *Limnocythere* and *Gomphocythere* both occur in large to very large lakes and in smaller (although permanent) water bodies, while also their tolerance ranges for conductivity/salinity, temperature etc. are very similar (see below).

The distribution pattern of *Gomphocythere* shows a clear affinity between the faunas of eastern and southern Africa, in this case including the Cape Province, as was already amply shown for other ostracod groups, such as *Limnocythere* (see

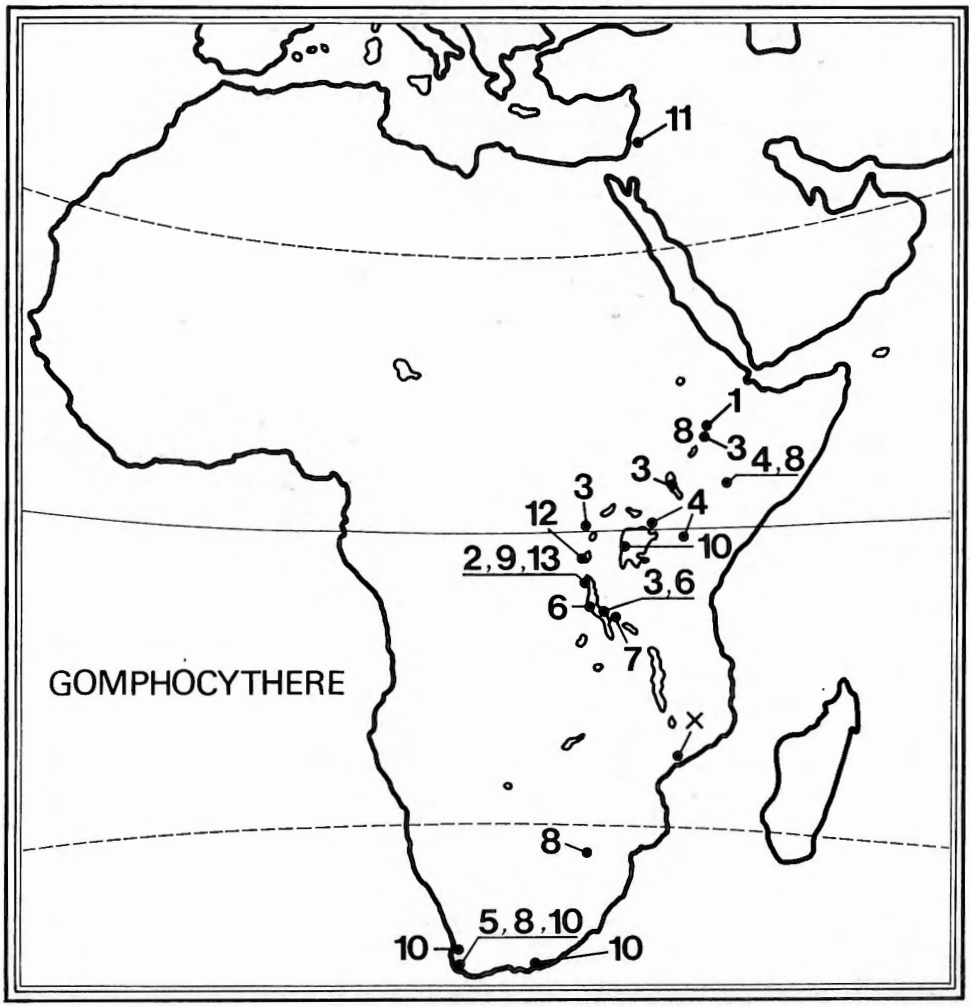

Fig. 6. — Distribution of *Gomphocythere* species.
1 = *G. aethiopis* ROME, 1970. 2 = *G. alata* ROME, 1962.. 3 = *G. angulata* LOWNDES, 1932. 4 = *G. angusta* KLIE, 1939. 5 = *G. capensis* (G. W. MÜLLER, 1914). 6 = *G. cristata* ROME, 1962. 7 = *G. curta* ROME, 1962. 8 = *G. expansa* SARS, 1924. 9 = *G. lenis* ROME, 1962. 10 = *G. obtusata* (SARS, 1910). 11 = *G. ortali* n.sp. 12 = *G. parcedilatata* ROME, 1977. 13 = *G. simplex* ROME, 1962. x = *G.* spec. indet.

MARTENS, 1990a), the Megalocypridinae (MARTENS and COOMANS, 1990) and the Cypridini (Martens, 1990b, 1992). These generalized patterns of the distribution of African ostracods again prove that East and South Africa indeed belong to the same zoogeographical province (TAYLOR, 1978 and others).

The presence of *Gomphocythere ortali* n.sp. in Nahal Dan is surprising and this mainly for two reasons. Firstly, the number of aquatic species of truly Ethiopian

origin in the Levant in general is rather limited and « ... whatever Ethiopian influence is felt in the inland waterfauna of the Levant, this is mostly limited to the south, i.e. to the Jordan Valley and the coastal plain of Israel ». (POR, 1989 : 137). The Nahal Dan and its spring belong, together with the Golan heights, to an area which is believed to be the southernmost limit of the Palaearctic, and the southern border of this region is termed the « Nehring-line » (POR, 1975 and others). Of the 156 taxa of aquatic animals, reported from the River Dan by ALLAN *et al.* (1988), the major part is Palaearctic in character. Only 7 of the identified species have an Ethiopian origin. All of these belong to the Hemiptera and the Chironomidae and are furthermore widely distributed in central and southern Israel. *Darwinula africana* KLIE, 1935 (MARTENS, unpublished data) and *Klieopsis horai* (KLIE) (in MARTENS *et al.*, 1991) are Ethiopian ostracods reported from Israel, but these species were found in springs around the Dead Sea, and this is the area where Ethiopian species can be expected.

Most of the other ostracods identified from the Nahal Dan (see above) are indeed also cold-stenothermal (even partly rheophylic) Palaearctic elements: *Psychrodromus* n.sp. (a second new (sub) species occurs in streams on the Golan heights) and a representative of the *Ilyocypris inermis*-group. *« Stenocypris » subterranea* HARTMANN is a cold-stenothermal species, typical of springs, rivers and subterranean aquatic habitats in the Eastern part of the Mediterranean.

There is also a second reason why the presence of *G. ortali* n.sp. in this area is surprising: the habitat itself, a cold water spring and river, is unusual for species of *Gomphocythere*. So even if we accept that *G. ortali* n.sp. is an endemic relict of a much wider ancient distribution of *Gomphocythere*, comprising the entire Afro-Syrian Rift valley — and this indeed seems to be the most logical explanation — then it is still not clear why this species survived in the cold running waters of the Nahal Dan and not, for example, in Lake Tiberias (LERNER-SEGGEV, 1968) or in Lake Hula (MARTENS 1993, DIMENTMAN *et al.* 1992), which would constitute more logical habitats. It should also be mentioned that the species does not occur in either Nahal Senir or Nahal Hermon, the other two main headwaters of the river Jordan.

Finally, to further complicate matters, Nahal Dan is in all probability not the original and/or only refuge of the species in this area, as this spring and river are supposed to be of postglacial origin (POR *et al.*, 1986). Other localities might for example be found in the Lebanon Mountains (POR, 1989).

Ecology

As was outlined above, species of *Gomphocythere* are generally confined to permanent lakes (large or small), where they can survive in a wide variety of ecological conditions, e.g. for pH (5.6 for *G. expansa* in Black Bosvlei, W. Cape — MARTENS, unpublished — and 9.5-10 for the Tanganyika species), conductivity (a range of 0-30,000 $\mu S.cm^{-1}$ for *G. angulata* — MARTENS, 1990a), etc. Most East African lakes have semi-permanent water temperatures of 20-25°C, but both the Ethiopian crater lakes and some of the vleis of the Cape Flats have considerably colder waters.

A number of *Gomphocythere* species have a maze of ridges on the ventral side of the carapace and this hinted towards a hyponeustic mode of life, in analogy with, for example, *Notodromas* and *Oncocypris*. However, several species of *Gomphocythere* (including *G. ortali* n.sp.) lack these ridges, and these taxa are certainly benthic species.

It is not impossible that certain species of this genus live in the subsurface (interstitially), but this would need further substantiation. It should finally be noted that Cytheridellini in all probability do not have dry resistant stages, which is the main reason why they are confined to permanent waterbodies. Of the Limnocytherinae, only some Leucocytherini are thus far known to have such dry resistant stages.

ACKNOWLEDGEMENTS

Mr J. Cillis and Mrs C. Behen offered technical assistance with the illustrations. Dr R. Ortal assisted in various field collections during my 1991 study visit and generally organized all logistics. This trip was financed by the scientific exchange programme of the cultural agreement between Belgium and Israel. Dr K. Wouters (Brussels) and Dr D. Danielopol (Mondsee) kindly read the manuscripts and suggested improvements.

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DESCRIPTION D'UNE NOUVELLE CREVETTE DE L'ÎLE DE LESBOS : *HIPPOLYTE SAPPHICA* SP. NOV. (CRUSTACEA, DECAPODA, CARIDEA, HIPPOLYTIDAE)

par

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RÉSUMÉ

Une nouvelle crevette Hippolytidae *Hippolyte sapphica* sp. nov. récoltée sur l'île de Lesbos, Nord-Est de la Mer Égée est décrite ici. Cette espèce est comparée avec *Hippolyte inermis* LEACH et avec *Hippolyte longirostris* (CZERNIAVSKY) avec lesquelles elle a été souvent confondue.

Mots-clés : Hippolyte sapphica, crevette, Crustacea, Decapoda, Caridea, Hippolytidae, Grèce, Méditerranée.

Description of a new shrimp from the Lesbos island : *Hippolyte sapphica* sp. nov. (Crustacea, Decapoda, Caridea, Hippolytidae)

SUMMARY

A new hippolytid shrimp, *Hippolyte sapphica* sp. nov. from the Lesbos island, Northeastern Aegean Sea, is described here. The new species is compared with *Hippolyte inermis* LEACH and *H. longirostris* (CZERNIAVSKY) with which it has often been confused so far.

Keywords : Hippolyte sapphica, shrimp, Crustacea, Decapoda, Caridea, Hippolitidae, Greece, Mediterranean.

INTRODUCTION

Des récoltes littorales de Crustacés Décapodes effectuées dans divers secteurs de l'île de Lesbos, Nord-Est de la Mer Egée, au cours du mois de juillet 1992 ont permis la capture d'une nouvelle espèce du genre *Hippolyte*. Les spécimens ont été capturés au moyen d'une épuisette garnie de mailles de 2.5 mm, tués par congélation puis fixés avec de l'éthanol 75 % contenant 5 % de glycérine. Le matériel type a été réparti entre l'Institut royal des Sciences Naturelles de Belgique, Bruxelles (I.G. :

27 867), le Nationaal Natuurhistorisch Museum, Leiden (RMNH D 42 359), le Muséum National d'Histoire Naturelle, Paris (MNHN — Na 12 117) et la Smithsonian Institution, Washington DC (USNM 256 951).

DESCRIPTION

Hippolyte sapphica sp. nov.

(Figs 1, 5, 7, 8, 9)

Hippolyte gracilis SOIKA, 1948 : p. 100, fig. 1, sauf le second et le septième rostre (= *H. longirostris*). Non *H. gracilis* HELLER, 1862

Hippolyte inermis BACESCU, 1967 : p. 137, fig. 71-73 ; GEISS, 1990 : p. 186, photo en couleur. Non *H. inermis Leach,* 1815

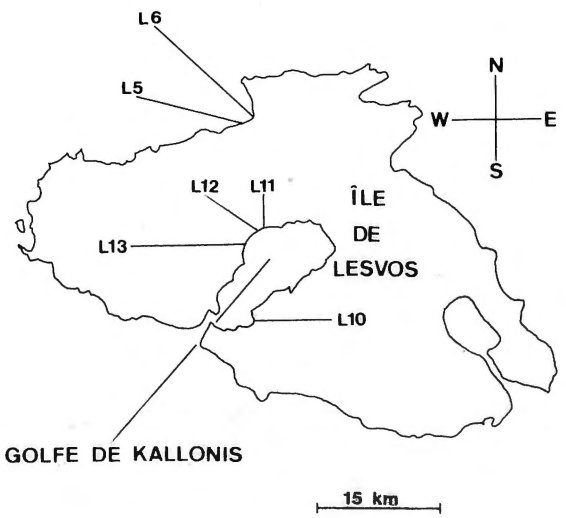
Hippolyte longirostris GELDIAY et KOCATAS, 1968 : p. 25, fig. 14, pl. V fig. 1 ; COTTIGLIA, 1983 : p. 61, fig. 21. Non *H. longirostris* (CZERNIAVSKY, 1868)

Hippolyte prideauxiana ? NIEZABITOWSKI, 1912 : p. 959, pl. XLV (pro parte) ; BACESCU, 1949 : p. 3, fig. 4-6. Non *H. prideauxiana* LEACH, 1817

Matériel examiné : Skala Polihnitou, 400 m au Nord du port, 17/VII/1992, 2 ♀♀ dont 1 ovigère — 20 m à l'Est du port de Skala Kallonis (localité type), 16/VII/1992, 1 ♂ et 73 ♀♀ dont de nombreuses ovigères — 1 km à l'Est du port de Skala Kallonis, 18/VII/1992, 1 ♂ et 35 ♀♀ dont de nombreuses ovigères — à équidistance entre Skala Kallonis et Parakila, 18/VII/1992, 2 ♀♀ dont 1 ovigère. [Tous les spécimens: herbiers de Zostéracées, 0.2-1 m].

Holotype : 1 ♀ ovigère déposée à l'Institut royal des Sciences Naturelles de Belgique.

Étymologie : sapphicus (adjectif latin), relatif à Sapho, célèbre poétesse qui habita l'île de Lesbos au sixième siècle avant Jésus-Christ.



Silhouette élancée. Carapace étroite. Épine hépatique nettement en retrait de l'épine supra-orbitaire. Distance séparant l'extrémité de l'épine hépatique de la marge antérieure de la carapace presque toujours aussi longue ou plus longue que la longueur de l'épine. Zone ptérygostomienne arrondie ou sub-anguleuse mais sans épine. Rostre terminé par une pointe unique, moyennement étroit, rectiligne, dirigé dans le prolongement de la carapace ou légèrement vers le bas, aussi long ou légèrement plus long que la carapace, dépassant largement le pédoncule antennulaire, atteignant presque ou dépassant très légèrement l'extrémité des scaphocérites. Formules rostrales (dents rostrales dorsales + dents post-rostrales / dents rostrales ventrales): 2+2/3 (2 ex.); 2+1/4 (4 ex.); 2+1/3 (54 ex.); 2+1/2 (50 ex.); 2+1/1 (1 ex.); 1+1/3 (1 ex.); 1+1/2 (1 ex.). Dents dorsales ne dépassant pas le quart proximal du rostre. Quand il y a 2 dents dorsales rostrales, la distance séparant celles-ci est toujours plus faible que la distance séparant la première dent dorsale rostrale de la dent post-rostrale. Dents ventrales régulièrement réparties sur la moitié distale du rostre.

Pléonite 3 à profil anguleux, d'autant plus marqué que le spécimen est grand. Pleurons des pléonites 1-5 arrondis et sans épines. Sur le telson, épines dorsales de la seconde paire à peu près à équidistance de la première paire et du bord postérieur du telson. Telson terminé par 6 ou parfois 8 épines; les 2 épines externes de longueur voisine de la moitié des 2 épines intermédiaires.

Cornée modérément dilatée, un peu plus large que la tige du pédoncule oculaire, environ 2 fois moins longue que celle-ci, dépassant légèrement l'extrémité des stylocérites.

Article basal du pédoncule antennulaire présentant une épine du côté interne (seulement visible en vue ventrale chez les crevettes entières) qui atteint presque l'extrémité du stylocérite (chez le spécimen disséqué). Stylocérites longs et très étroits, atteignant le niveau des 8/10 ou des 9/10 du premier article du pédoncule antennulaire et n'atteignant pas l'extrémité du pédoncule antennaire. Sur les crevettes entières, les stylocérites apparaissent habituellement comme nettement écartés de l'article basal antennulaire sur toute leur longueur (fig. 1b); lorsqu'on monte l'antennule entre lame et lamelle (fig. 1c), des déformations se produisent et les stylocérites peuvent apparaître comme contigus avec l'article basal antennulaire sur les 2/3 de leur longueur. Fouet robuste de l'antennule nettement plus court que le fouet gracile.

Article basal antennaire avec une épine distale externe. Scaphocérite environ 4 fois plus long que large, avec des marges latérales rectilignes et parallèles sur la plus grande partie de sa longueur, terminé par un lobe arrondi et séparé de l'épine externe par une échancrure bien distincte.

Pièces buccales comme figurées. Mandibule dépourvu de palpe, doté d'un processus molaire et d'un processus incisif avec 7 dents à sommet obtus à droite et 8 à gauche (chez le spécimen disséqué). Mxp3 atteignant l'extrémité du premier article du pédoncule antennulaire et dépassant légèrement le pédoncule antennaire; 10 épines mobiles sur la moitié distale de sa marge interne (chez le spécimen disséqué).

Pince de P1 portant 30 minuscules dents obtuses sur la marge tranchante du doigt mobile et 21 sur celle du doigt fixe (chez le spécimen disséqué). Premier article du carpe de P2 plus long que le troisième ; troisième article plus long que le second. Le premier article 4-6 fois plus long que large, le second 2 fois plus long que large et le troisième 2.5-3 fois. Mérus des P3-5 avec une seule épine, sub-distale, en position latéro-externe (manquant rarement sur P4-5). Carpe des P3-5 avec une épine sub-proximale latéro-externe (manquant parfois sur P4-5). Marge ventrale du propode des P3-5 avec 6-7 (parfois 4) paires d'épines accompagnées de quelques soies (dans le groupement proximal, les soies peuvent être seules présentes). Chez la femelle disséquée, la marge ventrale des dactyles est garnie de 14 épines mobiles sur les P3, 13 sur le P4D, 14 sur le P4G, 13 sur les P5. Chez les mâles, le propode des P3-5 est distalement un peu dilaté et le dactyle de ces mêmes péreiopodes peut se rabattre contre le propode. Le dactyle du P3 droit du mâle allotype ne porte que 11 épines. Étendu vers l'avant, le P3 atteint un niveau légèrement inférieur à celui de la moitié du scaphocérite.

Endopodite du pléopode 1 du mâle comme figuré. Appendix masculina un peu plus long que l'appendix interna et atteignant le niveau des 6/10 de l'endopodite. Appendix masculina terminé par des soies robustes, non plumeuses. Ces soies mesurent les 7/10 de la longueur externe de l'appendix masculina.

Six œufs embryonnés mesurés présentaient les dimensions suivantes: 0.72 × 0.50 mm ; 0.70 × 0.47 mm ; 0.67 × 0.52 mm ; 0.67 × 0.48 mm ; 0.67 × 0.48 mm ; 0.64 × 0.55 mm.

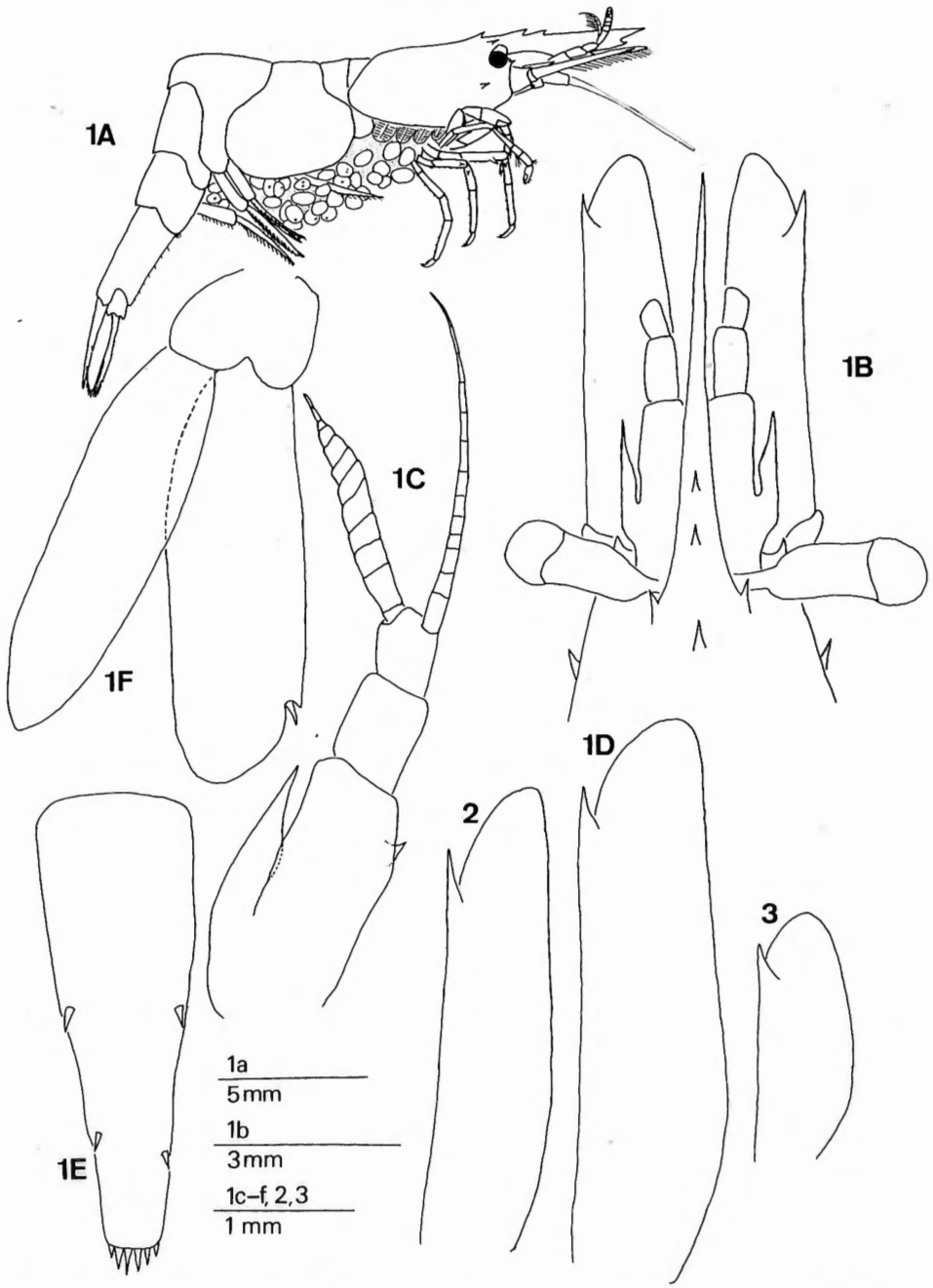
Coloration : Vert intense uni ou avec des taches ou des bandes longitudinales beige-grisâtres ou avec une fine ligne noire longitudinale sur chaque surface latérale du corps (holotype).

Longueur maximale : ♂ : carapace sans le rostre = 2 mm ; crevette entière = 12 mm. ♀ : carapace sans le rostre = 5 mm ; crevette entière = 23 mm.

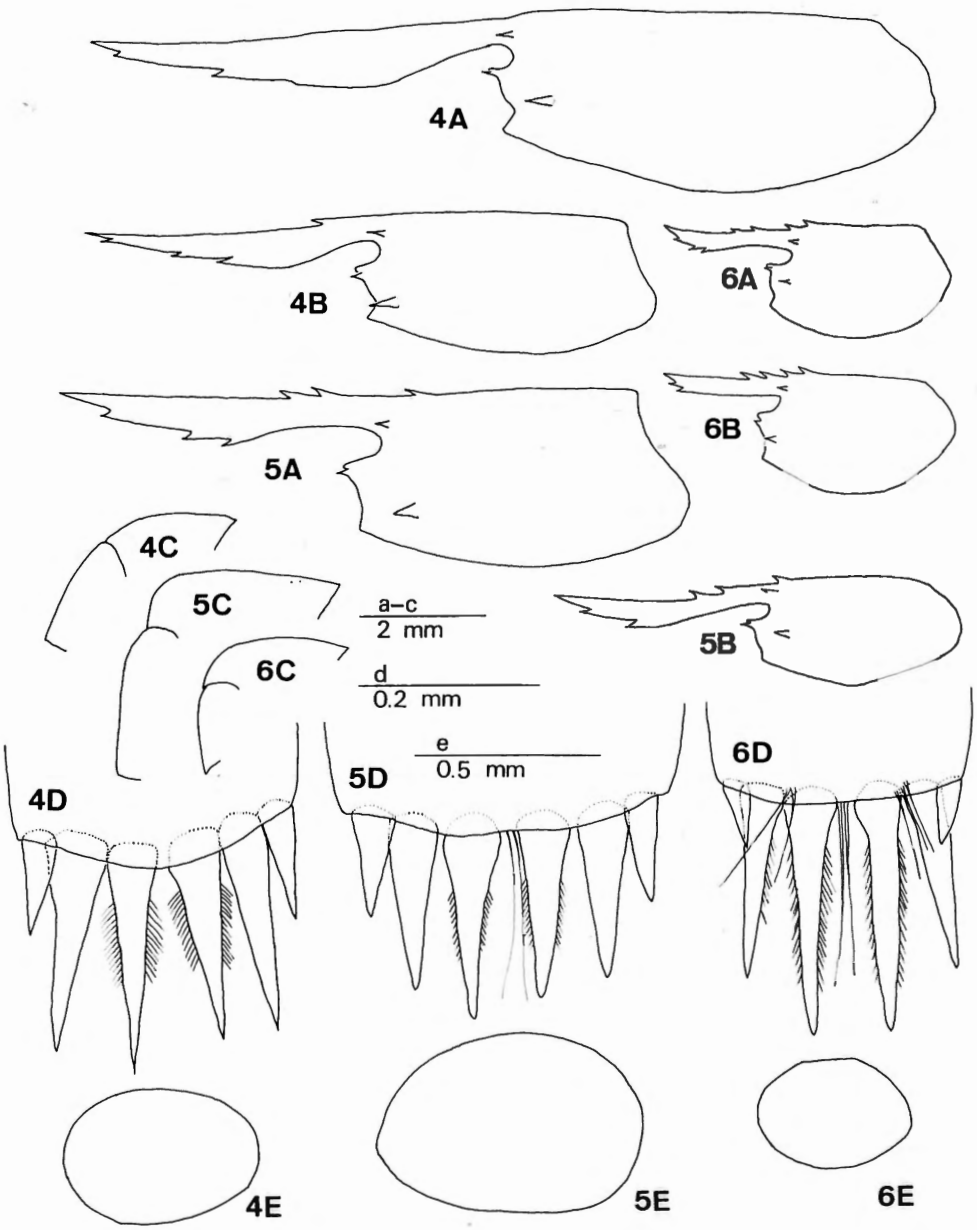
DISCUSSION

Comparaison avec *H. inermis* et *H. longirostris*

Hippolyte sapphica sp. nov. est facile à distinguer des autres représentants méditerranéens du genre actuellement connus: *H. holthuisi* ZARIQUIEY ALVAREZ, 1953, *H. hunti* (GOSSE, 1877), *H. inermis* LEACH, 1815, *H. leptocerus* (HELLER, 1863), *H. leptometrae* LEDOYER, 1969 et *H. longirostris* (CZERNIAVSKY, 1868). Cependant, comme elle a été confondue à plusieurs reprises avec *Hippolyte longirostris* (CZERNIAVSKY, 1868) et *Hippolyte inermis* LEACH, 1815, il a semblé nécessaire d'établir un tableau comparatif permettant de distinguer les 3 espèces (tableau 1). Ce tableau a été établi sur base de l'examen de *Hippolyte inermis* et *H. longirostris* de provenances très diverses (Atlantique et bassin méditerranéen) et de *H. sapphica* de Lesbos. Aux données de ce tableau, il convient d'ajouter que certaines figures de BACESCU (1949 et 1967) suggèrent que, chez les *H. sapphica* pontiques, l'épine post-



Figs 1-3. 1. *Hippolyte sapphica* sp. nov. : holotype ♀, crevette entière (1A) ; paratypes ♀♀, (1B-F) ; vue antérieure dorsale (1B) ; antennule gauche (1C) ; scaphocérite gauche (1D) ; telson (1E) ; uropode droit (1F). - 2. *Hippolyte inermis* ♀, Anaxos (île de Lesbos) : scaphocérite gauche. - 3. *Hippolyte longirostris* ♀, Skala Kallonis (île de Lesbos) : scaphocérite gauche.



Figs 4-6. — 4. *Hippolyte inermis* ♀♀, Anaxos (île de Lesbos). - 5. *Hippolyte sapphica* sp. nov. ♀♀ paratype. - 6. *Hippolyte longirostris* ♀♀, baie de Soude (Crète) (A) ; Skala Kallonis (île de Lesbos) (B-E). - (A, B : carapace. - C : jonction dorsale des pléonites 3 et 4. - D : extrémité du telson. - E : œuf embryonné).

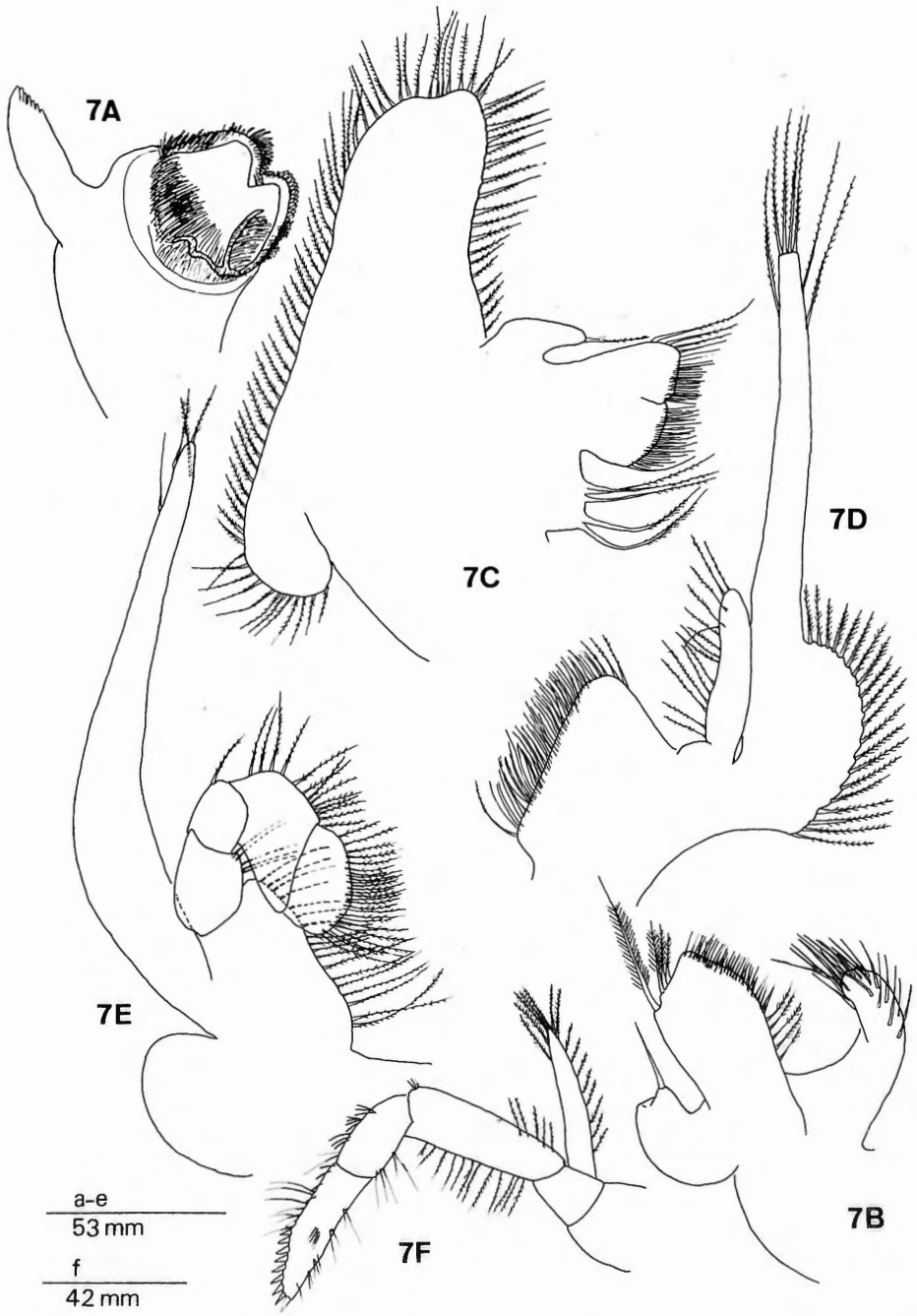
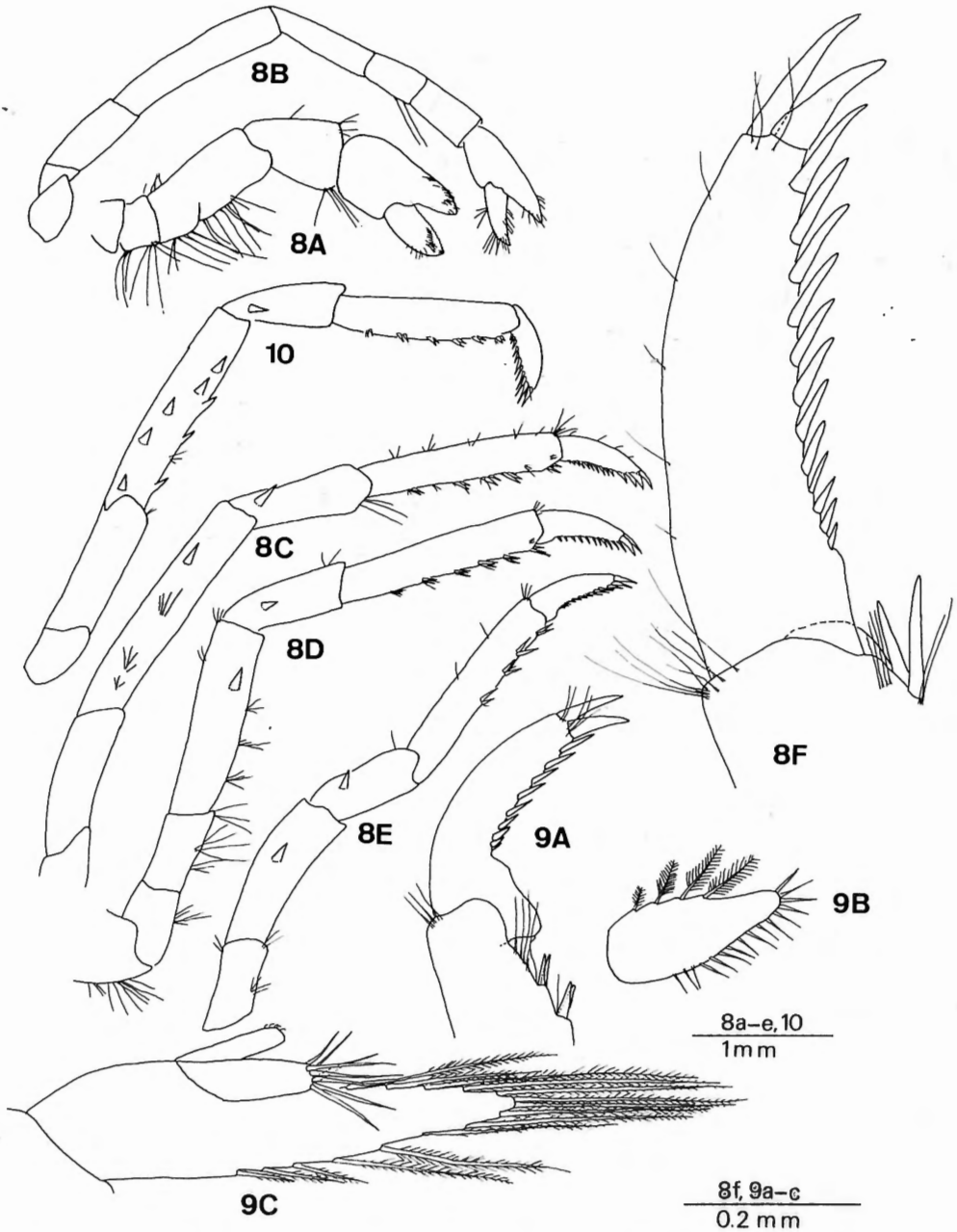


Fig. 7. — *Hippolyte sapphica* sp. nov. ♀ paratype. — A. Md droit. - B. Mx1 gauche. - C. Mx2 droite. - D. Mxp1 droit. - E. Mxp2 droit. - F. Mxp3 gauche.



Figs 8-10. — 8. *Hippolyte sapphica* sp. nov. ♀ paratype : P1D (8A) ; P2D (8B) ; P3D (8C) ; P4D (8D) ; P5D (8E) ; dactyle du P3D (8F). - 9. *Hippolyte sapphica* sp. nov. ♂ allotype : extrémité du P3D (9A) ; endopodite du pléopode 1 droit (9B) ; endopodite du pléopode 2 droit (9C). - 10. *Hippolyte inermis* ♀, Anaxos (île de Lesbos) : P3D.

rostrale puisse parfois manquer. Le tableau 1 indique que *H. sapphica* présente 5 caractères communs ou proches (caractères n° 4-5 et 14-16) avec *H. longirostris* et 11 avec *H. inermis* (caractères n° 1-3, 8-13 et 17-18), ce qui suggère une parenté plus étroite avec cette dernière. La possession chez *H. sapphica* et *H. inermis* de 7-9 dents au processus incisif mandibulaire, inhabituelle dans le genre *Hippolyte,* renforce cette supposition, de même que l'identité parfaite de leurs livrées chromatiques (jusque dans la forme des taches et des lignes longitudinales).

Biologie

Sur l'île de Lesbos, *H. sapphica* est présente et localement extrêmement abondante dans les herbiers de Zostéracées (*Cymodocea* ou *Zostera*) du golfe presque fermé de Kallonis où règnent des conditions semi-lagunaires. Par contre, elle est absente des herbiers de posidonies de la côte Nord-Est de Lesbos, où elle est remplacée par *H. inermis* (un seul spécimen de *H. inermis* fut trouvé dans le golfe de Kallonis, à Skala Polihnitou, station proche de son embouchure). Il semble donc que les 2 espèces aient tendance à s'exclure. Par contre, *H. longirostris* s'observe indifféremment dans le golfe et à l'extérieur de celui-ci. La baie d'Izmir, située à un peu moins de 100 km au Sud de Lesbos, où *H. sapphica* est aussi présente (GELDIAY et KOCATAS, 1968, comme *H. longirostris*) est également une baie très fermée où doivent régner des conditions analogues. La lagune de Venise et la Mer Noire où l'espèce existe aussi (SOIKA, 1948, comme *H. gracilis*; BACESCU, 1967, comme *H. inermis*) possèdent des eaux franchement dessalées. La petite taille des *H. sapphica* mâles s'expliquerait par le fait qu'ils changeraient très rapidement de sexe. Les très gros œufs de cette espèce suggèrent un développement larvaire raccourci, ce qui constitue un avantage en eau dessalée ou de salinité variable.

Distribution

Bien que des incertitudes subsistent, il semble que *H. sapphica* présente une aire de distribution limitée et sans doute morcelée. En Mer Egée, elle existe à Lesbos (données présentes) et dans le golfe d'Izmir (GELDIAY et KOCATAS, 1968) mais nous ne l'avons trouvée ni à Naxos, ni dans le Sud-Est du Péloponnèse ni en Crète, lors de récoltes littorales intensives menées en 1985-1987. Pourtant, les stations prospectées comprenaient deux baies très fermées à herbier dense de Zostéracées où régnaient des conditions assez comparables à celles du golfe de Kallonis: baie de Limani Geraka dans le Péloponnèse et baie de Souda en Crète. A Limani Geraka, les seules *Hippolyte* observées étaient des *H. longirostris* et, dans la baie de Souda des *H. longirostris* et des *H. inermis*. Il se peut que ces stations soient trop isolées d'autres milieux favorables pour avoir été colonisées par cette espèce ou d'une étendue trop petite pour assurer le maintien d'une population stable. Il est par contre possible que *H. sapphica* soit présente dans diverses enclaves semi-lagunaires de la moitié Nord de la Mer Egée, celles-ci devant être nombreuses d'après les cartes. En Adriatique, *H. sapphica* n'est connue avec certitude que de la lagune de Venise (SOIKA, 1948) mais sa distribution y est vraisemblablement plus étendue. En Mer Noire, elle est présente sur les côtes bulgares, très rare sur les côtes roumaines

TABLEAU 1

Caractères distinctifs de *Hippolyte inermis, H. sapphica* et *H. longirostris.*

	Caractères	*H. inermis* LEACH, 1815 (fig. 2,4,10)	*H. sapphica* sp. nov. (fig. 1,5,7,8,9)	*H. longirostris* (CZERNIAVSKY, 1868) (fig. 3,6)
1.	silhouette	très gracile	très gracile	très robuste
2.	longueur totale	habituellement > 15 mm	habituellement > 15 mm	habituellement < 15 mm
3.	rostre	aussi long ou plus long que la carapace	aussi long ou plus long que la carapace	habituellement plus court que la carapace
4.	dents post-rostrales	0	1 (rarement 2)	1-2 (souvent 0 chez les mâles pontiques)
5.	dents de la marge dorsale du rostre	0-1	2 (rarement 1)	1-3 (parfois 0 chez les mâles pontiques)
6.	dents dorsales (rostrales + post-rostrales)	0-1	3 (rarement 2 ou 4)	2-4 (souvent 1 et parfois 0 chez les mâles pontiques)
7.	extrémité de l'épine hépatique	un peu en retrait ou dépassant parfois légèrement la marge antérieure de la carapace	fortement en retrait de la marge antérieure de la carapace	dépassant légèrement ou un peu en retrait de la marge antérieure de la carapace
8.	stylocérites	atteignant les 8/10 ou les 9/10 du premier article de l'antennule	atteignant les 8/10 ou les 9/10 du premier article de l'antennule	atteignant les 6/10 ou les 8/10 du premier article de l'antennule
9.	fouet robuste des antennules	plus court que le fouet gracile	plus court que le fouet gracile	un peu plus long que le fouet gracile
10.	scaphocérites	environ 4.3 fois plus longs que larges	environ 3.8 fois plus longs que larges	environ 2.7 fois plus longs que larges
11.	processus incisif des mandibules	avec 7-9 dents (1 spécimen examiné)	avec 7-8 dents (1 spécimen examiné)	avec 4 dents (1 spécimen examiné)
12.	premier article du carpe de P2	4-6 fois plus long que large	4-6 fois plus long que large	2-3 fois plus long que large
13.	étendu vers l'avant, le P3 atteint	un niveau légèrement inférieur à la 1/2 des scaphocérites	un niveau légèrement inférieur à la 1/2 des scaphocérites	le niveau des 4/5 des scaphocérites
14.	mérus de P3	avec 6-9 épines latérales externes et ventrales	avec 1 épine latérale externe sub-distale	avec 1 (rarement 2) épines latérales externes sub-distales
15.	mérus de P4	avec 4-6 épines latérales externes et ventrales	avec 1 épine latérale externe sub-distale (parfois absente)	avec 1 épine latérale externe sub-distale (parfois absente)
16.	profil dorsal du pléonite 3	faiblement convexe	anguleux (surtout chez les grands spécimens)	fortement convexe, parfois sub-anguleux
17.	épines externes du telson	mesurant environ la 1/2 de la longueur des épines intermédiaires	mesurant environ la 1/2 de la longueur des épines intermédiaires	mesurant normalement moins que le 1/3 des épines intermédiaires
18.	diamètre moyen des œufs embryonnés montés au polylactophénol	0.50 x 0.39 mm (6 œufs mesurés)	0.68 x 0.50 mm (6 œufs mesurés)	0.43 x 0.30 mm (8 œufs mesurés)

(BACESCU, 1967) et pourrait être absente des côtes russes et ukrainiennes. En effet, aucune des nombreuses *Hippolyte* du Nord de la Mer Noire illustrées par CZERNIAVSKY (1868 et 1884) ne sont référables à cette espèce. Il se peut que *H. sapphica* aie une distribution plus large mais rien ne le laisse présager.

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ANATOMY AND FINE STRUCTURE OF THE METAPLEURAL GLAND IN *ATTA* (Hymenoptera, Formicidae)

by

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SUMMARY

Representatives of the ant genus *Atta* Fabricius possess metapleural glands that are characterized by a complex morphological organization. In contrast to most other glands in these ants a muscular supply nearby the glandular orifice is lacking. An hypothesis is postulated concerning the implication of the morphology of the glandular opening for the release of the secretion, probably facilitated by the existence of a cuticular ridge between the glandular opening and the hindlegs. In three species (*Atta bisphaerica, Atta laevigata* and *Atta sexdens*) the ultrastructure of the gland was studied. The elongated secretory cells possess a fairly developed granular endoplasmic reticulum. Very often we observed the presence of inclusions of lysosomal origin.

Key-words : Morphology, ultrastructure, metapleural gland, *Atta.*

INTRODUCTION

The paired metapleural glands are complex structures located at the posterolateral edge of the metathorax, each consisting of a cluster of glandular cells, with each cell draining through a duct into a common chitinous collecting sac. The collecting sac leads into the storage chamber or reservoir, which is a simple sclerotized cavity. Because of the presence of a narrow ridge inside the reservoir and the lack of a muscular supply, transport of the secretion to the outside by capillarity seems the most probable mechanism. Although we previously assumed that it would be difficult to obtain an overview of the complete glandular morphology, our trials demonstrated that accurate dissections, followed by SEM, can give satisfactory results. MASCHWITZ *et al.* (1970) were able to isolate a strongly acid secretion out of the metapleural gland reservoirs of some myrmicine workers, including *Atta sexdens* (L.). According to these authors, the secretion smells like honey and would be an effective antibiotic against *Escherichia coli, Staphylococcus*

aureus and *Penicillium glaucum*. The main compound displaying an antibiotic activity is phenyl acetic acid. A more detailed analysis (MASCHWITZ *et al.*, 1970 ; MASCHWITZ, 1974) of the *Atta* metapleural gland secretion showed that beta-indolyl acetic acid and beta-hydroxy-hexanoic, — octanoic and — decanoic acid also occur in the secretion.

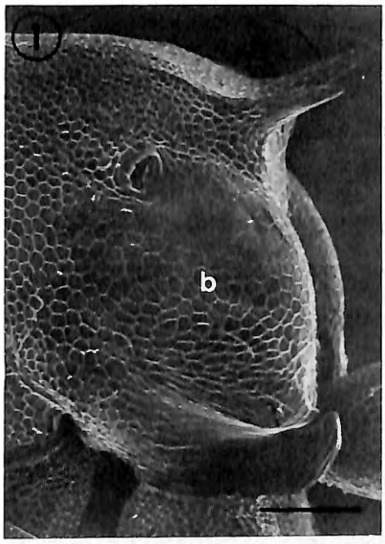
The morphology and ultrastructure of the metapleural gland in *Atta* are the subject of the current study. Until now, morphological studies on the metapleural gland are limited to those of HÖLLDOBLER and ENGEL-SIEGEL (1984), covering many ant species belonging to several subfamilies, and FANFANI and DAZZINI VALCURONE (1991), dealing with some dolichoderine species. Ultrastructural details have been reported by TULLOCH *et al.* (1962), Billen and VAN BOVEN (1987) for Old World army ants, and by SCHOETERS and BILLEN (1992) for *Diacamma* MAYR.

MATERIAL AND METHODS

The ants examined were collected from their nest in Viçosa, MG, Brasil (*Atta bisphaerica* FOREL soldiers, *Atta laevigata* (FR. SMITH) queen and soldiers), and from two laboratory colonies. An incipient colony of *Atta sexdens sexdens* (L.) was collected after the nuptial flight in December 1973 in Cayenne, French Guyana. A colony of *Atta sexdens rubropilosa* (FOREL) was likewise collected in Viçosa, MG, Brasil (nuptial flight in November 1987). Tissues were fixed in 2 % glutaraldehyde, buffered at pH 7.3 with 0.05M Na-cacodylate and 0.15M saccharose. After postfixation in 2 % osmium tetroxide, samples were dehydrated in acetone and embedded in Araldite. Thin sections for transmission EM were double stained with a LKB 2168 Ultrostainer, and examined with a Zeiss EM 900 electron microscope. Material for scanning EM was coated with gold and viewed with a Philips SEM 515 microscope.

Double-fixed glands for scanning EM of their internal anatomy were examined after critical point drying. Removal of tissue to see cuticular parts of the gland happened in a 5 % potassium hydroxide solution.

Figs. 1-6. — 1. Scanning electron micrograph of the bulla of the metapleural gland in a *A. sexdens sexdens* minor worker. b = bulla. (Scale 100 µm). - 2. Survey of the metapleural gland in a *A. bisphaerica* soldier, showing the internal anatomy. mf = muscle fibres, r = reservoir, SC = secretory cells. (Scale 200 µm). - 3. KOH-treated collecting chamber in a *A. laevigata* queen. Note the irregular surface. r = reservoir. (Scale 200 µm). - 4. Longitudinal semi-thin section through a *A. sexdens sexdens* metathorax (minima worker). The arrow points to the specialized cuticular ridge. (Scale 100 µm). - 5. Secretory cell clusters in a *A. sexdens rubropilosa* worker. (Scale 50 µm). - 6. Morphological adaptation towards an optimization of secretion transport once it is out of the reservoir (arrow) (*A. sexdens rubropilosa* minor worker). (Scale 50 µm).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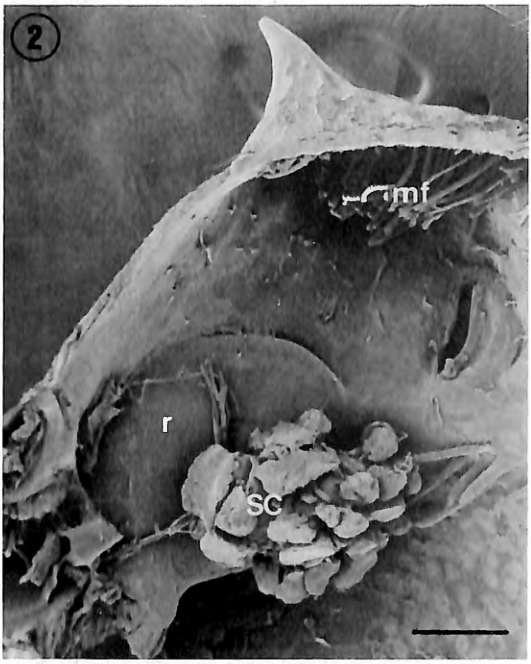
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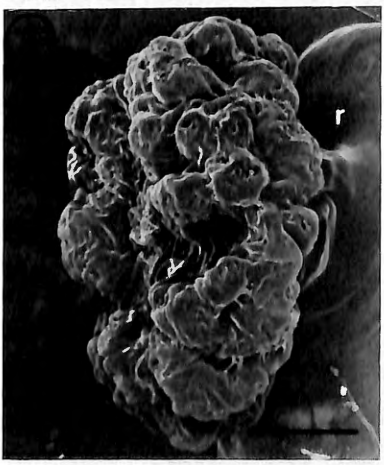
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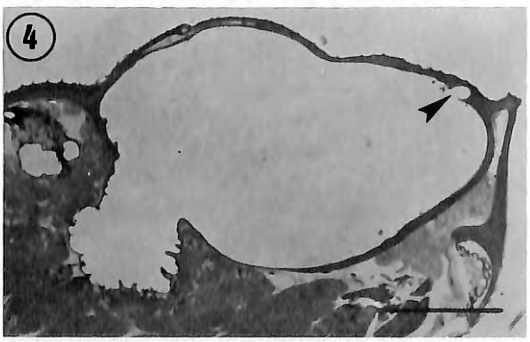
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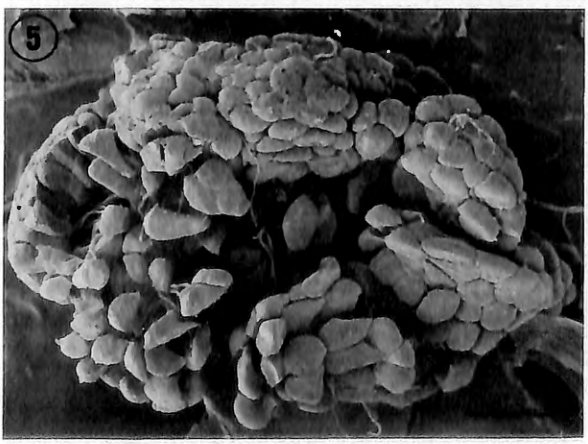
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6

RESULTS

In the case of *Atta,* the metapleural gland can externally be recognized by a bulla, and a curved slit-shaped opening to the outside (Fig. 1 and 6). Observations under a dissecting microscope make it possible to determine whether the reservoir is empty or not. The collecting chamber and the reservoir are clearly distinguishable, as can be seen on scanning micrographs of the sclerotized internal parts (Fig. 2). Scanning microscopy of the collecting chamber shows that its surface is very irregular (Fig. 3).

Scanning microscopy of the internal appearance of the metapleural gland in different *Atta* species invariably shows a large bean-shaped reservoir and several secretory cell clusters surrounding the collecting chamber (Fig. 2 and 5). Similar observations were done under the light-microscope (Fig. 4). The secretory cells are usually grouped into relatively large clusters of about 20 cells. This clustered organization can easily be recognized on scanning micrographs (Fig. 2 and 5). The well-defined clustered organization (sometimes up to 18 clusters) of the secretory cells surrounding this collecting chamber (Fig. 4 and 5) is different from the one found in other ant species. In other species we can readily distinguish fewer cell clusters, which are usually situated more closely to each other.

The externally visible part of the metapleural gland is a narrow, horizontally located slit-like opening near to the hindleg coxae (Fig. 7) on both sides. At its posterior end, each slit widens into a narrow semicircular slit. A non-sculptured chitinous area of the exoskeleton surrounds the opening of the gland (Fig. 6), which is even more conspicuous when comparing this smooth appearance with the characteristic reticulate body sculpture elsewhere on the body of the ant. The typical hexagonal pattern found there is lacking near the opening (Fig. 6).

The secretory cells

The number of remarkably white coloured glandular cells seems to vary considerably when comparing workers of different size. Giving precise cell numbers for all worker sizes is difficult, but it seems that about 400 cells occur in medium workers, soldiers and queens. Minor and minima workers possess fewer secretory cells, but in these castes the highest relative development of the gland was recorded (when referring to size of thorax). The secretory cells possess a large distal portion and a rather slender proximal part, the latter in direct connection with a duct cell. Each secretory cell is consistently equipped with a more straight zone in the proximal part of the cell, which becomes more curved in the distal part, corresponding with the so-called end apparatus. In semi-thin sections this end apparatus appears darker than the cytoplasm and is surrounded by a narrow microvillar lining which is usually only faintly stained (Fig. 4).

The rounded secretory cell nucleus is usually located in the broader distal part of the cell. It is characterized by several dark chromatin condensations. Several vesicle types were observed in the cytoplasm (Fig. 8). Also inclusions were found

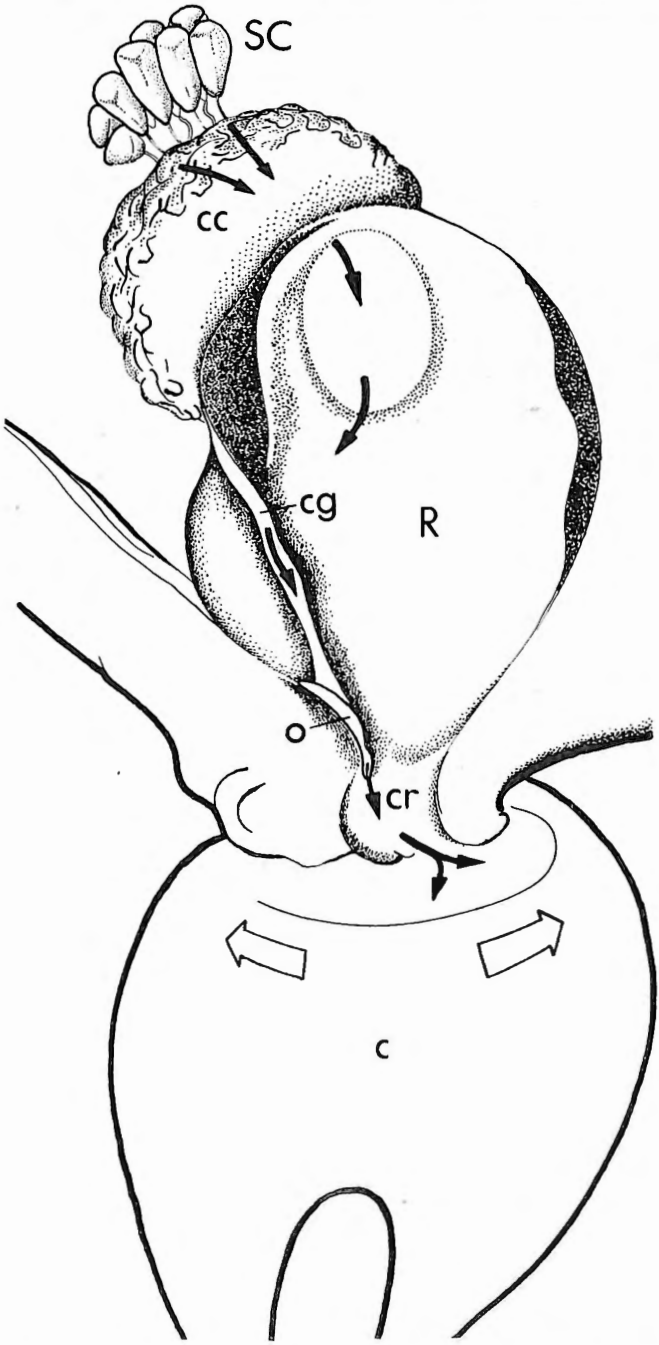
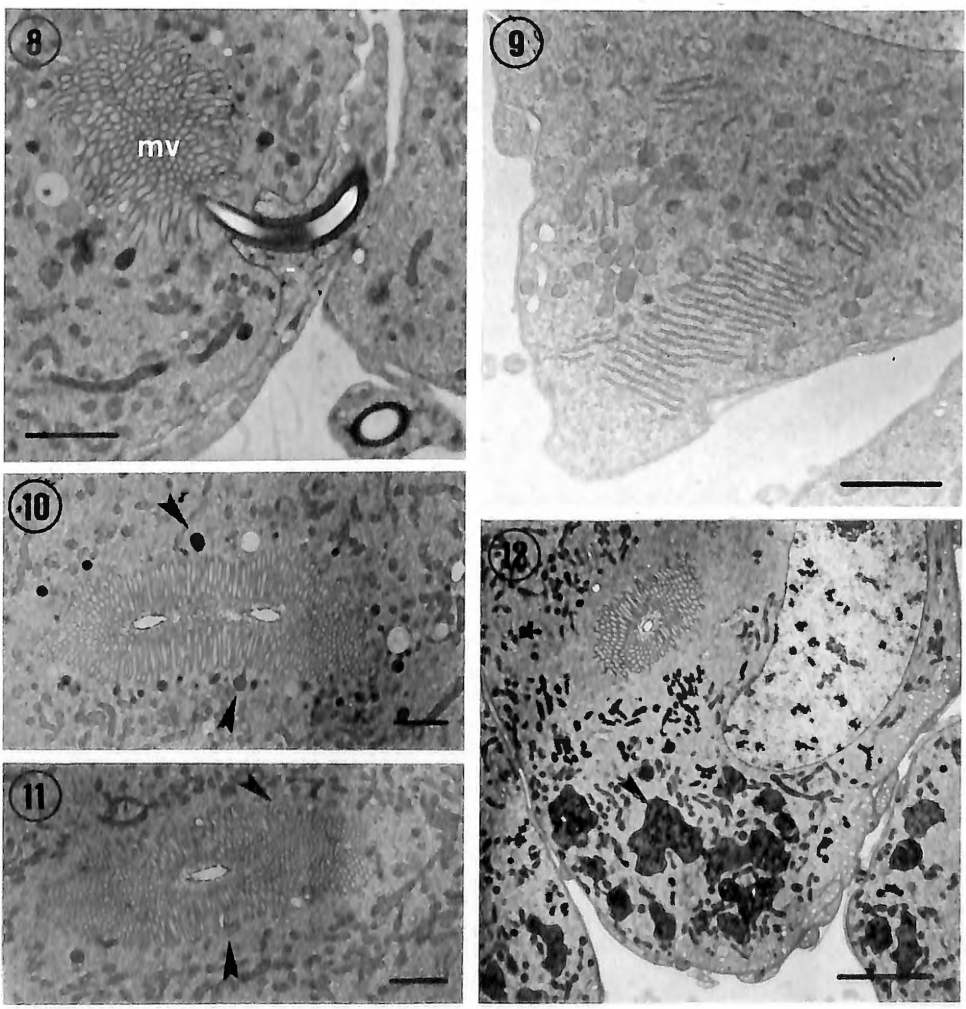


Fig. 7. — Reservoir morphology and presumed pathway the secretion follows (black arrows) before release into the outside world. White arrows indicate spreading of secretion through coxal movements. c = coxa of the hindleg, cc = collecting chamber, cg = cuticular gutter inside the reservoir, cr = cuticular ridge nearby hindleg coxa, o = glandular orifice, R = reservoir, SC = secretory cell.



Figs. 8-12. — Junction of the collecting canal and the conducting canal in a *A. sexdens rubropilosa* worker. mv = microvilli. Scale bar 2 µm. - RER in the vicinity of the nucleus of a secretory cell (*A. sexdens rubropilosa* worker). Scale bar 2 µm. - Electron-dense inclusions (arrows) surrounding the microvillar region of the collecting canal (*A. sexdens rubropilosa* worker). Scale bar 2 µm. - Lamellar inclusions (arrows) in the vicinity of the end-apparatus (*Atta sexdens rubropilosa* worker). Scale bar 2 µm. - Large inclusions of lysosomal origin (arrow), probably corresponding with autophagosomes, observed in a *A. laevigata* soldier. Scale bar 5 µm.

consisting of two separate subunits, apparently fusing. One of both subunits contains parallel lamellar structures (length 0.5 µm), resembling membranes. Microvilli with a length of about 1.7 µm form the lining of the end apparatus (Fig. 10 and 11). These microvilli project into an extracellular space, the width of which is rather small in the gland of a recently emerged worker (0.2 to 0.5 µm broad). In the vicinity of the microvilli electron-dense (diameter about 0.6 µm) and electron-lucent vesicles (diameter from 0.5 to 0.8 µm) were observed.

The amount of RER (Fig. 9) and vesicles, when considered over the whole cytoplasm, varies considerably. Most of the electron-dense vesicles were found surrounding the end apparatus. In the young workers, the RER stacks showed a broad lumen. The Golgi-apparatus was not very common. If present, the dictyosomes had a length of 0.3 to 0.9 µm. In general mitochondria are about 1.5 µm long.

The stacks of RER show a typical parallel organization (Fig. 9) and were found on various locations in the cell. These stacks sometimes show an orientation parallel to the nuclear membrane, whereas in other cases it is perpendicular to it. We also observed inclusions of lysosomal origin, probably corresponding to autophagosomes filled with remnants of membranes and undigested material. Such inclusions, which in the case of *Atta laevigata* do not seem to be spherical, were fairly abundant (Fig. 12). Their contents are clearly electron-dense. This is also the case for the numerous mitochondria.

DISCUSSION

Our observations concerning the variation of cell numbers and the gland development in relation to the size of the ant correspond with the results of WILSON (1980). The occurrence of a large number of secretory cells in each metapleural gland of *Atta* makes it an important source of both volatile and non-volatile chemicals (own observations), in all the (sub)species investigated. The cellular organization of the metapleural gland is consistent with the bicellular secretory unit, with a secretory cell and a duct cell (NOIROT and QUENNEDEY, 1974; BILLEN, 1987; BILLEN, 1991). The microvillar lining in the end apparatus showed little variation in its appearance, and certainly between secretory cells within the same individual. The presumable absence of enlarged extracellular spaces and the corresponding lack of creation of additional volume for the storage of secretion, could be explained by the presence of a large collecting chamber and an even larger atrium. The production of secretion is not likely to happen under neuroendocrine control, since no nerve fibres were seen in association with the glandular cell clusters.

The obvious occurrence of inclusions of lysosomal origin, such as autophagosomes, could lead to the assumption that the cells in which they were found are degenerating. As a consequence, these cells could be in an advanced stage of their secretory cycle (BAZIRE-BÉNAZET and ZYLBERBERG, 1979). Our observations on the presence of end-apparatuses without signs of microvillar distortion in cells with plenty of the inclusions mentioned above, however, probably indicate that signs of

cellular degeneration and end-apparatuses with dilated extracellular spaces do not necessarily have to occur together.

The presence of the glandular orifice so close to the hindleg coxa suggests, in combination with movements of the latter, a role in making the secretion accessible for spreading over the body. The pathway the secretion follows is obviously compartmentalized. A first barrier the secretory molecules have to pass is the end apparatus. After passage through the duct the secretion is accumulated in the collecting chamber that is morphologically separated from the glandular reservoir. Although this has not been elucidated yet, there must be a well defined reason for the compartmentalization of the glandular reservoir. Compartmentalization of structures involved in secretion is also found in the venom gland (with its convoluted gland part). After temporary storage in the collecting chamber, the secretion passes through a more or less circular opening in order to get into the reservoir and reach the glandular orifice to the outside. The reservoir wall is provided with a narrow channel, coming from the reservoir region close to the collecting chamber and going to the slitlike opening. This well defined morphological adaptation strongly suggests a secretion transport towards the gland opening, possibly by capillarity. Once the secretion is out of the reservoir, its spreading could be enhanced by coxal movements. Our observations concerning the appearance of the glandular bulla correspond to those reported by HÖLLDOBLER and ENGEL-SIEGEL (1984).

In the secretory cells of a callow *A. sexdens rubropilosa* (FOREL) worker we observed the presence of a fairly well developed RER. Until now, the few ultrastructural observations on this gland in other species revealed the developed smooth endoplasmic reticulum, probably in agreement with the production of more or less volatile substances. This leads us to the assumption that proteinaceous substances could be present in the secretion. An observation we made outside the nest (under a binocular microscope) is that the secretion quickly loses its volatile constituents and that a sticky residual mass is left on the substrate (for weeks, or even months). So, the most remarkable aspects recorded in our investigation are the presence of a well developed RER and the sticky residu found in the glandular reservoirs. These two aspects do not seem to be contradictory.

This investigation forms part of a comparative research on the metapleural gland in ants. When we compare our morphological data with those obtained for the metapleural gland in other representatives of the Formicidae, we are able to emphasize the following characteristics of this gland in *Atta*. The gland is very well developed in all workers, ranging from minima to soldier, as well as in females. The glandular opening is located relatively close to the hindleg coxae, whereas more primitive species (belonging to Nothomyrmeciinae, Myrmeciinae and Ponerinae) usually display glandular openings that are located further away from the coxae and lack the adaptation for further transport of secretion towards the coxae. Cuticular hairs associated with the gland opening are totally absent, whereas in some ant species these are obviously present outside (*Cephalotes* LATREILLE, *Lasius* FABRICIUS) or inside the reservoir (*Amblyopone* Erichson). The cuticular collecting chamber of the metapleural gland in *Atta* reaches one of the highest degrees of development in ants. In some Ponerinae and Formicinae the elaboration of the

collecting chamber seems to be less important. Apparently the presence, size, and activity of the metapleural glands in *Atta* are to be considered as important characters contributing to the dominance of these ants in the neotropical region.

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POINTS OF VIEW

DIFFERENTIATION : « KEEP THE GENOME CONSTANT BUT CHANGE OVER AND OVER AGAIN ITS IONIC AND/OR MACROMOLECULAR ENVIRONMENT » ? A CONCEPTUAL SYNTHESIS

by

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SUMMARY

The unifying concept that differentiation in animals is the stepwise formation of cells or cell clusters which differ primarily in their plasma membrane-cytoskeletal properties and which mostly become organised into a variety of epithelia, lining different compartments, is formulated. As a consequence of these differences in plasma membrane-cytoskeletal complex properties — this causal relationship being the very point — the distinct emerging cell types will be able to display a differential pattern of protein synthesis, cellular morphology and physiology, notwithstanding the fact that they have identical genomes and similar basic mechanisms of protein synthesis and processing. Differences in the plasma membrane-cytoskeletal complex, of which the structure furnishes the cells with a differential three-dimensional molecular scaffold, are usually achieved first and are of necessity followed by differential protein synthesis and pattern formation. This can be also succinctly stated as : form precedes function. In addition to this widely used principle, secondary mechanisms for controlling the expression of specific genes in specific cell types may operate. The major « strategy » used in differentiation of somatic cells seems to be to keep the genome constant (GURDON's experiments, 1962) but to change its « environment » over and over again. This environment comprises two sets of constituents, ionic and macromolecular ones, acting in complementary ways. The first one may be more appropriate for the coarse tuning of gene expression/protein synthesis and the second (especially the trans-acting factors) for the fine tuning. Consideration of animal development in terms of differential epithelium formation may make a major contribution to the unification of developmental biology of animals.

Key words : differentiation, development, gene expression, epigenetics, cytoskeleton, nuclear matrix, plasma membrane, epithelium, pattern formation.

INTRODUCTION

The question as to whether there is a universal principle underlying differentiation is fundamental to the developmental biology of animals. Although intensively sought, no such principle has yet been discovered and there is considerable doubt whether it exists at all. In view of the large number of possible mechanisms already shown to be instrumental to differentiation (GILBERT, 1991 ; GURDON, 1992), such doubt is not unwarranted.

A great number of classical and recent papers on development and control of gene expression-protein synthesis has been screened for mechanisms (*e.g.* differential protein synthesis) which generate functional asymmetry. All those that were not operational in all experimental animal species so far used were eliminated, in the hope of finding one or more universally valid asymmetry generating principles.

DATA FROM THE LITERATURE

We can make the general statement that the different cell types of a differentiated organism all have the same genome although there are a few exceptions such as antibody producing cells, and X-chromosome inactivation in mammals which may be disregarded here. There is no evidence from the literature survey of any mechanism operating directly at the level of the genome, transcription, translation or protein processing, which generates functional cellular asymmetry in all animal species. This is not incompatible with the fact that some of these mechanisms are undoubtly instrumental in differentiation in some species or cell types (GILBERT, 1991). Several sets of data, all relevant to the generation of asymmetry, and all linked in some way to the properties of the plasma membrane-cytoskeleton complex, did emerge :

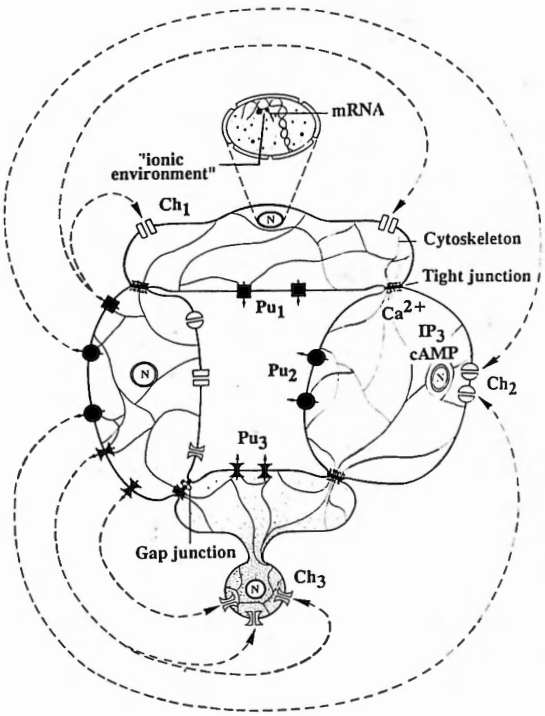
1. In contrast to the situation in unactivated fucoid eggs (QUATRANO, 1990), no description was found of an animal cell that is spherically symmetrical with respect to its membrane-cytoskeletal complex. All animal cell types have a built in asymmetry : *they all differ in their plasma membrane-cytoskeletal complex and they all have the means of segregating membrane proteins* such as receptors and ion transporting proteins, in different domains (*e.g.* by specialisations of the cytoskeleton), thereby facilitating transcellular transport and, where it occurs, self electrophoresis (Fig. 1). Non-random (asymmetrical) distribution of at least some of the membrane-bound proteins present is the rule, random distribution of all proteins present in the membranes (spherical symmetry) — if it exists at all — the exception. This is also true for zygotes.
2. The cytoskeleton, apparently never spherically symmetrical in animal cells, can serve as an anchoring site for the nucleus, for some types of RNA (SINGER, 1992), for some membrane proteins *etc.* (Fig. 1). There are types of zygote in which some maternal mRNAs seem to be associated with specific domains of the cytoskeleton. The nucleus has its own skeleton (nuclear matrix, nuclear scaffold), the form of which is flexible and probably cell- and tissue specific. It acts as an

anchoring site for DNA : genes which are actively being transcribed are associated with it (GETZENBERG *et al.*, 1991). It allows compartmentalisation of several nuclear functions (replication, transcription, RNA processing, RNA transport) for which it seems to be essential (VAN DRIEL *et al.*, 1991 ; BEREZNEY, 1991).

3. *Cleavage patterns sooner or later all yield blastomeres which differ (individually or groupwise) in at least their plasma membrane-cytoskeletal complex* (Fig. 2). This implies a causal relationship with differences in the distribution of some membrane proteins and perhaps also of some maternal mRNA's. It leads to the generalisation that all the different cell types of which differentiated organisms exist must differ in their plasma membrane-cytoskeletal complex. It follows that the « ionic environment » is likely to differ from cell type to cell type.
4. *This complex can be instrumental,* be it mostly indirectly, in a variety of ways *in controlling differential gene expression-protein synthesis* (DE LOOF *et al.*, 1992).
5. The definition of an animal as an organism that develops from a blastula refers to an important but often overlooked property of every animal : in its development it must unavoidably pass through the stage of being a closed simple (monolayer) *epithelium, the blastoderm* (WILEY *et al.*, 1990 ; DE LOOF, 1992). Furthermore, the vast majority of animal cells becomes organised in a variety of epithelia with different characteristics, sometimes highly folded, lining a number of different compartments. *Compartmentalisation by epithelia is an inherent and essential property of animal development and physiology,* just as the intracellular compartmentalisation by membrane-limited organelles is essential to eukaryotic cell physiology (DE LOOF, 1992). Cells cannot engage in epithelium formation without first generating polarity in their plasma membrane/cytoskeletal complexes. This can also be succinctly stated as : form precedes function. Epithelial organisation and *de novo* protein synthesis may be causally linked. Before blastoderm formation embryos largely depend on maternal RNAs ; substantial de novo protein synthesis starts only after epithelial organisation has become established. Such a causal link, however, has not yet been experimentally investigated, mainly because in the literature the blastula has only rarely been thought of as an epithelium.
6. The fact that proteins, for all the profound importance they undoubtedly have on the structure and biochemistry of organisms, cannot by themselves specify design and pattern (PENMAN, 1991), is frequently overlooked although this is so selfevident and has been stressed many times. This function needs the help of a three-dimensional scaffold, the cytoskeleton/nuclear matrix, itself proteinaceous in nature.

A UNIFYING PRINCIPLE

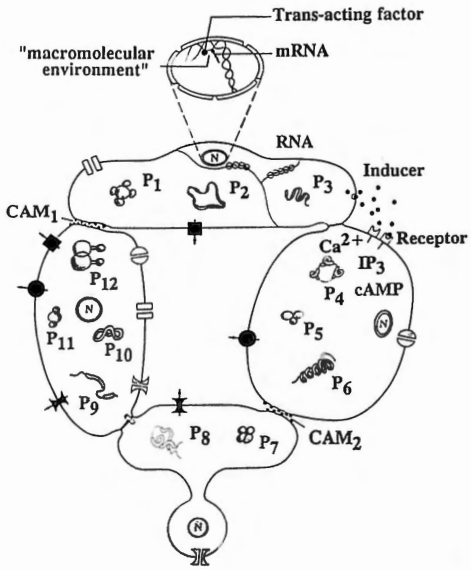
The above considerations suggest that a universal principle underlying differentiation does exist and that it is probably a simple one : it seems to be a logical consequence of some of the properties of the cytoskeleton and of biological membranes



1

Definition: Differentiation is the generation of cells which differ in their plasmamembrane-cytoskeletal complex, this being indirectly instrumental to differential protein synthesis.

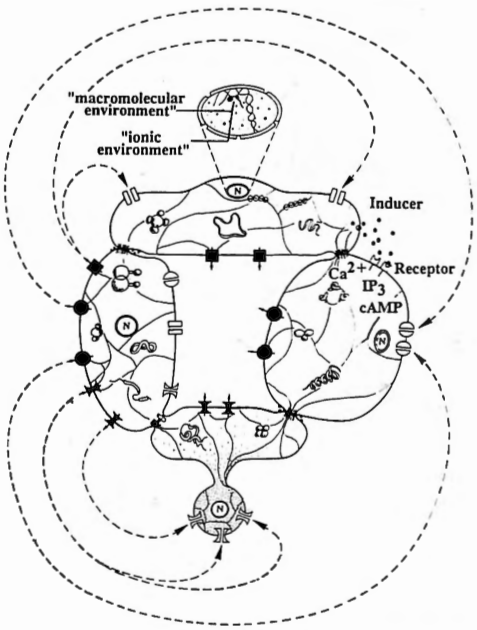
Principle: keep the genome constant but change its (ionic) environment.



2

Definition: Differentiation is the generation of cells which differ in the sets of proteins they synthesise with differential cellular physiology as a result.

Principle: keep the genome constant but change its (macromolecular) environment.



3

Principle : keep the genome constant but change again and again its macromolecular and/or ionic environment.

Figs 1-3: — Cross sections through a hypothetical 4-celled, epithelially organised animal, in which the major mechanisms instrumental to differential protein synthesis are depicted.

1. Features, definition of differentiation and underlying principle particularly relevant to membrane physiology.

The different cell types have the ability to segregate proteins such as ion pumps and channels, in their plasma membranes. As a result they can drive an (electroneutral or electrogenic) ion flux through themselves and through the organism as a whole (the dotted lines in A and C). In cell D an intracellular gradient of macromolecules is generated as the result of self electrophoresis. The cytoskeleton is instrumental in anchoring and segregating ion transporting molecules in the membrane, in forming tight junctions and in keeping the nucleus (N) in a well-defined position. Chromatin structure partially depends on the ionic « environment » in the nucleus. It is not a priori excluded that in certain circumstances the nuclear pore complexes could be closed so that the ionic composition of the nucleoplasm might be different from that of the cytoplasm. Gap junctions, when open, allow free passage of ions and small molecules. Secondary messengers are needed for transduction of some signals. The structures with an arrow through them represent ion pumps (Pu_1-Pu_3), those with a hole, ion channels (Ch_1-Ch_3). For reasons of simplicity, only one type of receptor, ion pump and channel and one direction of ion pumping is represented in cell A, B and C; in the normal situation an array of such membrane proteins is present. Molecules can evidently not be drawn to scale.

2. Features, definition of differentiation and the underlying principle particularly relevant for molecular biology.

The different cell types display differential patterns of protein synthesis, mainly as the result of differences in trans-acting factors present in the nuclei (N). As the result of different protein sets (P_1-P_{12}), the cells can have different physiological functions. There are different types of cell adhesion molecules (CAM_1-CAM_2). Intercellular communication can be realised by inducers, hormones etc. Secondary messengers are needed for transduction of some signals. The cytoskeleton can be instrumental for anchoring mRNA.

3. Superposition of 1 and 2 showing the universal principle underlying differentation.

on the one hand, and of the necessity of epithelial compartmentalisation on the other. In my opinion it can be formulated as follows :

> Differentiation in animals is the stepwise formation of cells or cell clusters which differ primarily in their plasma membrane-cytoskeletal properties and which mostly become organised into a variety of epithelia, lining different compartments. These differences in plasma membrane-cytoskeletal complex properties cause the distinct emerging cell types to display different patterns of protein synthesis, cellular morphology and physiology, notwithstanding the fact that they have an identical genome and similar mechanisms of protein synthesis and processing.

To summarize : differences in the plasma membrane-cytoskeletal complex, the structure of which confers a differential three-dimensional molecular scaffold on the cells, are first brought about ; differential protein synthesis and pattern formation follow (of necessity) : form precedes function. The major « strategy » used in differentiation of somatic cells seems to be to keep the genome constant (GURDON's experiments, 1962) but to change its « environment » over and over again. This environment has ionic and macromolecular constituents, which act in complementary fashions. The first may be more appropriate for the coarse tuning of gene expression/protein synthesis (WILDON *et al.*, 1992 ; VANDEN BROECK and DE LOOF, 1993), the second (especially the trans-acting factors) for the fine tuning.

THE IMPORTANCE OF EPITHELIUM FORMATION IN ANIMAL DEVELOPMENT

In the Five Kingdoms classification system, animals are defined as organisms developing from a blastula, while plants develop from an embryo. This definition may itself explain why the polarity in the plasma membrane/cytoskeletal complex is so important and why this has to be brought about so early. An early embryo becomes a blastula at the moment when its cells become organised into a simple *epithelium* enclosing a fluid compartment (DE LOOF, 1992). Each epithelium consists of cells which are *necessarily polarised* with respect to their plasma membrane/cytoskeletal complex. Thus, the introduction of polarity in the complex must be achieved early, otherwise neither the organisation into an epithelium nor proper functioning would be possible. An epithelium, however, is not necessarily homogeneous and such heterogeneity may also be true for a blastula. Next, the blastular epithelium starts folding, forming the archenteron. As development proceeds, more and more compartments are formed, all lined by epithelia consisting of cells with a specific plasma membrane/cytoskeletal complex, which enclose a fluid compartment with a specific ionic composition. In fact, all animals are, to a large extent, organised as a variety of more or less folded epithelia each enclosing a number of fluid compartments. Since by definition animals are to a large extent epithelially organised, the role of the plama membrane/cytoskeletal complex cannot be other than crucial. Indeed, an improper organisation of the plasma membrane/cytoskeletal complex in a given epithelium would cause malfunctioning. This may

also apply to many non-epithelially organised cell types such as muscle cells. Epithelial cells are specialised in transcellular transport of solutes, especially of ions. In my opinion, taking into consideration that function follows form, a system in which the expression of a number of epithelium-specific genes is somehow causally linked to the specific transcellular solute transport makes sense (VANDEN BROECK and DE LOOF, 1993).

WHAT COMES FIRST ?

Can different differentiated cell types of an organism engage in differential protein synthesis (which is one aspect of differential physiology) without *first* having introduced differences in their plasma membrane/cytoskeletal complexes ? A more general form of this question is : can cells having identical genomes, identical protein synthesis mechanisms and identical plasma membrane/cytoskeletal complexes engage in differential protein synthesis ? The answer is not clear cut. It might be positive if the cells were subjected to extracellular environments which differ markedly in their composition (ions, inducers *etc.*).

Imagine two cells which originated by symmetrical cytokinesis from a stem cell and which have the same number of plasma membrane- or nuclear receptors for a given inducer molecule. One cell is incubated in a medium with an inducer concentration just sufficient to ensure occupation of all the receptors for this ligand. The other cell is incubated in a medium with a much lower inducer concentration resulting in partial occupancy of the receptors by their ligand. Depending on the mode of action of the inducer, differential protein synthesis might result. This situation may occur in regions where an inducer gradient is present. Differences in ionic composition in the two media may — under certain conditions — also result in differential protein synthesis (DE LOOF *et al.*, 1992 ; VANDEN BROECK *et al.*, 1993). Differences in ionic composition of the extracellular environment can be achieved by epithelial compartmentalisation, a very commonly used mechanism in animal development (DE LOOF, 1992). There are still other possibilities. The general rule is that the further away the cells are from each other in developing organisms, the more likely it is that this strategy for generating differential protein synthesis will succeed.

When the cells are very close to each other, they would be expected to experience the same or at least very similar extracellular environments. Can cells with an identical genome, identical mechanisms of protein synthesis and an identical plasma membrane/cytoskeletal complex and furthermore experiencing the same extracellular environment engage in differential protein synthesis ? The answer is probably negative as long as none of the above parameters changes. If the genome, the mechanisms of protein synthesis and the extracellular environment are kept unchanged as is very often the case in developing embryos, the plasma membrane/cytoskeletal complex becomes the preferential parameter for introducing differential

protein synthesis. There are, indeed, several ways in which this complex can be instrumental in differential protein synthesis (DE LOOF *et al.*, 1992).
The generation of asymmetry during early development would not be easy if the cytoskeleton could not function as an anchoring site for some plasma membrane proteins and/or some maternal mRNAs or if it were spherically symmetrical. Furthermore, the organisation into epithelia would not be possible without specialisations and polarisation of the cytoskeleton.

THE GENERATION OF DIFFERENCES IN PLASMA MEMBRANE CYTOSKELETAL COMPLEXES

At the moment of oviposition or upon fertilisation, almost all animal eggs already display some form of polarity, that of a non-spherically symmetrical plasma membrane-cytoskeletal complex. This in combination with cleavage in planes other than from that of bilateral symmetry (the double asymmetry principle, DE LOOF, 1986 ; GURDON, 1992 ; HORVITZ and HERSKOWITZ, 1992) results in blastomeres which differ in their plasma membrane/cytoskeletal complexes without the necessity of first engaging in differential protein synthesis (Fig. 2). This is a very common mechanism in the Animal Kingdom.

It is striking that in all animal organisms studied in this respect, differences in plasma membrane/cytoskeletal complex are introduced very early in development, not later than the third cleavage, thus while the cells are still very close to each other. This suggests -but does not prove- that this is an important mechanism instrumental to differentiation. Some maternal mRNAs, on being translated, could also play a role, directly (when they code for membrane proteins) or indirectly (when they code for factors that influence the activity of membrane proteins already present) (Fig. 2).

A very nice example of the possibility of inducing polarity in the plasma membrane of a spherically symmetrical egg comes from outside the Animal Kingdom, namely from fucoid eggs. Initially « fresh » eggs are not polarised. This can be deduced from the observation that the thallus/rhizoid axis can develop in any direction following an appropriate physical stimulus such as light. Illumination causes directed rearrangemants in the plasma membrane properties resulting in the segregation of ion pump/channel activity : the activated egg then starts to drive an ion flux, especially of Ca^{+2}, through itself. Soon thereafter polarity becomes morphologically visible (JAFFE, 1966 ; QUATRANO, 1990). There are no indications that this requires protein synthesis first. Changes in the cytoskeleton can under some conditions be achieved by polymerisation from a pool of preformed monomers or by depolymerisation. This proves that differences in plasma membrane/cytoskeletal complex can be induced without prior differential protein synthesis.

Once *de novo* protein synthesis starts, there are evidently many ways of influencing membrane properties and physiological processes, which in their turn may again influence gene expression and so on : the system becomes « self-propelling » and

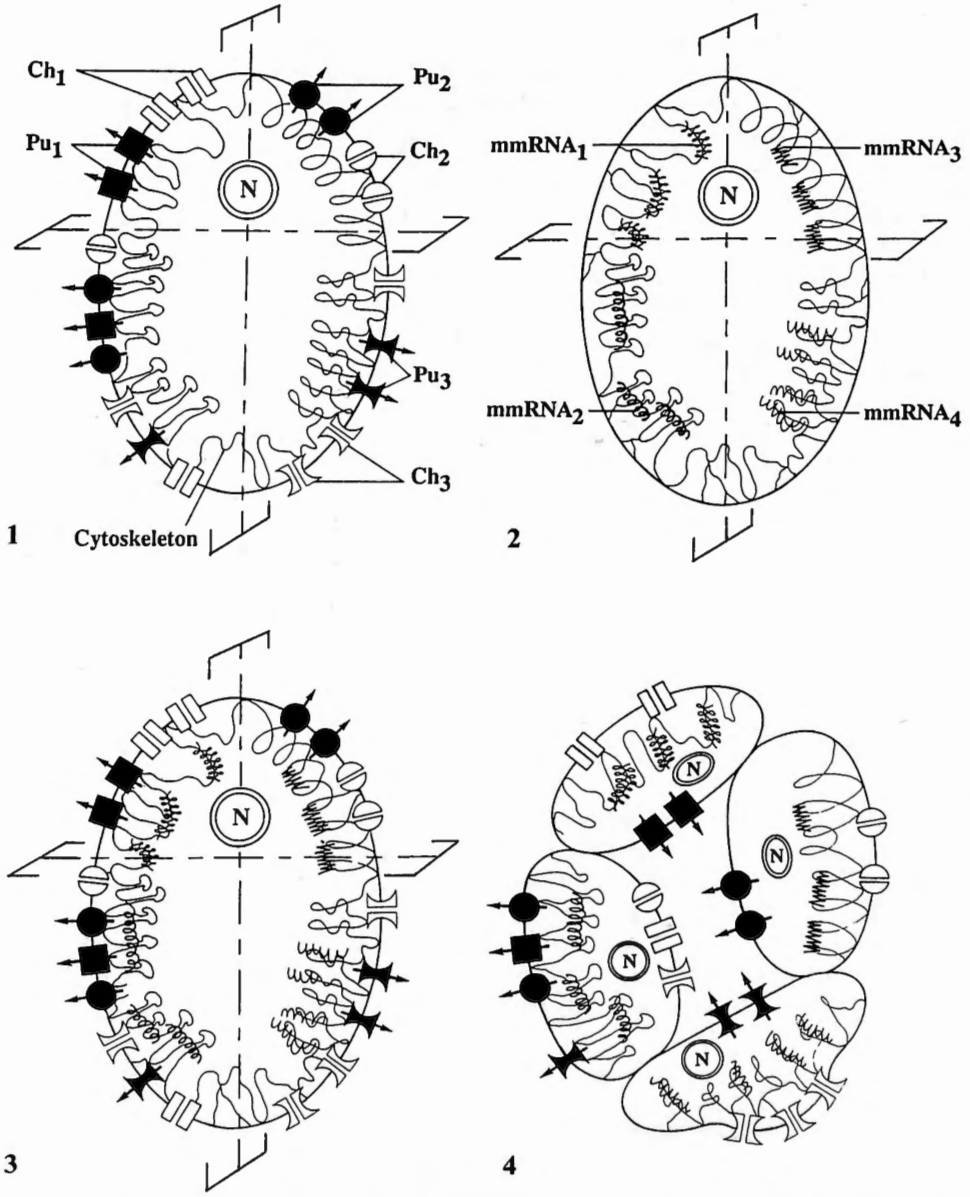


Fig. 2. — Mechanisms instrumental in the generation of functional asymmetry in a hypothetical 4-celled, epithelially organised animal. Cross sections through a « stem cell » (or more generally, a zygote), illustrating the possible role of the cytoskeleton in anchoring membrane proteins and/or maternal mRNAs.

1. Features particularly relevant to membrane physiology. Membrane proteins, here depicted as ion pumps (Pu_1-Pu_3) and channels (Ch_1-Ch_3), are asymmetrically distributed over the plasma membrane and held in place by some means, *e.g.* the the cytoskeleton. If some of the

cause and result become difficult to distinguish between (membrane impression and gene expression : BRUNNER, 1977).

There is no contradiction between the fact that the differences in the plasma membrane/cytoskeletal complex in differentiated cells are a result of this differentiation and the fact that these differences are themselves instrumental in differential protein synthesis.

Furthermore, differences in macromolecular trans-acting factors among the differentiating cells will as well play an important role. There are several possible ways to generate such differences. Some trans-acting factors could be coded for by maternal mRNAs and anchored to specific parts of an asymmetrical cytoskeleton. If cleavage does not occur along a plane of bilateral symmetry, blastomeres will be formed with different populations of maternal mRNAs (here again the double asymmetry principle). Another possibility is that a given maternal mRNA, or the protein it codes for, is not anchored but freely diffusible. If the molecule is charged and if the zygote drives an electrogenic ionic flux through itself, as is the case in *e.g.* the *Drosophila* egg (OVERALL and JAFFE, 1985), preferential localisation could result. There is so far no experimental evidence in favour of such a mechanism. Another possibility is that no such maternal mRNAs are present in the zygote but that as the result of differences arising in ionic environments, specific trans-acting factors or complexes of such factors are formed in some cells as the result of *de novo* protein synthesis.

DISCUSSION

The concept I propose here is based mainly on the following observations. Firstly, animal development and epithelial compartimentalisation are intrinsically connected. Secondly, not only epithelial cells but probably many other cell types as well, must elaborate their own specific plasma membrane-cytoskeletal complex before they can start functioning properly. Thirdly, all the differentiated cell types of any animal probably differ in their plasma membrane/cytoskeletal complex. It follows that the intracellular « ionic environment » is likely to differ from cell type

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early cleavages occur elsewhere than along the plane of bilateral symmetry, progeny cells will be formed which differ in their plasma membrane properties. N : nucleus.

2. Cross section through the same « stem cell » or zygote, showing features relevant for direct macromolecular control of gene expression/differentiation.
Maternal mRNA's ($mmRNA_1$-$mmRNA_4$), here depicted as strands of different form, are asymmetrically distributed in the cytoplasm and are held in place by some means such as the cytoskeleton. If some of the early cleavages occur elsewhere than along the plane of bilateral symmetry, progeny cells will be formed which differ in their « stem cell » mRNA's properties.

3. Superposition of 1 and 2.

4. As the result of the double asymmetry principle, 4 different cell types are formed.

to cell type. In the past, differences in the plasma membrane/cytoskeletal complex have usually but not always been considered to be a result of differentiation, which is evidently correct. In my opinion, the possibilities of the plasma membrane/cytoskeletal complex operating as a powerful driving force for differential physiology/protein synthesis, although well documented from physiological studies, have been largely undervalued in developmental biology.

The variability in properties of membranes (plasma membrane and perhaps also membranes of some cell organelles) is primarily, but not exclusively, due to differences in the nature and distribution of membrane-associated proteins, the relevant ones for development being receptors, ion transporting proteins, cell adhesion molecules/receptors and some cytoskeletal proteins which extend into the plasma membrane. Each cell type has its own set of membrane proteins and its own specific ionic environment. Although only relatively few (mostly inorganic) ionic species (the major ones being H^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^- and organic ions) participate in the ionic environment in all species, the complexity of this environment should not be underestimated. Indeed, it comprises ionic concentrations/activities, ionic/voltage gradients, gradients of charged macromolecules which can arise as the result of selfelectrophoresis (WOODRUFF and TELFER, 1980) in some systems, and ionic/electrical compartmentalisation of at least some membrane-limited cell organelles. The number of different ionic environments which can be generated with these universally present inorganic ions is very large and is sufficient to allow each of the several hundred different cell types of which complex higher animals exist, to have its own specific ionic environment. Changes in ionic environment or electrical activity are potentially instrumental in the « coarse » control of gene expession by changing the conformation and/or activity of some proteins (*e.g.* enzymes), the form of the cytoskeleton/nuclear scaffold, the interaction between the cytoskeleton and membrane proteins or RNA, between DNA and DNA binding proteins in general (chromatin structure, LEZZI, 1970) or between some other macromolecules *etc.* (DE LOOF, 1986, 1992 ; DE LOOF *et al.*, 1992 ; VANDEN BROECK *et al.*, 1993). For the fine tuning of specific gene expression, macromolecular transacting factors are evidently more appropriate (BIGGIN and TJAN, 1989 ; KARIN, 1990) although electrical control is also a possibility for some genes (VANDEN BROECK *et al.*, 1993). One should not overlook the fact that almost all membrane-limited cell organelles have been reported to be able to establish their own specific intraorganelle ionic environment (DE LOOF *et al.*, 1992). Whether or not this is also true for the nucleus is still controversial (DINGWALL, 1991 ; DE LOOF, 1992). For the moment it is not known whether the properties of some cell organelles, such as the nuclear envelope and the Golgi complex, might also differ slightly from cell type to cell type or whether they are subject to change in a given cell type and contribute to differential gene expression.

Mechanisms of making cells aggregate in specific ways (adherins, specialisations of the cytoskeleton etc., EDELMAN, 1986 ; TAKEICHI, 1991 ; HYNES and LANDER, 1992) are essential especially in an epithelium-type organisation (GUMBINER, 1990 ; RODRIGUEZ-BOULAN and NELSON, 1989). Transembryonic ionic (electric) currents and selfelectrophoresis, which have been observed in several developing systems

(*e.g.* JAFFE and STERN, 1979 ; ROBINSON and STUMP, 1984) are also a logical consequence of epithelial organisation and inherent to that of the segregation (*e.g.* by anchoring them to some proteins of the cytoskeleton) of ion pumps and channels. They are thought to be more than just epiphenomena (JAFFE and NUCCITELLI, 1977 ; NUCCITELLI, 1986).

Intercellular communication by gap junctions, another type of specialisation of the plasma membrane, allows not only exchange of small organic molecules but also adjustments in ionic environments. Disruption of this flexible system can lead to disruption of the normal differentiation patterning (GUTHRIE, 1987 ; FRASER *et al.*, 1987).

The principle formulated here may appear unrealistically simplistic, especially if it is confronted with the array of mechanisms known to be instrumental in controlling the expression of specific genes in specific cell types. Many workers in the field of contemporary developmental biology take the view that there is no need for a universal principle underlying differentiation since so many mechanisms can lead to differential protein synthesis in different cell types. I am very reluctant to accept this defeatist way of thinking, which certainly looks plausible at first sight. My thesis is that, because of the universal epithelial organisation of animals, the generation of differences in plasma membrane/cytoskeletal complex is *necessarily* used by almost all differentiating animal cell types (at least the epithelially organised ones), but that in *addition* some cell types may use one, or perhaps more, *secondary* specialised mechanism(s) for controlling the expression of some of its genes. In my opinion this is rather the rule than the exception for many cell types. Indeed, each differentiated cell type expresses so many genes that the use of combinations of control mechanisms is more likely to ensure a correct expression pattern than would a single mechanism. The more cell types and genes an organism has, the more *superimposed* control mechanisms may be needed. All these mechanisms lead to generating differences in the ionic and/or macromolecular environment of the genome.

This simple holistic principle -keep the genome constant but repeatedly change its macromolecular and/or ionic environment- can reconcile and integrate all established mechanisms of epigenetic (LOVTRUP, 1974) and of genetic control of differentiatiation as well as a number of postulated ones : environmental cues can be interpreted by the plasma membrane's making use of different secondary/tertiary signal transducing systems, while the membrane proteins are coded for by the genome. Of basic importance in the principle is that there are two complementary tuning mechanisms controlling gene expression : one in which the inorganic ions are instrumental (organic ions like polyamines are also important, FEUERSTEIN *et al.*, 1991) and the other with macromolecular trans-acting factors. Both allow a very large variability and number of different combinations. The principle also stresses the importance of taking the whole cellular infrastructure into account in order to understand cellular functioning (CLEGG, 1984 ; 1992) and development.

Because of its very nature (changes in membrane properties, ion permeability etc., usually cause electrophysiological and biochemical cascade effects ; the symmetry or asymmetry of the fragile cytoskeleton/nuclear scaffold cannot be easily

demonstrated), the principle can at present hardly be fully proven or disproven experimentally : there are too many variables. However, it gains plausibility from the vast number of data which fit into it. It may stimulate the design of new lines of research both in animal systems and in plant and fungal ones, where the concept is probably applicable as well. It may also contribute to a better understanding of the mechanisms of epigenetic-, tissue specific- and multifactorial regulation of gene expression. It certainly will require the study of the activity of genes in their natural nuclear environment : this is a challenge for the future. Thinking about animal development in terms of differential epithelium formation may bring more unity in developmental biology.

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WHY DO FEMALES ENGAGE IN EXTRA-PAIR COPULATIONS? A REVIEW OF HYPOTHESES AND THEIR PREDICTIONS

by

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SUMMARY

Behavioural ecologists have become aware that extra-pair copulations form a major element of avian mating behaviour. Previously, attention was focused primarily on the benefits of this behaviour to males. However now that it is realized that females are not just passive participants, but instead may be largely in control of which male fathers their young, the major goal is to understand what females gain from extra-pair copulations. In this paper we summarize some of the existing hypotheses and we advance and discuss some new predictions that may open perspectives for future research.

Key-words : Reproductive behaviour, extra-pair copulations, paternity, female choice, review.

INTRODUCTION

Copulations outside the social pair bond (known as « extrapair copulations » or EPCs) are now documented for a wide range of bird species (reviews in WESTNEAT *et al.*, 1990 ; BIRKHEAD and MØLLER, 1992) and form an important alternative reproductive behaviour by which males can increase their reproductive success [« mixed reproductive strategy » (TRIVERS, 1972)]. The benefit for males could be proven, using genetic markers, by showing that EPCs can result in fertilizations (e.g. WESTNEAT, 1987 ; LANK *et al.*, 1989 ; BIRKHEAD *et al.*, 1990). Although there are also potential costs for males performing EPCs (reviews in BIRKHEAD and MØLLER, 1992), obviously the benefit of fathering more offspring is very important and males can gain a substantial part of their reproductive success through EPCs (GIBBS *et al.*, 1990 ; MORTON *et al.*, 1990 ; WESTNEAT, 1990).

However, recent studies of different bird species show that in fact extra-pair paternity is likely to be largely controlled by the female (WAGNER, 1991a ; KEMPENAERS *et al.*, 1992 ; LIFJELD and ROBERTSON, 1992 ; BIRKHEAD and MØLLER,

1993a). In most bird species males lack an intromittent organ, and therefore the cooperation of the female might be necessary to make a successful copulation (with sperm transfer) possible (FITCH and SHUGART, 1984), although there is still debate over this topic (discussion in WAGNER, 1991a ; BIRKHEAD and MØLLER, 1992). Nevertheless, a number of observers have reported or implied that EPCs require female cooperation (WAGNER, 1991a and references therein). Moreover, in many species it has been observed that females are actively seeking or soliciting EPCs from certain males [e.g. northern fulmar *Fulmarus glacialis* (HATCH, 1987) ; zebra finch *Taeniopygia guttata* (BIRKHEAD *et al.*, 1988) ; black-capped chickadees *Parus atricapillus* (SMITH, 1988) ; house sparrow *Passer domesticus* (MØLLER, 1990) ; blue tit *Parus caeruleus* (KEMPENAERS *et al.*, 1992)]. Good experimental evidence for female control of extra-pair paternity stems from a recent study of tree swallows *Tachycineta bicolor* (LIFJELD and ROBERTSON, 1992). Since the data suggest that females play an active role in getting EPCs, they should also get some benefits from them.

HYPOTHESES AND PREDICTIONS

Many hypothetical benefits of EPCs for females have been proposed (reviews in WESTNEAT *et al.*, 1990 ; BIRKHEAD and MØLLER, 1992) but there are only a few studies that provide good evidence for one or another hypothesis. Good evidence is lacking because of three main reasons. (1) There are still few studies that combine both detailed behavioural work with DNA-fingerprinting results (paternity data) for the same individuals, (2) in the past most people have focused their attention on male behaviour and (3) for several of the hypotheses clear predictions are lacking and only a few attempts have been made to differentiate between the hypotheses (notable exceptions are : WAGNER, 1992a ; BIRKHEAD and MØLLER, 1993b ; LIFJELD *et al.*, 1993).

BIRKHEAD and MØLLER (1992) reviewed the different hypothetical benefits and discussed the existing evidence for these hypotheses. However, they did not propose any predictions to test the hypotheses. In this paper we will limit ourselves to the discussion of what we think are the seven most likely hypothetical benefits of EPCs for females. The aim of this paper is only to propose and discuss some predictions to differentiate between the hypotheses. It should be noted that the hypotheses are not exclusive and thus the same female might obtain several benefits or different females might benefit in different ways. To understand how EPC behaviour might have evolved, one should also consider the costs of this behaviour to females, but this is beyond the scope of this paper (for a discussion see WESTNEAT *et al.*, 1990).

In general there are two types of female benefits from EPCs : phenotypic or direct effects and genotypic effects (WESTNEAT *et al.*, 1990). There is one major difference between them. Females can only receive genetic benefits if the EPCs lead to extra-pair young, while for the non-genetic benefits to occur, the EPCs do not have to result in the fertilization of an egg. Therefore, important differences in female behaviour can be expected, depending on the type of benefit.

Hypothesis 1 : courtship feeding before copulation

This hypothesis can only explain why females engage in EPCs if males do indeed feed the female before the EPC. TASKER and MILLS (1981) showed that in red-billed gulls (*Larus novaehollandiae scopulinus*) within-pair copulation success increased when males provided courtship feeding before the copulation attempt, suggesting that in some species females indeed trade copulations against food. Observations of EPCs should make it clear whether this benefit can occur.

Hypothesis 2 : male parental care

This hypothesis states that females try to obtain extra help for feeding their young, by copulating with other males. It is based on the idea that males will provide parental care in relation to their certainty of paternity (cue is access to the fertile female). This idea is supported by copulation patterns and male help in the polyandrous mating system of the dunnock *Prunella modularis* (BURKE *et al.*, 1989). Apart from this, the only existing evidence is the observation that after females are widowed, they start copulating with new males showing interest and this has been interpreted as the females trying to deceive these males into helping them (GJERSHAUG *et al.*, 1989 ; MEEK and ROBERTSON, 1991). However, so far there is no evidence that EPCs result in male help. Broods fathered by different males can thus also be a result of such « rapid mate switching » (PINXTEN *et al.*, in press).

Regardless of the fact that one should indeed observe that females receive help from extra males (with which they copulated), one can make the following predictions. (1) EPCs should be performed with males that are likely to give parental care (e.g. unpaired males, males that lost their mate or nest). (2) Those females that engage in EPCs should badly need the extra help or expect little male help later (e.g. secondary females of polygynous males). Also, the extra help should result in an increase of the female's reproductive success. This is more likely in situations where food is scarce and where providing parental care is costly.

Hypothesis 3 : mate appraisal and acquisition

Females may engage in EPCs in order to appraise and acquire future mates (COLWELL and ORING, 1989 ; WAGNER, 1991b). This hypothesis does not require an insemination or fertilization, so EPCs could take place outside the fertile period. One should be able to show that females that have performed EPCs with certain males, will later choose these males as a partner. This may be in the same year (second breeding attempt or new male of polyandrous female) or in consecutive years (e.g. WAGNER, 1991b).

Hypothesis 4 : avoiding rejection costs

Females might passively accept EPCs to avoid a potentially larger cost of rejecting a persistent male. In this case females do not benefit from the EPCs, but from not refusing them. This hypothesis cannot be valid if one observes females actively

seeking or soliciting EPCs. In species where the male closely guards the female, it is unlikely that females cannot avoid an EPC by warning their mate when another male approaches them, unless the copulation attempts are made by multiple males [e.g. sexual chases in bank swallows *Riparia riparia* (BEECHER and BEECHER, 1979); communal displays in house sparrows (MØLLER, 1987a)]. However, in colonial species with a high level of nest-site competition or where nests can be destroyed, nest guarding may prevent continuous mate guarding (BIRKHEAD and MØLLER, 1992), and females that are left alone may be attacked by males [e.g. white ibis *Eudocimus albus* (FREDERICK, 1987)]. This hypothesis is also likely to be valid for species where males have an intromittent organ and can forcibly copulate [e.g. « rape » in waterfowl where females might even be killed during an EPC attempt (MCKINNEY *et al.*, 1983)]. In some species the threat of infanticide can also be a rejection cost (ROBERTSON, 1990).

Hypothesis 5 : insurance against male infertility

In the first place females want their eggs to be fertilized. Therefore, they could copulate with more males as an insurance against the possible infertility of their own mate. This hypothesis is not easy to test because (1) male infertility is difficult to examine (BIRKHEAD and MØLLER, 1992) and (2) the hypothesis may predict both a positive and a negative association between extra-pair paternity and the occurrence of infertile eggs. WETTON and PARKIN (1991) argued that for a male with low viable sperm counts there will be an increased risk of his mate laying infertile eggs. At the same time, the male might have a reduced success in sperm competition resulting in an increased likelihood of extra- pair fertilization. Alternatively, females that engage in extra-pair copulation should enjoy a higher fertilization success than those who do not, resulting in fewer, not more, infertile eggs in nests with extra-pair offspring. Some confusion may also arise because one can make different starting assumptions. (1) It is possible that males are only temporarily infertile (or less fertile) because they copulated at a high frequency [sperm depletion hypothesis (WETTON and PARKIN, 1991)]. However, sperm depletion through frequent copulation could also be « organized » by the female, because then she has more control over paternity. In this case the benefits have to come from either « good genes » or genetic diversity (see further). (2) Females are aware of the fertility status of their male. If this is the case, one expects only some females to perform EPCs (those females paired to infertile/less fertile males) and one should find that most nests contain no extra-pair young, while in a few nests all young are sired by an extra-pair male. (3) Females are unaware of the fertility status of their male. In this case all females should perform at least some EPCs. The resulting pattern of extra-pair paternity would then be that again a few nests contain only extra-pair young (those of infertile males), but a lot of nests should contain a low proportion of extra-pair young (assuming that infertility is rare).

To further examine this hypothesis, we should have an idea of the occurrence of male (and female) infertility in wild populations and of the effect of copulation

frequency on sperm depletion (see BIRKHEAD, 1991 ; BIRKHEAD and FLETCHER, 1992).

Hypothesis 6 : genetic diversity

In an unpredictable environment females might benefit from genetically diverse offspring (WILLIAMS, 1975 ; GLADSTONE, 1979). The following predictions can be made for this hypothesis.

(1) Pattern of extra-pair paternity in the population : few extra-pair young per nest in most nests or, if many extra- pair young per nest, they should all have different fathers. In principle, a female could have each egg fertilized by a different male, but there are actually few examples from DNA fingerprinting studies which record more than two males as the fathers of chicks within a single brood (BIRKHEAD and MØLLER, 1993b). Extra-pair young could be randomly distributed over the nests. (2) If genetic diversity is important, females should perform EPCs both with neighbours and with floater males and they should not refuse EPCs from certain males. In conclusion, females should not be selective. (3) Most or all females should engage in EPCs. (4) Nests with extra-pair young (high genetic diversity) should result in more recruits than nests without extra-pair young if genetic diversity is really beneficial to the female. One could also argue that the females perform EPCs as an « insurance » against an unpredictably fluctuating environment. (5) The variance in male reproductive success in the population should not increase, because males should both lose paternity and father young in other nests. (6) Extra-pair paternity should be more common if pair members are relatives, because then the genetic diversity obtained through sexual reproduction is lower and inbreeding can be costly [e.g. splendid fairy-wren Malurus splendens (BROOKER *et al.*, 1990 ; ROWLEY and RUSSELL, 1990)].

Hypothesis 7 : genetic quality

This hypothesis states that females benefit from EPCs if their offspring are fathered by males with « good genes », either genes for general vigor or genes for attractiveness (BIRKHEAD and MØLLER, 1992).

Two versions of this hypothesis should be considered.

A. Under the assumption that all females have identical preferences, we can make the following predictions. (1) General pattern of extra-pair paternity : bimodal distribution of extra-pair young, i.e. no extra-pair young in some nests, a lot of extra-pair young in other nests. In an extreme case, when assuming that (a) it is very important to have your offspring fathered by a « good » male and (b) all females are able to copulate with the same male(s), one could find a lot of extra-pair young in a lot of nests [e.g. Reed Bunting *Emberiza schoeniclus* (DIXON, A. pers. comm.)]. Thus, extra- pair young are not randomly distributed amongst nests and they are never reciprocal. (2) Females should be selective in that they only perform EPCs with certain (« better ») males. Unless females can judge the quality of a male very quickly (if males have honest indicators of genetic quality), they should

only perform EPCs with known males (e.g. territorial neighbours). (3) Females paired to low quality males should engage in EPCs while females paired to high quality males should refuse EPCs (or cooperate in mate guarding). Thus, some females refuse EPCs or do not solicit them. On the other hand females might resist extra-pair matings as a ploy to test the quality of males or to incite male-male competition (McKINNEY *et al.*, 1983). (4) Some males should be clearly preferred for EPCs and this should result in these males obtaining a higher reproductive success both through within- and extra-pair young. Thus, the variance in reproductive success between males increases. (5) In nests with extra-pair young, the extra-pair young should recruit better than the legal (within-pair) young or should have a higher reproductive success when breeding later, because they live longer or attract more females. Also, offspring of males that get EPCs should recruit better than offspring of other males. However, the last prediction is much more difficult to test, because of the many confounding variables that might play an important role (female quality, territory quality, parental investment). The first comparison is easier to make because extra-pair and within-pair young have the same mother and are raised together in the same nest. (6) Males that have fathered extra-pair young should be of high quality and thus for example live longer or attract more females (polygyny) and they should recruit more young from their own nest than males that suffer lost paternity.

B. The alternative assumption is that females have variable preferences, for example if genetic complementarity is important. Variation in female preference has often been neglected and is poorly studied (see KIRKPATRICK and RYAN, 1991), but causes a difference in the above predictions. Extra-pair paternity may be reciprocal, males may be cuckolded with one female but not with another and the variance in male mating success may not be as big as for identical preferences.

A special case of the good genes hypothesis is direct sperm competition to allow the best sperm to fertilize the eggs (e.g. study on adders, *Vipera berus* (MADSEN *et al.*, 1992)). If the female has no way of telling the genetic quality of a male, she could engage in multiple copulations and let the sperm « decide » which is the best male through competition in the female reproductive tract. The sperm fertilizing her eggs are (by definition) the best at sperm competition. Since many aspects of ejaculate/copulation behaviour are probably highly heritable, the female will tend to have sons who are also good at sperm competition. MADSEN *et al.* (1992) suggested that sperm that is more successful in fertilizing the ova could also be more effective in producing viable offspring. If females benefit from direct sperm competition, one predicts that all females engage in EPCs (see also LIFJELD *et al.*, 1993) and that this still leads to a few males getting all the extra-pair young.

THE STUDY OF MALE BEHAVIOUR

The behaviour of the male, which has received most attention up until now because of the obvious male benefits, might give few insights to explain the detected pattern of extra-pair paternity. This is because all males clearly benefit from having

extra-pair young, and one can thus expect that all males will try to get them when the possibilities arise and that all males will also try to avoid being cuckolded themselves. If females control copulations, then differences in the benefits of EPCs to different females will result in different female behaviour and might then determine the pattern of extra-pair paternity.

MATE GUARDING AND OTHER PATERNITY PROTECTION BEHAVIOURS

Whatever the benefits females might obtain, if the EPCs lead to the fertilization of one or more eggs, they are very costly to the cuckolded male. Therefore males should always adopt behaviour that minimizes the risk of being cuckolded, either through guarding the fertile partner, frequent copulation or retaliatory copulations (reviewed in BIRKHEAD and MØLLER, 1992). One can expect to find a negative correlation between the intensity of mate guarding and the number of extra-pair young in the nest if mate guarding is an effective protection against extra-pair paternity. However, if the male quality hypothesis is true, then high quality males that have no better quality neighbours should not invest in mate guarding, because their female will not engage in EPCs anyway. Then a positive relation between mate guarding and extra-pair paternity could be found [e.g. eastern bluebirds *Sialia sialis* (GOWATY and BRIDGES, 1991)]. This does not mean that mate guarding is not important, but that it is not very effective [as shown for the blue tit (KEMPENAERS *et al.*, 1992)]. In that case, males of lower quality that guard their mate are probably making the best of a bad job. Frequent copulation is perhaps more effective, but it is also possible, given that last male sperm precedence seems to be the mechanism of sperm competition in birds (BIRKHEAD and MØLLER, 1992), that the timing of the copulation is more important than the number of copulations. In that case, the female has a strong possibility of control. In some species the anti-cuckoldry tactics seem to be remarkably ineffective. Despite close mate guarding or high within-pair copulation rates, one can find a high rate of extra-pair paternity in e.g. eastern bluebirds (GOWATY *et al.*, 1989 ; GOWATY and BRIDGES, 1991), swallows *Hirundo rustica* (MØLLER, 1987b ; 1989), tree swallows (VENIER and ROBERTSON, 1991 ; LIFJELD *et al.*, 1993), house sparrows (MØLLER, 1987a ; WETTON and PARKIN, 1991). It thus seems that in some species females are a step in front in this sexual conflict.

EXTRA-PAIR PATERNITY AND FEMALE CHOICE

Females of many bird species are known to be choosy in that they show clear preferences for certain males (e.g. MØLLER, 1988 ; ANDERSSON, 1992) or for males holding certain high value resources [e.g. high quality territories (VERNER, 1964 ; ALATALO *et al.*, 1986). This preference for certain males can lead to these males pairing earlier (e.g. CATCHPOLE, 1980 ; MØLLER, 1988), getting more females (e.g. VERNER, 1964), having more broods within one season (e.g. MØLLER, 1988) and get-

ting more copulations (e.g. HOGLUND and LUNDBERG, 1987). And now recent studies clearly suggest that females are also exerting some choice over which male(s) father their offspring through EPCs (KEMPENAERS *et al.*, 1992; LIFJELD and ROBERTSON, 1992).

The study of female benefits from EPCs could perhaps also shed more light on the paradox of the lek, because one can draw a number of important parallels between female choice in a lek and female choice for EPCs. (1) In both cases females choose from among a number of males (assembled in a lek, members of the colony or territorial neighbours), and in both cases the females seem to receive little else than ejaculates. Females often show strong unanimity in their choice of copulation partner. The result of this unanimity among females is that a few males acquire most of the matings (e.g. HOGLUND and LUNDBERG, 1987). In a recent study of razorbills (*Alca torda*) WAGNER (1992b) describes the EPC behaviour occurring on mating arenas outside the colony and explicitly refers to it as lekking behaviour. He argues that in this species lekking is a secondary mating system. In a lot of lekking species, female preference has already been studied in great detail and it has been shown that male characteristics such as tail length (ANDERSSON, 1992), tail morphology (HOGLUND *et al.*, 1990), and fighting ability (ALATALO *et al.*, 1991) determine male mating success. For EPCs it is far from clear what male characteristics females might be choosing (KEMPENAERS *et al.*, 1992; LIFJELD and ROBERTSON, 1992) and this should be a priority in future research.

CONCLUSION

We hope that the above discussion will help to unravel the problem of female benefits from EPCs. As already pointed out by WAGNER (1991b), one of the major problems in testing these hypotheses is that they make overlapping predictions. Therefore, we think that it is essential to collect data on male and especially female behaviour and extra-pair paternity for the same individuals. Only a combination of these data and eventually data on offspring survival can give a clear picture.

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