Belg. J. Zool. - Volume 127 (1997) - issue 2 - pages 105-106 - Brussels 1997

IN MEMORIAM
JOZEF K.A. VAN BOVEN (1915-1997)

On 7 April 1997, the Belgian Zoological Society lost one of its eminent entomologists with the passing of Professor van Boven.

Jozef van Boven was born on 19 May 1915 in Roermond. Even as a young boy, he was impressed by the diversity of animal life he found in the rural fields in this area of the Netherlands. Especially the variety of insects left a deep impression, and formed the basis of his later career as an entomologist. After completing secondary school, he entered the catholic seminary in Roermond, where he received his priest ordination on 10 April 1943. Priesthood brought him in contact with the Jesuit fathers Erich Wasmann and Hermann Schmitz, who were both well-known myrmecologists. The need to hide during the war, however, brought him by coincidence to Leuven, where he came in touch with Jezuit father Albert Raignier, also a renowned myrmecologist. From his diocese in the Netherlands, he got permission to enrol as a student of biology at the university in Leuven. Here, the professional contact with the ant world began, and led, in 1957, to the completion of his PhD-thesis on allometry and polymorphism in ants. This same year, he was appointed as Professor of zoology at the K.U.Leuven.

For his main research on the taxonomy and biology of the Dorylinae, he carried out extensive field work in the former «Belgian Congo». The taxonomy of the Dorylinae remained his main field of expertise with annual expeditions to Africa, and several tens of articles published in this field. In addition, the ants of Belgium and the Netherlands were also the subject of his research, and led in 1976 to the publication in «Acta Zoologica et Pathologica Antverpiensia» of the very valuable De *mierenfauna van België*, that introduces the fascinating life of ants and includes a key to the Belgian Formicidae. As a Dutchman, he kept in touch with the Museum of Natural History in Maastricht, where he was curator of the Wasmann collection from 1963 until 1973. From 1967 to 1973 he was president of the «Natuurhistorisch Genootschap Limburg».

During his long academic career in Leuven, he taught entomology as well as an introductory course on the behaviour of social insects to hundreds of students. Professor van Boven was an extraordinarily talented teacher, who understood perfectly the art of grip-

ping the audience – his classes were even attended by students that did not have to take his courses, but that were just fascinated by his charming way of teaching!

Until his retirement in 1984, he was head of the division of animal ecology and systematics in the Zoological Institute, and for several years was member of the zoological committee of the National Fund for Scientific Research. When he retired, he devided his very professional entomological collection into three parts: the research collection with army ants went to the Royal Museum of Central Africa in Tervuren, the general ant collection moved to the Natural History Museum in Maastricht to join the Wasmann collection, while the general insect collection remained in the Laboratory of Entomology at Leuven.

After retiring, professor van Boven moved from Leuven to Kortrijk, where he exchanged scientific research for an active and serving pastoral life in the St. Maarten and Our Lady parishes. In addition to celebrating mass at noon, he would also walk to church to say mass at 7 am every morning, five days a week. On a dark and grey morning in November 1991, trying to escape from the wind and drizzling rain, a severe fall on his way to church resulted in a broken hip followed by a long and slow recovery period in hospital. His health became more fragile thereafter, but his spirit remained strong as ever. During his retired years in Kortrijk, he took part in three pilgrimage trips to Lourdes, the last time in August 1995 shortly after another stay in hospital.

Jozef van Boven will be remembred as an extremely charming and modest personality, a most respected colleague and wonderful friend.

Johan BILLEN
Zoological Institute
University of Leuven
Naamsestraat 59
B-3000 Leuven, Belgium

Belg. J. Zool. - Volume 127 (1997) - issue 2 - pages 107-114 - Brussels 1997

Received : 14 February 1997

GRIPHOMYIA (DIPTERA, DOLICHOPODIDAE, PELOROPEODINAE) A NEW GENUS FROM SOUTHERN THAILAND

PATRICK GROOTAERT AND HENK J.G. MEUFFELS

Department of Entomology, Koninklijk Belgisch Instituut voor Natuurwetenschappen, Vautierstraat 29, B-1000 Brussels (Belgium)

e-mail: grootaert@kbinirsnb.be

Abstract. *Griphomyia* gen. nov. with *G. gravicaudata* sp. nov. as type species, is described from rainforest in southern Thailand. Although it possesses a stalked hypopygium which is considered as an ancestral feature, the presence of symmetrically sclerotized pseudotracheae, denticulate epipharyngeal prongs, and a wing boss, all indicate that the new genus is quite derived. The morphology of the male genitalia places it rather in the Peloropeodinae with encapsulated hypopygium than in the Sympycninae with an apical, sessile hypopygium.

Key words: Diptera, *Griphomyia* n. gen, Thailand.

INTRODUCTION

The empidoid fauna of South Asia is very poorly known. As an example only 9 species of Dolichopodidae have been reported up to now from Thailand (DYTE, 1975), a figure which should be at least 50 times higher. It is not surprising, therefore, that even small collections yield numerous undescribed taxa.

The present study is a first contribution to the dolichopodid fauna of Thailand. Here we describe a new species belonging to a new genus. The stalked male genitalia would at first suggest that the genus is medeterine but examination of the mouthparts and other somatic characters shows that it is not. In fact considering the indistinct delineation of many subfamilies (ROBINSON, 1970; ULRICH, 1980), it is very difficult to place. The morphology of the male genitalia place it rather in the Peloropeodinae with encapsulated hypopygium than in the Sympycninae with an apical, sessile hypopygium.

MATERIAL AND METHODS

Material was collected by the first author in South Thailand by net sweeping along the banks of small rivers in rainforests. The specimens are preserved in alcohol in the collections of the Koninklijk Belgisch Instituut voor Natuurwetenschappen in Brussels. In the description of the hypopygium, the terms dorsal (D) and ventral (V) refer to the morphological position prior to the rotation of the male genitalia.

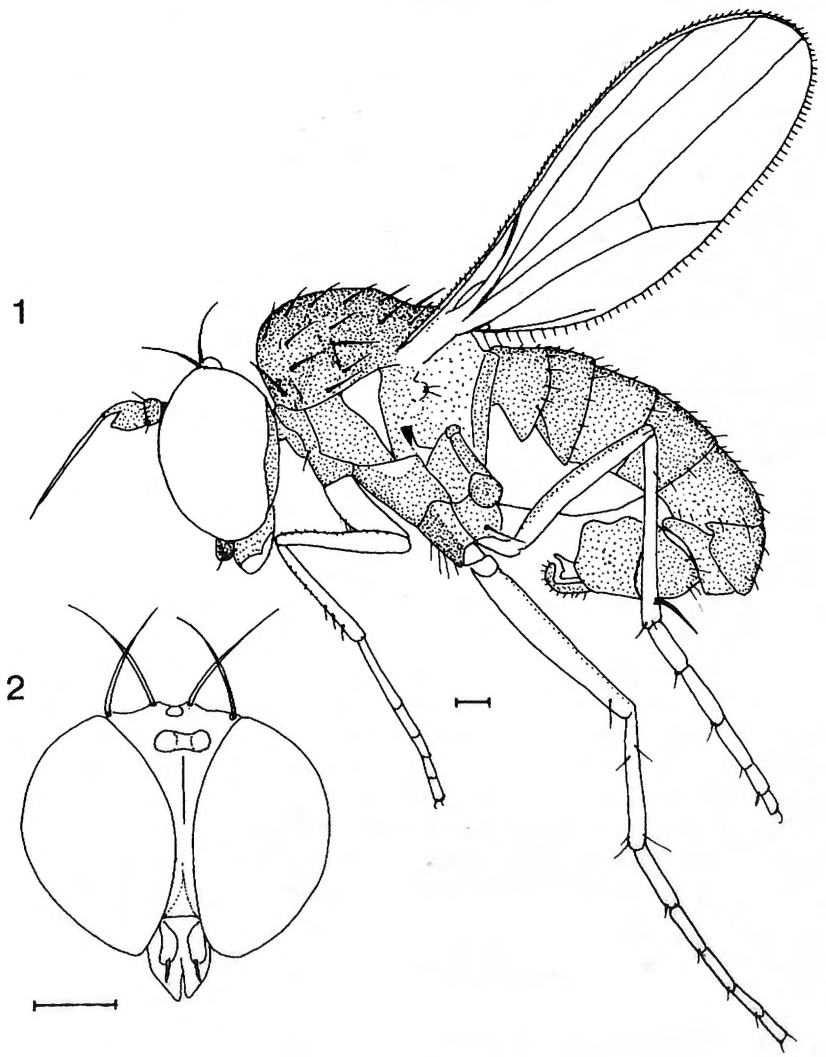
SYSTEMATICS

Griphomyia, gen. nov., Peloropeodinae

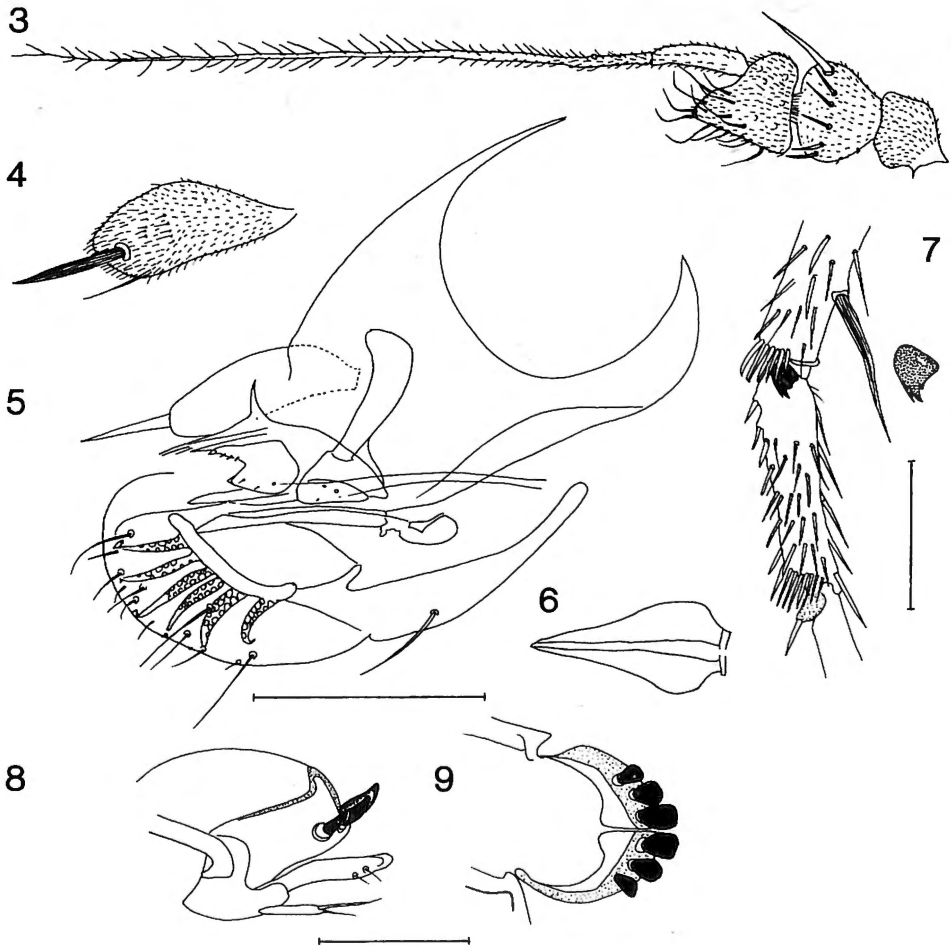
Figs 1-15

[Gender feminine; derivatio nominis: Gr. γρῖφος, a riddle]

Type species: *Griphomyia gravicaudata* sp. nov.



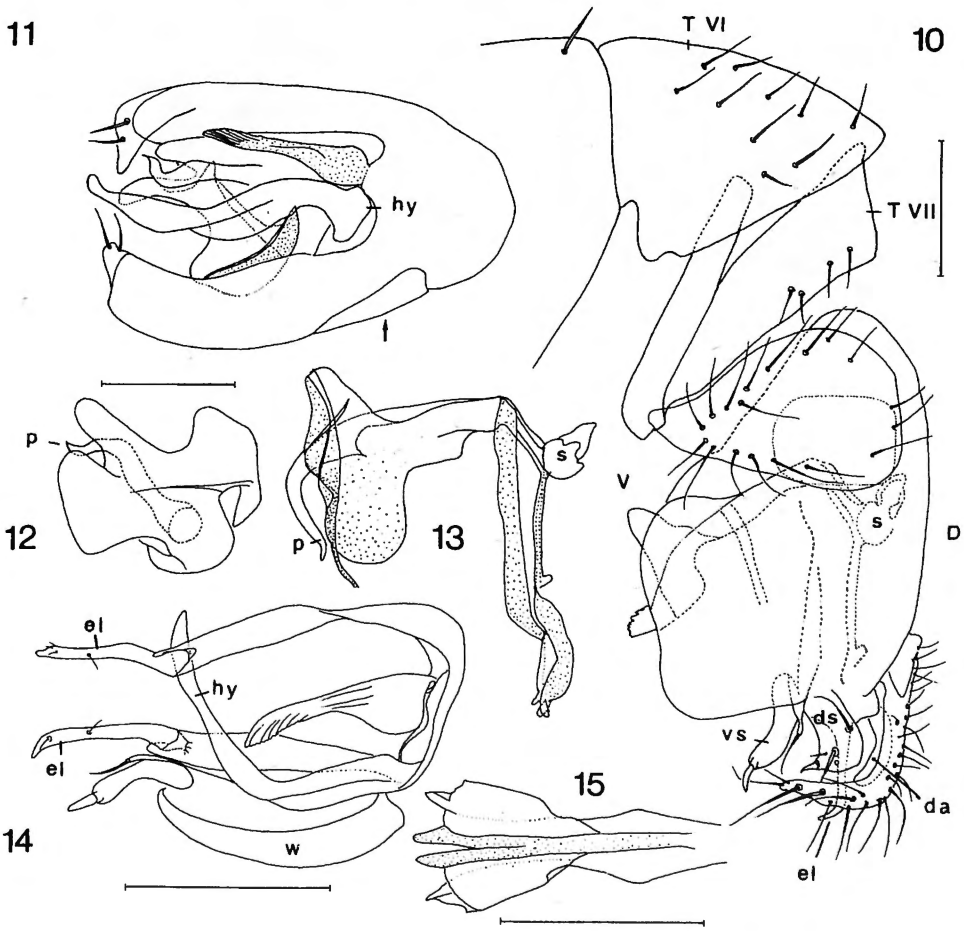
Figs 1-2. – *Griphomyia gravicaudata* gen. nov. sp. nov., male – 1. habitus. – 2. head in front. (Scale: 0.1 mm)



Figs 3-9. – *Griphomyia gravicaudata* male – 3. antenna – 4. palp – 5. mouthparts – 6. hypopharynx – 7. tip of hind tibia and metatarsus posteriorly with detail of multidenticulate plate; female – 8. ovipositor laterally – 9. tip ovipositor dorsally. (Scale: 0.1 mm)

Diagnosis

Small flies (less than 2 mm) with a rather hunch-backed appearance and very sparely bristled legs. Vertex rounded, occiput flat. Pseudotracheae symmetrically sclerotized. Antennae short; arista dorsal. Thorax with a flattened area in front of scutellum. Acrostichals very short, uniseriate; 5 dorsocentrals. Hind coxa with a feeble exterior bristle. Mid femur with a distinct anterior preapical; hind femur with a weak anterior preapical. First segment of hind tarsus shorter than second segment. Hypopygium relatively large, stalked. Segment VII long, tergum bristled.



Figs 10-15. – *Griphomyia gravicaudata*, male genitalia – 10. stalked hypopygium laterally (D: dorsal; V: ventral) – 11. hypopygium ventrally, arrow indicates foramen – 12. aedeagus dorsally – 13. aedeagus laterally, connected to sperm pump (S) and dorsal appendage – 14. epandrium ventrally – 15. tip of dorsal appendage ventrally (da: dorsal appendage; el: epandrial lobes; hy: hypandrium; p: penis; s: sperm pump; vs: ventral surstylus; w: wall of epandrium). (Scale: 0.1 mm)

Description

Head, seen from in front, nearly round (Fig. 2). Vertex evenly rounded, surpassing upper level of eyes, with a low ocellar callus. Upper postcranium convex above neck, flattened upwards. Frons moderately wide, narrowing towards antennae. Face narrowing downwards beneath antennae and slightly widening again towards clypeus. Palpi and rostrum small. Pseudotracheae symmetrically sclerotized (Fig. 5). Hypopharynx straight in profile; its base widened (Fig. 6). Epipharyngeal sclerites with 3 prongs. 2 not very long, diverging ocellars; 2 converging verticals, about as long as the ocellars, inserted high on

vertex near eye margins; 2 tiny postocellars; no postverticals. Postoculars very short, uniseriate. *Antennae* short (Fig. 3). First segment small, deeper than long, bare. Second segment larger and deeper than first segment, deeper than long, with very short marginal bristlets, that are longest on upper side. Third segment short, triangular, with blunt apex; arista dorsal.

Thorax high-vaulted, with a flattened area in front of scutellum. Acrostichals very short, uniseriate. 5 dorsocentrals, the hindmost of which is a little longer than the four preceding ones, that are nearly equal in length. Humerus with 1 bristle and 1 small hair. 2 notopleurals, 1 posthumeral, 2 presuturals, 2 supraalars, 1 postalar. Scutellum with 2 not very long marginals, without laterals. One propleural bristle.

Legs long, slender, very sparely bristled. Hind coxa with a thin, weak exterior bristle. A distinct anterior preapical on mid femur, and a weak anterior preapical bristle on hind femur. Mid tibia with 1 anterodorsal and 1 posterodorsal near base. Legs of ♂ unadorned. First segment of hind tarsus shorter than second segment.

Wing relatively short, with rounded apex. Apical part of m1+2 angled slightly upwards after tp, running from there to wing tip with a very feeble upward curvature; wing boss at middle of apical part of m1+2. r4+5 nearly straight, apically more or less parallel to m1+2. Distance between m1+2 and r4+5 greater than distance between r4+5 and r2+3. Anal vein present, running close to wing margin. Axillary lobe not developed (Fig. 1).

Abdomen of ♂ with 7, of ♀ with 5 visible segments, cylindrical, longer than thorax. Hypopygium rather large, stalked (Figs 1, 10). Segment VII long with tergum bristled. Epandrial foramen left lateral (Fig. 11). Base of epandrium (Fig. 14) with paired appendages, one presumed to be the hypandrium. Dorsad of these appendages a second set of long, extensions, probably homologous with the epandrial lobes, each composed of a basal segment articulating with an apical segment. Hypandrium articulating with base of epandrium (Fig. 14). Aedeagus irregularly multilobed with a central, twisted penis (Fig. 12). Dorsal and ventral surstyli present, attached to tip of epandrium. Dorsal appendage long, with an enlarged tip bearing spines (Fig. 15). Cerci centrally separated, square-shaped. Oviscapt with a row of 6 short, broad acanthae (Figs 8, 9).

Differential diagnosis:

The new genus can be distinguished from the other Peloropeodinae by the presence of a distinct anal vein which runs parallel to the hind margin of the wing, an undeveloped axillary wing lobe and a stalked hypopygium which lies free under the preabdomen.

***Griphomyia gravicaudata*, spec. nov.**

(Figs 1-15)

Male

Body length 1.7 mm; wing length 1.3 mm.

Head. Frons and face with blackish ground colour. Eyes nearly touching each other below middle of face (Fig. 2). Palpi brown, each with a small, black apical bristlet. Rostrum dark brown. Occiput black. Postoculars all black. *Antennae* (Fig. 3) dark brown. Third segment about as long as deep, triangular, with a rather blunt apex. Arista dorsal, more than 3 times as long as antenna, shortly pubescent; basal aristal segment more than half as long as third antennal segment.

Thorax. Mesoscutum and scutellum greenish black, feebly shining. Pleurae brownish black to dark brown, partly with a green metallic gloss.

Legs. Fore coxa yellow, mid and hind coxae brown. Legs, including trochanters, yellow. Tarsi indistinctly darkened towards their tips. Fore leg: coxa anteriorly with few, very short, dark hairs; at apex a few short and weak bristles. Trochanter bare. Femur and tibia practically without bristles; tibia about as long as femur. Length of tibia and tarsal segments (in mm): 0.35: 0.21: 0.08: 0.07: 0.04: 0.05. Mid leg: coxa anteriorly with few, very short hairs. Trochanter with a very small bristlet. Femur without bristles, except for a distinct anterior preapical and 1 or 2 tiny posteroventral preapical bristles. Tibia slightly longer than femur; 1 anterodorsal and 1 posterodorsal at basal fourth; a few short and thin apicals. Length of tibia and tarsal segments (in mm): 0.55: 0.24: 0.15: 0.11: 0.08: 0.07. Hind leg: coxa with a black exterior bristle; in some specimens a tiny second bristle. Trochanter bare. Femur without bristles, except for a tiny anterior preapical. Tibia slightly longer than femur; a tiny anterodorsal at basal fourth; 1 dorsal bristle near apex, preceded by some lengthened hairs. Tip of tibia with the usual posteroventral preapical comb and in addition a black denticulate plate (Fig. 7). Length of tibia and tarsal segments (in mm): 0.53: 0.16: 0.2: 0.15: 0.1: 0.07.

Wing. Hyaline, very feebly brownish tinged. Tip about half as long as apical part of m3+4. Halters yellow. Squamae yellow, with black border, and black cilia.

Abdomen. Above blackish brown, feebly shining; sterna brownish; unsclerotized parts yellowish. Hairs on terga minute, black; hindmarginal bristles extremely short, black, longer only on first tergum. Hypopygium brownish black; cerci small, brown.

Female

Body length 1.55 mm; wing length 1.3 mm.

Resembles the male. Face at its narrowest point about as wide as the distance between the ocellar bristles. Palpi and rostrum slightly larger than in ♂ and more yellowish brown. Oviscapt yellow, with a row of 6 small black acanthae (in allotype: 2 black acanthae on each side, and 2 yellowish acanthae in the middle).

Type material:

Holotype male and allotype female: Thailand, Phang-nga, Ao Luk, 15.iv.1996 (swept along brook and swamp in rainforest, sample n° 96076; leg. P. GROOTAERT).

Paratypes. 11 ♂, 5 ♀ from same sample as holotype; Phang-nga, Sa Nangmanora, 14.iv.1996, 11 ♂, 5 ♀ (along water falls; sample n° 96074), 15.iv.1996, 10 ♂ (sample n° 96075); Bok kai waterfalls, 4.iv.1996, 2 ♂ (sample n° 96022).

DISCUSSION

It is not easy to determine the exact phylogenetic position of the new genus since it is commonly known that the division of the Dolichopodidae into subfamilies (ROBINSON, 1970; ULRICH, 1980) is far from satisfactory and the phylogeny of the subfamilies has hardly been studied. The definition of the Medeterinae (BICKEL, 1987a; GROOTAERT & MEUFFELS, 1997) is now well delineated although in the genus *Medetera* problems have still to be resolved. Also the Sciapodinae and their position have been clarified by BICKEL (1994). The morphology of the mouthparts helps also in the classification of the subfamilies (SATO, 1991).

The presence of a dorsal arista, symmetrically sclerotized pseudotracheae, epipharyngeal sclerites consisting of three prongs, and the presence of a wing boss all indicate that *Griphomyia* is a quite derived genus and so it does not belong to the ancestral groups such as the Medeterinae (BICKEL, 1987a), Achalcinae (GROOTAERT & MEUFFELS, 1997b) nor the Babindellinae (BICKEL, 1987b). Nevertheless, *Griphomyia* possesses some plesiomorphic characters such as the presence of a well sclerotized anal vein, a stalked hypopygium and the mesoscutum being flattened in front of the scutellum. These symplesiomorphies should not confuse the phylogeny. They simply persisted.

We assume that the presence of a wing boss is a unique apomorphic character which originated only once as a result of the disappearance of vein M2 (BICKEL, 1994: 28). This vein probably disappeared in other groups as well, but, as in the Medeterinae, without resulting in a wing boss. In its turn, the wing boss may disappear as well, as it does in the genus *Teuchophorus*, the sistergroup of *Sympycnus*, where the boss probably moved towards the cross vein resulting in a turning up of the base of the apical portion of vein M (MEUFFELS & GROOTAERT, 1986).

Griphomyia seems to fit best in the subfamily Peloropeodinae as proposed by ROBEINSON (1970: 56). The latter subfamily is however not accepted by ULRICH (1980), who merged it again with the Sympycninae. The stalked hypopygium of *Griphomyia* is a plesiomorphic feature in comparison to the apical, sessile hypopygium of many Sympycninae and therefore it would be confusing to include *Griphomyia* into the Sympycninae. ROBINSON (1970) remarks that in the Peloropeodinae, Enliniinae and Medeterinae the penis-aedeagus mechanism is usually more distorted in shape and restricted to the middle and apical parts of the hypopygium. The Diaphorinae and Sympycninae, related sistergroups, have a rounded basal and ventral surface along which runs the penis-aedeagus system. A thorough revision of the more than 50 genera of the Sympycninae is urgently needed in order to redefine the subfamily and its boundaries.

NEGROBOV (1991) still accepts the Peloropeodinae and places even the genus *Acropsilus* in that subfamily. The latter genus which is very proliferic in the Asiatic region, has comparable mouthparts and also a stalked hypopygium, the stalk composed of a long abdominal segment VII. Although there are many differences with *Griphomyia*, both genera have a curious dorsal preapical bristle on the hind tibia (GROOTAERT & MEUFFELS, in litt.) in common.

ACKNOWLEDGEMENTS

The first author (P. G.) acknowledges a grant from the National Fund for Scientific Research (F.K.F.O. project n° 2.9008.90). Dr. Marc POLLET critically reviewed the manuscript.

REFERENCES

BICKEL, D.J. (1987a) – A Revision of the Oriental and Australasian *Medetera* (Diptera: Dolichopodidae). *Records of the Australian Museum* , **39**: 195-259.

BICKEL, D. J. (1987b) – Babindellidae, a new subfamily of Dolichopodidae (Diptera) from Australia, with a discussion of symmetry in the dipteran male postabdomen. *Entomologica scandinavica* **18**: 97-103.

BICKEL, D.J. (1994) – The Australian Sciapodinae (Diptera: Dolichopodidae); with a Review of the Oriental and Australasian Faunas, and a World Conspectus of the Subfamily. *Records of the Australian Museum*, supplement **21**: 1-394.

DYTE, C.E. (1975) – Family Dolichopodidae. In M.D. DELFINADO and D.E. HARDY (Eds), *A Catalog of the Diptera of the Oriental Region*, Volume II: 212-258.

GROOTAERT, P. & H.J.G. MEUFFELS (1997) – Dolichopodidae (Diptera) from Papua New Guinea XIV. *Paramedetera*, a new genus in the Medeterinae. *Invertebrate Taxonomy* , **11**: 309-319.

GROOTAERT, P. & H.J.G. MEUFFELS (1997b) – Dolichopodidae (Diptera) from Papua New Guinea XV. *Scepastopyga* gen. nov. and the establishment of a new subfamily, Achalcinae. *Journal of Natural History,* **31**: 1587-1600.

MEUFFELS, H.J.G. & P. GROOTAERT (1986) – Dolichopodidae (Diptera) from Papua New Guinea IV. New species of the genus *Teuchophorus* Loew, 1857. *Indo-Malayan Zoology* , **3**: 217-262.

NEGROBOV, O.P. (1991) – Family Dolichopodidae. In A. SOOS & L. PAPP (Eds), *Catalogue of Palaearctic Diptera*: 11-139.

ROBINSON, H. (1970) – The subfamilies of the Family Dolichopodidae in North and South America (Diptera). *Papéis Avulsos de Zoologia* , **23**: 53-62.

SATO, M. (1991) – Comparative Morphology of the Mouthparts of the Family Dolichopodidae (Diptera). *Insecta Matsumurana* , **45**: 49-75.

ULRICH, H. (1980) – Zur systematischen Gliederung der Dolichopodiden (Diptera). *Bonner zoologische Beiträge* , **31**(3-4): 385-402.

Belg. J. Zool. - Volume 127 (1997) - issue 2 - pages 115-122 - Brussels 1997

Received : 12 February 1997

PRESENCE OF DOUBLE SPINES ON THE SECOND PREURAL CENTRUM OF THE TURBOT (SCOPHTHALMUS MAXIMUS L., PLEURONECTIFORMES: SCOPHTHALMIDAE)

BRUNO CHANET(1) AND FRANCE WAGEMANS(2)

(1)Ressources halieutiques – Poissons marins – Faculté des sciences,
Université de Bretagne Occidentale, 6 avenue Le Gorgeu, BP 809, 29285 Brest cedex, France.
(2)Service de morphologie fonctionnelle, Institut de Zoologie,
22 quai de van Beneden, B-4020 Liège, Belgium.
e-mail: chan2837@eurobretagne.fr

Abstract. The literature concerning the presence of double spines on the second preural centrum in flatfishes is being reviewed and the different hypotheses proposed to account for this phenomenon are presented. A study using laboratory-reared specimens of the development of the caudal endoskeleton of the turbot on gives new data to explain the presence of this feature. Such double spines seem to be the result of a fusion during the ontogeny between the third preural centrum and the second preural centrum.

Key words: Pleuronectiformes, turbot, caudal endoskeleton, development.

INTRODUCTION

The caudal endoskeleton of pleuronectiform fishes has been studied by a large number of authors (BARRINGTON, 1937; MONOD, 1968; HENSLEY & AHLSTROM, 1984; CHAPLEAU, 1993 and further references therein). Normally, the second preural centrum possesses one neural spine and one haemal spine (Fig. 1), these spines often being broader than the other ones. But many authors showed the frequent occurrence of double spines on this centrum, i.e. the second preural centrum (PU2) possesses two neural spines and/or two haemal spines (CHABANAUD, 1937; BARRINGTON, 1937; FUTCH, 1977; HENSLEY & AHLSTROM, 1984). This feature is common in Pleuronectiformes (HENSLEY & AHLSTROM, 1984) and different hypotheses have been proposed to explain it:

1) these double spines are the result of the fusion of one epural and one hypural with respectively the neural spine and the haemal spine of the centrum (COLE & JOHNSTONE, 1902).

2) these double spines are the result of the fusion of the two last neural arches together on the one hand, and of the two last haemal arches on the other hand (BARRINGTON, 1937).

3) these double spines are the result of the fusion of the two last preural centra (HENSLEY & AHLSTROM, 1984, following ROSEN, 1973).

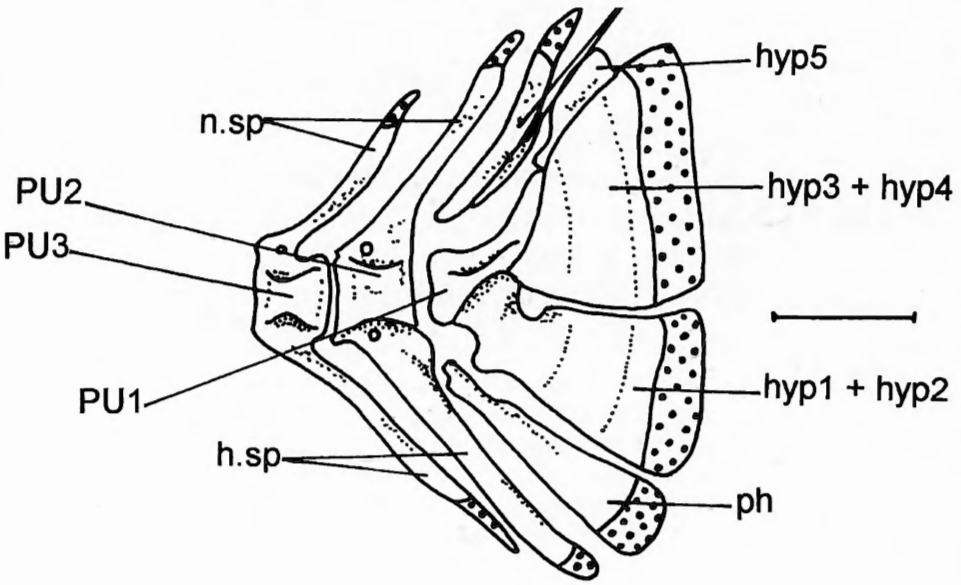


Fig. 1. – Caudal endoskeleton of a young turbot (*Scophthalmus maximus*), 29th day of development (SL=16 mm), left lateral view. The centrum of PU2 bears one neural spine and one haemal spine. The scale indicates 1 mm. The areas with black circles are cartilaginous regions. [ep: epural; h.sp: haemal spine; hyp: hypural; n.sp: neural spine; PU: preural centrum].

HENSLEY & AHLSTROM (1984) stressed that a detailed survey of this feature was needed. Recently, we have had the opportunity to study the development of the caudal endoskeleton of a pleuronectiform fish: the turbot, *Scophthalmus maximus* L., 1758, Scophthalmidae. Some specimens showed double spines on the second preural centrum. The purpose of this paper is to present our results and to give data in order to corroborate or to invalidate these hypotheses and thus to try to explain the occurrence of these double spines.

MATERIAL AND METHODS

Scophthalmus maximus fry were raised in the aquaculture-station of France Turbot-NATA (Noirmoutiers, France) at 15°C. Batches of 40 fry were sampled on days 0 to 31 and batches of 30 fry on days 33 to 61 post hatching. The fry were fixed in a $CaCO_3$ buffered 10% formalin solution and were cleared in trypsin. Some of them were stained with alcian blue to reveal the cartilages and others with alizarine, which stains calcified bones, according to Taylor and Van Dyke's method (1985). It was possible to stain the most young stages simultaneously with alizarine and alcian. Finally, the fry were stored in glycerin. A 6 month-old specimen was cleared in trypsin, stained with alizarine and stored in glycerin according to Taylor and Van Dyke's method. The specimens, 270 larval and

juvenile turbots, have been studied with a binocular Wild M10 Leica dissecting microscope at 8x magnification, and a drawing tube. The length from the tip of the snout to the posterior margin of hypural elements(standard length - SL) was measured for each specimen.

RESULTS

The caudal skeleton elements can be detected as early as the 14th day after hatching. At this stage, they are merely cartilaginous buds. They develop progressively and their ossification begins on the 22nd day. This process first occurs in the centra, at the base of the spines and in the median part of the hypurals, consequently, the latter still have both extremities, proximal and distal, made of cartilage (Figs 1-2-3). This cartilaginous proximal part is gradually replaced by bone, whereas the cartilaginous distal part remains for a longer time. 123 of our specimens (45%) possess only one haemal spine and one neural spine on the PU2 centrum at every stage of the development (Figs 1-4-5). They are long, slender; the neural spines are postero-dorsally directed, whereas the haemal spines are postero-ventrally orientated. 55% of the studied specimens show double spines on the second preural centrum (Figs 2-3). On these specimens, the PU2 centrum bears two neural

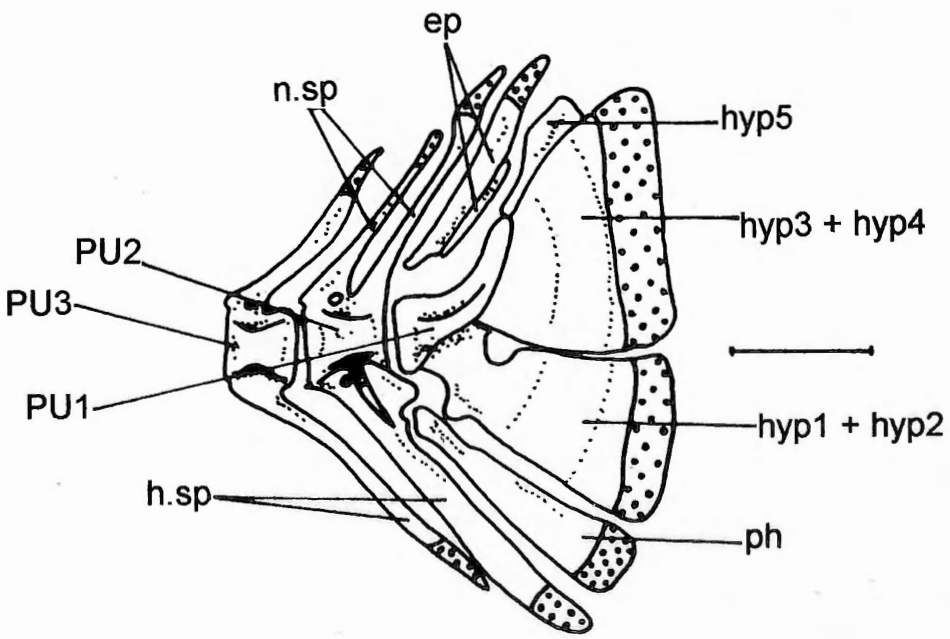


Fig. 2. – Caudal endoskeleton of a young turbot (*Scophthalmus maximus*), 29th day of development (SL=17 mm), left lateral view. The centrum of PU2 bears double neural spines and a broader haemal spine. The scale indicates 1 mm. The areas with black circles are cartilaginous regions. [ep: epural; h.sp: haemal spine; hyp: hypural; n.sp: neural spine; PU: preural centrum].

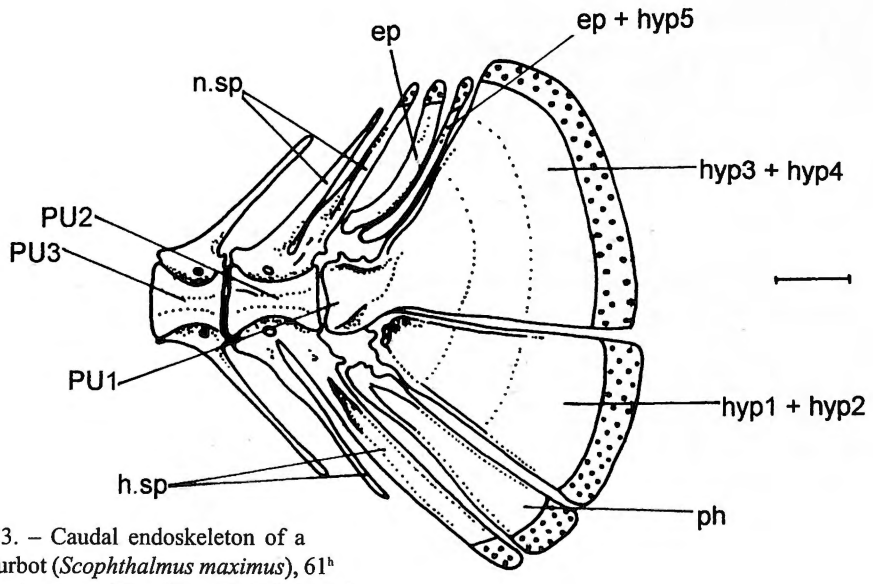


Fig. 3. – Caudal endoskeleton of a young turbot (*Scophthalmus maximus*), 61h day of development (SL =28 mm), left lateral view. The centrum of PU2 bears double neural and haemal spines. The scale indicates 1 mm. The areas with black circles are cartilaginous regions. [ep: epural; h.sp: haemal spine; hyp: hypural; n.sp: neural spine; PU: preural centrum].

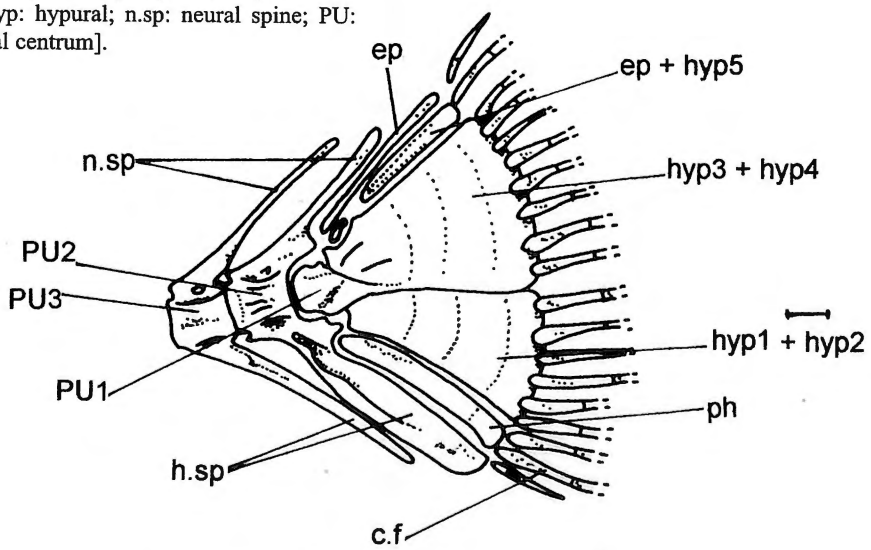


Fig. 4. – Caudal endoskeleton of a young turbot (*Scophthalmus maximus*), 6 month of development (SL= 68 mm), left lateral view. The centrum of PU2 bears one neural spine and one haemal spine. The scale indicates 1 mm. The areas with black circles are cartilaginous regions. [c.f: caudal fin ray; ep: epural; h.sp: haemal spine; hyp: hypural; n.sp: neural spine; PU: preural centrum].

spines and two distinct haemal spines or two neural spines and a very broad haemal spine (Figs 2-3). The bases of these double spines are very close together. These specimens are not only different from the others by the presence of these double spines, but they also differ in the number of vertebrae. The specimens bearing one neural spine and one haemal spine on the PU2 centrum possess 10-11 precaudal vertebrae and 20 caudal vertebrae. These numbers are constant, but each specimen which possesses double spines on the second preural centrum has only 19 caudal vertebrae. 25 of the studied specimens (9%) show no double spines and have 19 caudal vertebrae (Fig. 5). Moreover, the caudal endoskeleton of all the specimens is built on the same pattern. They possess five hypurals, one parhypural, and two epurals. Hypurals 1 and 2, on the one hand, and hypurals 3 and 4 on the other hand, are fused in two distinct plates (Figs 1-2-4) and the upper hypural plate (formed by the fusion of hypurals 3 and 4) is fused with the centrum of PU1. Before day 60, each of our young specimens had two epurals (Fig. 2) whereas in older fish, the more posterior epural becomes fused with hypural 5 (Fig. 4), as FUTCH (1977) described it in *Trichopsetta ventralis* (GOODE AND BEAN) 1885 (Bothidae).

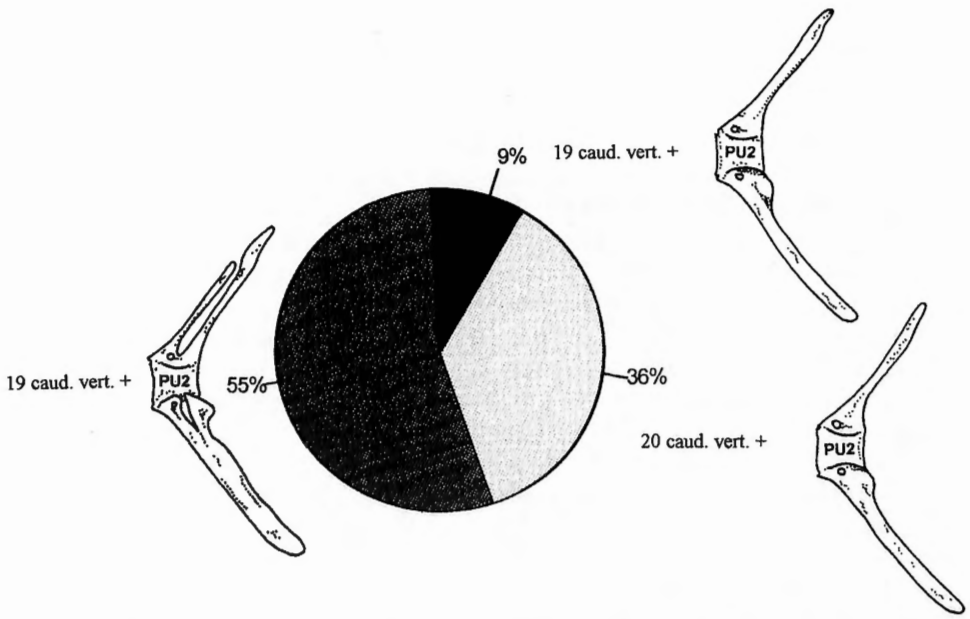


Fig. 5. – Representation of the numerical percentage of the number of the caudal vertebrae related to the number of neural and haemal spines on the centrum of PU2 in *Scophthalmus maximus*.

DISCUSSION

The presence of double spines on the second preural centrum has been mentioned by many authors, with several explanations proposed. COLE & JOHNSTONE (1902: 194) noticed in the plaice (*Pleuronectes platessa* L. 1758, Pleuronectinae) that: «the posterior

shafts (of the second preural centrum spines) so closely resemble the succeeding epural and hypural respectively as to suggest that one epural above and an hypural below have fused to the laminate portions, which latter are undoubtedly similar to and perhaps represent the neural and haemal spines in front. As, however, we have no positive evidence for such a fusion, the spines in question are here described as simple neural and haemal spines». BARRINGTON (1937:468), in his study about the development of the tail in the plaice and the cod (*Gadus morrhua* L. 1758, Gadidae), showed that: «the penultimate vertebra (= second preural centrum) comes to bear two dorsal and two ventral arches as a result of their fusion during development». HENSLEY & AHLSTROM (1984:676) reviewed the relationships of flatfishes and considered that the presence of two neural spines on the second preural centrum is the result of « a fusion of this vertebra with an anterior one bearing a spine». Actually, they followed ROSEN (1973:499): «the fusion of the preural caudal centra may explain the presence of two neural spines on the PU2 centrum». ROSEN (1973) stressed that this kind of event is frequent within higher euteleostean groups, but HENSLEY & AHLSTROM (1984) provided no ontogenetic data to corroborate this vertebral fusion. Are these double neural spines or double haemal spines anomalies? The former authors stressed the fact that this feature was not so rare and that a detailed survey of their occurrences was needed. A review of the bibliography shows that this feature has been already observed by several authors. CHABANAUD (1937:377) represented the caudal skeleton of *Samaris cristatus* GRAY 1831 (Samaridae), with two neural spines on the PU2 centrum. In the same article (:378), a caudal skeleton of *Solea solea* (= *Solea vulgaris* QUENSEL 1806, Soleidae) is shown with double neural and haemal spines. Usually in this species, the centrum bears only one neural spine and one haemal spine (FABRE-DOMERGUE & BIÉTRIX, 1905; CHAPLEAU & KEAST, 1988). When BERG (1941) described *Eobothus vialovi* BERG 1941, a probable fossil flatfish from the Lower Eocene of Uzbekistan (CHANET & SCHULTZ, 1994), he mentioned two haemal arches and two haemal spines on the PU3 and PU2 centra. In 1969, AMAOKA, in his study about the Japanese sinistral flatfishes, showed doubles spines (haemal and neural) on the PU2 centrum of three bothid species: *Taeniopsetta ocellata* (GÜNTHER 1880), *Parabothus coarctatus* (GILBERT 1905) and *Laeops kitaharae* (SMITH & POPE 1906). HENSLEY (1977:696) mentioned a particular larva of *Engyophrys senta* GINSBURG 1933 (Bothidae): «one 7.0 mm larva has two neural spines (unossified) associated with the area of the notochord where the second preural centrum develops». SAKAMOTO (1984) showed that some flatfish species possessed two haemal spines on the PU2 centrum: one Pleuronectinae (*Hippoglossus stenolepis* SCHMIDT 1904), one Rhombosoleinae (*Ammotretis elongatus* MC CULLOCH 1914) and one Samaridae (*Samariscus latus* MATSUBARA & TAKAMUKI, 1951). FUTCH (1977) indicated that one specimen of *Trichopsetta ventralis* (Bothidae) showed double neural spines on the PU2 centrum. MUNROE (1996) explained the presence of multiple neural spines (from one to four) on the caudal centra of one reversal specimen of *Symphurus vanmelleae* CHABANAUD 1952 (Cynoglossidae) as the result of fusion between caudal vertebrae.

Our data show that, in the turbot, the presence of double spines on the PU2 centrum seems to be the result of a fusion between two centra: the antepenultimate (PU3) and the penultimate (PU2) vertebrae, as ROSEN (1973) and HENSLEY & AHLSTROM (1984) thought. This phenomenon is not just the result of the fusion between the arches as BARRINGTON (1937) indicated. And what about a probable capture of one hypural and one epural ele-

ment as COLE & JOHNSTONE (1902) proposed? If such a mechanism occurred the numbers of epurals and hypurals would have been affected and the fishes bearing double spines would have possessed a peculiar caudal endoskeleton. But, none of the specimens we have examined shows such differences. Although young turbot have two epurals, older fish have five hypurals and one epural, and hypural 5 present in the adult is the result of fusion between the posterior epural and hypural 5.

The study of the development of the turbot gives arguments to say that the occurrence of double spines on the second preural centrum is the result of the fusion of the third preural centrum and the second preural centrum. Such an hypothesis seems to be valid to explain the presence of these double spines in the turbot. But, because it is possible to think that the double spines present on the penultimate centrum of the plaice (COLE & JOHNSTONE, 1902; BARRINGTON, 1937) or *Trichopsetta ventralis* (FUTCH, 1977) may be the result of different mechanisms occurring during the development of these fishes, this hypothesis has to be confirmed by ontogenetic studies on some other flatfish species. One can argue that these abnormalities are the consequence of the fact that the studied specimens were not reared in natural conditions but in laboratory conditions. Many authors reported numerous incidences of morphological and skeleton abnormalities associated with aquacultural practices for flatfishes (HOUDE, 1971; HEAP & THORPE, 1987; LAGARDÈRE *et al.*, 1993). In field sampled specimens, however the presence of double spines on the PU2 centrum is not rare. Pending new evidence, the simplest solution to explain the presence of double spines on the PU2 centrum in flatfishes is to suppose that these features have been formed by the same mechanism as proposed here for the turbot.

ACKNOWLEDGEMENTS

We have the opportunity here of thank: Mr Leclercq (France Turbot, Noirmoutiers, France), Mrs Lebègue (NATA, Noirmoutiers, France) for the rearing of the specimens, P. Vandewalle (Institut de Zoologie, Liège, Belgium), F. Meunier (MNHN, Paris, France) for their advice, D. Baron (Brest, France) for editing our English first text, Prof. E. Schokaert (Limburgs Universitair Centrum, Diepenbeek, Belgium) and two anonymous reviewer who greatly improved the quality of this article. FW is a researcher at the Fonds pour la Recherche dans l'Industrie et l'Agriculture.

REFERENCES

AMAOKA, K. (1969) – Studies on the sinistral flounders found in the waters around Japan.- Taxonomy, anatomy and phylogeny. *J. Shimonosoki Univ. Fish.*, **18**: 65-340.

BARRINGTON, E.J.W. (1937) – The structure and development of the tail in the plaice (*Pleuronectes platessa*) and the cod (*Gadus morrhua*). *Quart. J. Microsc. Sci.*, **79**: 447-469.

BERG, L.S. (1941) – *Eobothus vialovi* n. sp. An Eocene flatfish from the western Than-Shan. – *Bull. Acad. Sci. URSS, cl. Sci. Biol.*, **3**: 480-485.

CHABANAUD, P. (1937) – L'extension prorse périphérique, la contraction axiale post-hypophysaire et l'anisoconie rhachidienne des téléostéens dyssymétriques. *Bull. Soc. zool. Fr.*, **62**: 368-385.

CHANET, B. & O. SCHULTZ (1994) – Pleuronectiform fishes from the Upper Badenian (Middle Miocene) of St. Margarethen (Austria). *Ann. Naturhist. Mus. Wien*, **96A**: 95-115.

CHAPLEAU, F. (1993) – Pleuronectiform relationships: a cladistic reassessment. *Bull. Mar. Sci.*, **52** (1): 516-540.

CHAPLEAU, F. & A. KEAST (1988) – A phylogenetic reassessment of monophyletic status of the family Soleidae, with comments on the suborder Soleoidei (Pisces, Pleuronectiforms). *Can. J. Zool.*, **66**: 2797-2810.

COLE, F.J. & J. JOHNSTONE (1902) – *Pleuronectes*: the Plaice. *Trans. Liverpool Mar. Biol. Soc.*, **8**: 145-396.

FABRE-DOMERGUE E. & E. BIETRIX (1905) – Développement de la sole (*Solea vulgaris*). Introduction à l'étude de la pisciculture marine. Vuibert et Nony, Paris 247 pp.

FUTCH, C.R. (1977) – Larvae of *Trichopsetta ventralis* (Pisces: Bothidae), with comments on intergeneric realtionships within the Bothidae. *Bull. Mar. Sci.*, **27** (4): 740-757.

HEAP, S.P. & J.P. THORPE (1987) – A preliminary study of comparative growth rates in O. group malpigmented and normally pigmented turbot, *Scophthalmus maximus* (L.), and turbot-brill hybrids *S. maximus, S. rhombus* (L.). *Aquaculture*, **60**: 251-264.

HENSLEY, D.A. (1977) – Larval development of *Engyophrys senta* (Bothidae), with comments on intermuscular bones in flatfishes. – *Bull. Mar. Sci.*, **27**: 681-703.

HENSLEY, D.A. & E.H. AHLSTROM (1984) –Pleuronectiforms: Relationships. In: Ontogeny and Systematics of Fishes (Moser H. G., W. J. Richards, A. W. Kendall, M. P. Fahay, S. L. Richardson and D. M. Cohen, eds.). *Sp. Pub., Am. Soc. Ichth. Herp.*, **1**: 670-687.

HOUDE, E.D. (1971) – Developmental abnormalities of the flatfish *Achirus lineatus* reared in the laboratory. *U. S. Fish. Bull.*, **69**: 537-544.

LAGARDÈRE, F., M. BOULHIC & T. BÜRGIN (1993) – Anomalies in the cephalic area laboratory-reared larvae and juveniles of the common sole, *Solea solea*: oral jaw apparatus, dermal papillae and pigmentation. *Env. biol. fish.*, **36**: 35-46.

MONOD, T. (1968) –Le complexe urophore des poissons téléostéens. *Mém. Inst. Afr. N.*, **81**: 1-705.

MUNROE, T.A. (1996) – First record of reversal in *Symphurus vanmelleae* (Pleuronectiformes: Cynoglossidae), a deep-water tongue fish from the tropical eastern Atlantic. *Cybium,* **20(1)**: 47-53.

ROSEN, D.E. (1973) – Interrelationships of higher euteleostean fishes. In Interrelationships of fishes, P.H. Greenwood, R.S. Lies and C. Patterson, eds. *Zool. J. Linnean Soc.*, suppl. 1, **53**: 397-536.

SAKAMOTO, K. (1984) – Interrelationships of the family Pleuronectidae (Pisces: Pleuronectiformes). *Mem. Fac. Fish. Hokkaïdo University*, **31**: 95-215.

TAYLOR, W.R. & G.C. VAN DYKE (1985) – Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium,* **9**: 107-121.

Belg. J. Zool. - Volume 127 (1997) - issue 2 - pages 123-138 - Brussels 1997

Received : 18 February 1997

LES AGAMIDAE (REPTILIA, SQUAMATA) DU PALEOGENE D'EUROPE OCCIDENTALE

MARC AUGE (1) ET RICHARD SMITH (2)

(1) Laboratoire de Paléontologie des Vertébrés, Case 106, Université Paris VI, 4 place Jussieu, F-75252, Paris cedex 05 (France)

(2) Laekenveld 6, B-1780 Wemmel (Belgique)

Résumé. Un nouveau lézard agamidé, *Tinosaurus europeocaenus*, est décrit dans l'Eocène inférieur du gisement de Dormaal (Belgique). D'autres Agamidae de l'Oligocène français *Uromastyx europaeus* et *Agama galliae* sont révisés et leur diagnose amendée. Ainsi, le genre *Quercygama* nov. gen. est proposé pour *A. galliae*. Les fossiles d'Agamidae en Europe sont peu nombreux et peu diversifiés et la présence de cette famille sur ce continent peut être expliquée par une série d'invasions depuis l'Asie (ou l'Afrique).

Mots clefs: Agamidae, *Tinosaurus*, Eocène, Oligocène, Europe.

THE AGAMIDAE (REPTILIA, SQUAMATA) FROM THE PALEOGENE OF WESTERN EUROPE

Abstract. A new agamid lizard, *Tinosaurus europeocaenus* is described in the lower Eocene from Dormaal (Belgium). Other agamids from the French Oligocene, *Uromastyx europaeus* and *Agama galliae* are revised and their diagnoses are emended. The new genus *Quercygama* is proposed for *A. galliae*. The agamid fossil record of Europe is generally less diversified than in other lizard families and it is argued that the presence of Agamidae in Europe results from recurrent asian (or african) dispersals.

Key words: Agamidae, *Tinosaurus*, Eocene, Oligocene, Europe.

INTRODUCTION

On connaît des restes d'Agamidae dans l'Eocène inférieur européen depuis les travaux de HECHT & HOFFSTETTER (1962) (voir aussi GODINOT *et al.*, 1978). HECHT & HOFFSTETTER attribuèrent ces fossiles au genre *Tinosaurus* Marsh, 1872 pour lequel on ne dispose pas actuellement de diagnose bien établie, quoique l'on ait défini plusieurs espèces à l'intérieur du genre, notamment dans le Paléogène (surtout l'Eocène) d'Amérique du Nord et d'Asie (MARSH, 1872; GILMORE, 1943; HOU, 1974; DONG, 1965; LI, 1991a). Ultérieurement, on a retrouvé des restes d'Agamidae, toujours attribués au genre *Tinosaurus*, dans d'autres gisements de l'Eocène inférieur d'Europe occidentale: Condé-en-Brie, Avenay, Mutigny, Sézanne (MP8+9; AUGÉ, 1990) et Prémontré (MP10; RAGE & AUGÉ, 1993). Dans le nouveau matériel de Dormaal, récolté par R. SMITH, figu-

rent quelques mâchoires d'Agamidae en bon état, des dentaires notamment, qui permettent de préciser la position systématique de ce lézard. Ces nouveaux fossiles se prêtent à des comparaisons, notamment avec les espèces déjà décrites et attribuées à *Tinosaurus*. Une diagnose de ce genre est proposée ici à partir de ces comparaisons et on revoit brièvement la validité de l'ensemble des attributions d'espèces proposées dans le genre *Tinosaurus*. Après un hiatus qui commence au début de l'Eocène moyen et se prolonge au-delà de la Grande Coupure Eocène/Oligocène, on retrouve des Agamidae dans l'Oligocène des Phosphorites du Quercy où deux espèces ont été décrites, *Agama galliae* Filhol, 1877 et *Uromastyx europaeus* de Stefano, 1903. Cette dernière espèce a déjà fait l'objet d'une révision (AUGÉ, 1988) et elle ne sera que succintement évoquée. Des doutes récurrents ont été émis sur l'identité générique de *Agama galliae* (MOODY, 1980; ESTES, 1983a) et cette question est abordée dans ce travail.

Les Agamidae font partie de la super-famille des Acrodonta, qui comprend, selon ESTES *et al* (1988) et FROST & ETHERIDGE (1989) les Chamaeleonidae, les Agamidae et les Priscagamidae du Crétacé asiatique (BORSUK-BIALYNICKA & MOODY, 1984; ALIFANOV, 1989). La monophylie des Agamidae n'est pas acquise, ce taxon pourrait être para- ou polyphylétique, bien que l'une des deux versions du cladogramme des Acrodonta proposé par FROST & ETHERIDGE (1989), «topology 2» supporte la monophylie des Agamidae.

ABRÉVIATIONS

IRScNB: Institut royal des Sciences naturelles de Belgique, Bruxelles.
MNHN: Museum national d'Histoire naturelle (Paris).
UCBL: Université Claude Bernard, Lyon.
USTL: Université des Sciences et Techniques du Languedoc, Montpellier.

ÉTUDE SYSTÉMATIQUE

– ORDRE SQUAMATA OPPEL, 1811
– SUPER-FAMILLE ACRODONTA COPE, 1864
– FAMILLE AGAMIDAE GRAY, 1827

Genre *Tinosaurus* Marsh, 1872

Espèce type: *Tinosaurus stenodon* Marsh, 1872

Diagnose amendée. Dentaire de forme générale allongée et assez grêle. Dentition postérieure acrodonte subacrodonte avec l'apex des dents triconodonte. Présence de plusieurs dents pleurodontes à l'avant du dentaire, l'une d'elles pouvant être caniniforme. Sur le dentaire et sous la rangée dentaire existe un plateau dentaire bien marqué mais la base des dents acrodontes ne s'implante pas jusqu'à lui. Le sulcus Meckeli est ouvert à la fois mésialement et ventralement, au moins à l'avant du dentaire. Sur le maxillaire, la partie antérieure des processus prémaxillaires ne se relève pas. Le processus palatin du maxillaire est bien développé.

Composition spécifique. T. stenodon Marsh, 1872; *T. pristinus* Leidy, 1872; *T. doumuensis* Hou, 1974; *T. europeocaenus* nov. sp.; ?*T. lushihensis* Dong, 1965; ?*T. yuanquensis* Li, 1991a.

***Tinosaurus europeocaenus* nov. sp.**

(Fig. 1)

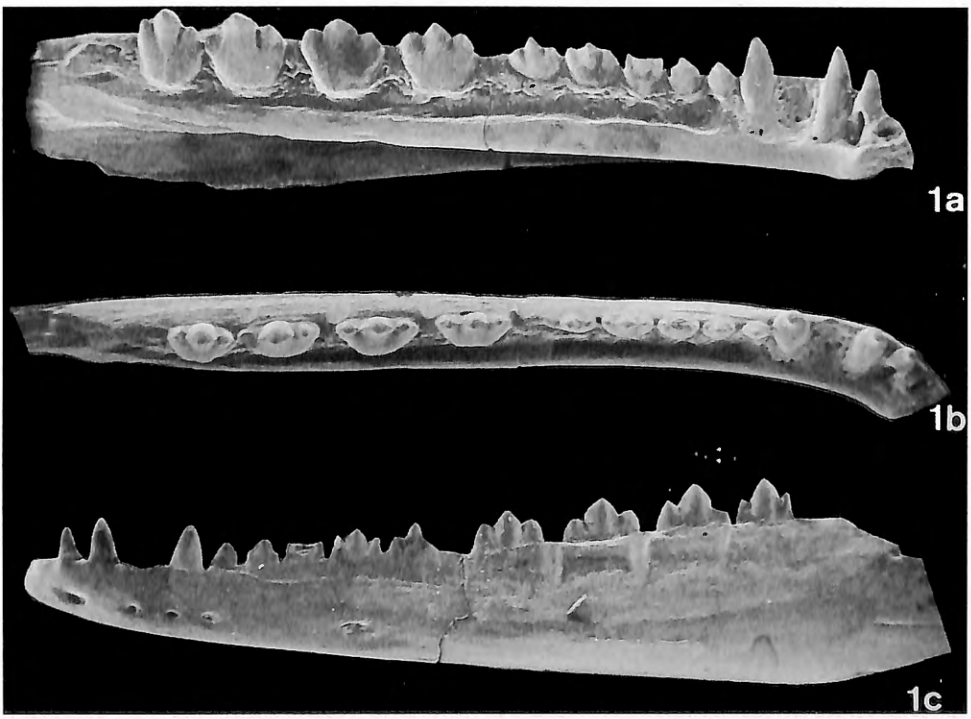


Fig. 1. – *Tinosaurus europeocaenus* nov. sp., dentaire, holotype, Eocène inférieur, Dormaal, IRScNB, n° R 202. 1a: vue linguale; 1b: vue dorsale; 1c: vue labiale. X 12,5

Holotype. Un dentaire gauche, partie postérieure abîmée, IRScNB R202, Fig. 1 a-c.

Localité type et âge. Dormaal, Belgique. Eocène inférieur, niveau MP7 de l'échelle biochronologique des mammifères paléogènes d'Europe (voir SMITH & SMITH, 1996).

Extension géographique et stratigraphique. Nord-Ouest de l'Europe, gisements de Dormaal (MP7), Avenay (MP8+9), Mutigny (MP8+9), Condé-en-Brie (MP8+9), Prémontré (MP10). Eocène inférieur, du niveau standard de Dormaal (MP7) à celui de Grauves (MP10).

Derivatio nominis. Contraction de Europe et Eocène, qui indique la répartition géographique et stratigrapique de l'espèce.

Matériel. Dormaal (MP7), collection R. Smith, un dentaire gauche complet, Fig. 1 a-c (IRScNB R202), trois dentaires gauches incomplets, un dentaire droit incomplet (DIII S), un dentaire droit incomplet, un maxillaire gauche incomplet (DIII RS), treize fragments de mâchoires dont trois dentaires gauches incomplets, trois dentaires droits incomplets, un maxillaire incomplet (DIIC RS). Dormaal, collection D. Delsate, un fragment de dentaire gauche. Avenay (MP8+9), partie antérieure d'un dentaire gauche, MNHN, AV 16464, fig. 3 *in* AUGÉ (1990). Mutigny (MP8+9), un maxillaire droit incomplet, MNHN, MU 16466, fig. 4 *in* AUGÉ (1990). Condé-en-Brie (MP8+9), partie postérieure d'un dentaire droit, MNHN, CB 16437, fig. 2 *in* AUGÉ (1990); un dentaire gauche et un maxillaire fragmentaires. Prémontré (MP10), un maxillaire incomplet.

Diagnose. T. europeocaenus diffère des autres membres du genre *Tinosaurus* par la présence d'un sulcus Meckeli assez largement ouvert à l'arrière du dentaire; par le faible développement du rebord mésial au niveau du bord ventral du dentaire; par la forme très allongée du dentaire. La morphologie des dents acrodontes sépare aussi *T. europeocaenus* des autres espèces du genre: ces dents portent des cuspides latérales importantes et sont largement séparées l'une de l'autre chez *T. europeocaenus.*

Description

Dentaire. La description concerne principalement l'holotype de l'espèce. Le bord ventral du dentaire est droit alors que le bord dorsal s'élève régulièrement de l'avant vers l'arrière. Autrement dit, le dentaire s'élargit régulièrement de l'avant vers l'arrière, bien que sa forme générale reste effilée. Il existe cinq foramens labiaux, le dernier est le plus important et il s'ouvre sous la neuvième position dentaire (comptée à partir de l'avant). Labialement, une ligne concave vers le haut sépare une surface inférieure, convexe vers l'extérieur, d'un plan supérieur, d'aspect rugeux, incliné labio-ventralement à linguo-dorsalement. Des sillons occlusaux, verticaux, peu profonds et situés entre les dents, divisent incomplètement cette surface. Postérieurement, le dentaire s'étend bien au-delà de la rangée dentaire. Quelques fossiles incomplets de Dormaal (niveaux DIII et DIIC) permettent d'observer la terminaison dorso-postérieure de l'os. Après la dernière dent, la paroi dorsale du dentaire se relève légérement pour former une ébauche de processus coronoïde qui ne s'élève pas au-delà de l'apex des dents. Une dépression importante occupe toute la face labio-postérieure du dentaire, après le niveau de la dernière dent et correspond certainement au contact avec les os post-dentaires. En vue linguale, le bord ventral du dentaire ne produit qu'un léger rebord interne, ce qui laisse le sulcus Meckeli exposé ventralement sur toute son étendue. Le sulcus Meckeli s'ouvre largement à l'arrière et il se rétrécit très graduellement jusqu'à la symphyse. D'abord dirigé ventro-mésialement, il prend une orientation purement ventrale sous la symphyse. Au-dessus du sulcus Meckeli, on observe un relief arrondi, représentant une lame horizontale réduite. A l'arrière, cette lame horizontale est interrompue par une surface verticale qui correspond certainement à un bord de contact avec le processus antérieur du coronoïde qui devait donc largement déborder sur le dentaire du côté lingual. La partie dorsale de la lame horizontale forme une sorte de plateau dentaire peu étendu mésialement.

La dentition, de type acrodonte, compte quatorze emplacements dentaires mais la rangée dentaire n'est peut-être pas complète. A l'avant subsistent quatre dents pleurodontes, à l'apex pointu, les deux premières étant recourbées vers l'arrière et elles prennent une morphologie caniniforme. La quatrième et la cinquième dents conservées représentent des formes de passage entre les types pleurodonte et acrodonte. Les dents suivantes, nettement tricuspides, peuvent être qualifiées d'acrodontes bien que leur implantation soit fortement décalée vers le bord lingual du dentaire. Plutôt que de type acrodonte, on pourrait parler de morphologie «subacrodonte» (ALIFANOV, 1989), les bases des dents étant toujours beaucoup plus exposées lingualement que labialement sur les fossiles de Dormaal. Les dents adjacentes restent largement séparées l'une de l'autre et les bases dentaires ne se confondent pas. Elles se soudent à la surface linguale du dentaire, sans jamais atteindre le plateau dentaire vers le bas. La taille des dents augmente régulièrement de l'avant vers l'arrière.

Maxillaire. Aucun des maxillaires disponibles n'est complet et on n'en connaît l'extrémité antérieure et l'extrémité postérieure que par les descriptions de HECHT & HOFFSTETTER (1962). La partie antérieure du processus dorsal, partiellement conservée, montre qu'il devait s'élever en pente douce à partir de l'avant du maxillaire. En vue dorsale, le bord supérieur de la lame horizontale se réduit à un profond sillon dans sa partie antérieure, alors qu'il s'élargit pour former une sorte de plateau mésial dans la partie centrale du maxillaire où se développe un processus palatin bien marqué. La dentition, ne diffère pas de celle du dentaire, tout au moins si l'on se réfère à la morphologie des dents acrodontes. Une seule dent pleurodonte semble conservée à l'avant de la rangée dentaire (spécimen DIIIR12), mais celle-ci n'est peut-être pas complète. HECHT & HOFFSTETTER (1962) ont donné une description détaillée de la partie antérieure (processus prémaxillaire) d'un maxillaire de Dormaal, aujourd'hui non disponible. L'essentiel en est repris ici : à l'avant, le maxillaire émet une lame interne subhorizontale, légèrement concave dorsalement, qui fournit une large articulation transversale pour le prémaxillaire. Ajoutons que ce maxillaire portait treize dents, apparemment sans canines à l'avant.

Variations. L'ensemble des fossiles disponibles a une morphologie remarquablement homogène. Cependant, un dentaire incomplet de Dormaal (niveau DIIC) porte une importante protubérance sur sa face ventrale, près de la symphyse et s'étendant partiellement sur la face labiale. Cette pachyostose est anormale et paraît liée, soit à une anomalie du développement, soit à un processus de régénération après lésion.

Discussion

L'existence de dents pleurodontes, caniniformes, à l'avant du dentaire des espèces de *Tinosaurus*, écarte ce genre des Chamaeleonidae. Il reste deux familles auxquelles pourrait appartenir *T. europeocaenus*, les Agamidae et les Priscagamidae, ces derniers connus uniquement à l'état fossile dans le Crétacé supérieur asiatique. *T. europeocaenus* présente quelques caractères qui semblent le rapprocher des Priscagamidae, avec son sulcus Meckeli assez largement ouvert postérieurement, l'existence d'une lame horizontale et d'un plateau dentaire bien définis. Mais on retrouve une morphologie semblable, voir identique chez certains Agamidae actuels (MOODY, 1980). Le splénial des Priscagamidae

est très étendu vers l'avant, mais cet os manque dans le matériel de *T. europeocaenus*. D'ailleurs, l'absence du splénial chez *T. europeocaenus* fait plutôt pressentir qu'il ne devait pas être très développé et que sa surface de contact avec le dentaire était limitée. Aussi, nous considérons que le genre *Tinosaurus* appartient aux Agamidae, opinion adoptée par tous les auteurs qui l'ont étudié précédemment (voir ESTES, 1983a).

L'absence de processus coronoïde bien formé sur le dentaire exclut une affinité étroite entre *T. europeocaenus* et le genre actuel *Uromastix*, ainsi qu'avec *Pseudotinosaurus*, défini par ALIFANOV (1993) à partir de l'espèce *Tinosaurus asiaticus* Gilmore, 1943.

T. europeocaenus diffère de tous les Agamidae actuels par la large ouverture du sulcus Meckeli à l'arrière du dentaire, par la quasi-absence de rebord mésial au niveau de la partie ventrale du dentaire, amenant une ouverture ventro-linguale du sulcus Meckeli et, selon HECHT & HOFFSTETTER (1962), par l'absence de bord montant à l'avant des processus prémaxillaires du maxillaire. D'autres caractères, en partie observables sur quelques Agamidae actuels, paraissent très développés chez *T. europeocaenus*. Il s'agit de la présence de dents fortement triconodontes à l'arrière de la rangée dentaire; de l'implantation subacrodonte de ces dents; de l'existence d'un net plateau dentaire sous la rangée dentaire, bien que la base des dents acrodontes ne viennent pas s'implanter sur lui; de l'importance du processus palatin du maxillaire. L'ensemble de ces dispositions nous conduit à proposer la diagnose du genre *Tinosaurus* et celle de l'espèce de Dormaal.

Deux espèces, *Tinosaurus pristinus* Leidy, 1872 et *T. stenodon* Marsh, 1872 ont été décrites dans l'Eocène d'Amérique du Nord. Selon les figures de GILMORE (1928, plate II, fig. 16) et de HECHT (1959, fig. 10C, p. 55 *in* ESTES, 1983a), *T. pristinus* et *T. stenodon* ont un dentaire assez effilé, des dents acrodontes tricuspides et un plateau dentaire nettement détaché sur la face linguale du dentaire bien que la présence de dents antérieures caniniformes ne puisse être vérifiée. Ces deux espèces entrent donc effectivement dans le domaine de définition du genre *Tinosaurus*. Le dentaire de *T. doumuensis* Hou, 1974, du Paléocène chinois paraît très proche de celui de *T. europeocaenus*; seule l'absence d'un plan orienté ventro-labialement à dorso-lingualement sur la face labiale du dentaire chez *T. doumuensis* sépare ces deux espèces, si l'on s'en tient aux caractères visibles sur les figures de HOU (1974, fig. 6, p. 198). Quant à *T. lushihensis* Dong, 1965 de l'Eocène de Chine, sa dentition serait particulière, avec des dents acrodontes comprimées transversalement. L'absence de dents caniniformes sur le dentaire et le maxillaire exclut cette dernière espèce du genre *Tinosaurus*, ce que ESTES (1983a) avait déjà remarqué. Cependant LI (1991b) a revu la mâchoire holotype de *T. lushihensis* et il écrit que ses dents acrodontes sont compressées latéralement, non transversalement, donc semblables à celles des *Tinosaurus* spp. et de l'ensemble des Agamidae.

LI (1991a) définit une nouvelle espèce, *T. yuanquensis* dans l'Eocène supérieur chinois. La forme effilée de la partie antérieure du dentaire, seule conservée, la présence de quatre dents pleurodontes, dont la dernière caniniforme, ainsi que les dents acrodontes, nettement tricuspides, indiquent que cette espèce appartient effectivement au genre *Tinosaurus*. Les caractères retenus dans la diagnose de l'espèce *T. yuanquensis* paraissent néanmoins discutables et ils sont plesiomorphes à l'intérieur du genre *Tinosaurus*, ce qui conduit à mettre en doute la validité de ce taxon.

Genre *Uromastyx* Merrem, 1820

Espèce type: *Uromastyx spinipes* Merrem, 1820

Uromastyx europaeus (de Stefano, 1903)

1903: *Palaeochamaeleo europaeus;* DE STEFANO, p. 391-393, Tav. IX, fig. 7-12.

1955: *Uromastyx*?; HOFFSTETTER, p. 618.

1962: *Uromastyx*?; HOFFSTETTER, p. 252.

1980: *Uromastyx europaeus;* MOODY, p. 338.

1980: *Palaeochamaeleo;* MOODY & ROCEK, p. 85-86.

1983a: *nomen dubium;* ESTES, p. 56.

1986: *Uromastyx europaeus;* AUGE, p. 30, fig. 15-16, p. 42.

1988: *Uromastyx europaeus;* AUGE, p. 317-325, fig. 1-3.

1993: *Palaeochamaeleo;* ALIFANOV, p. 150.

1993: *Uromastyx europaeus;* RAGE & AUGE, p. 200.

Lectotype. Un dentaire droit, fig. 12 *in* DE STEFANO (1903) anciennes collections du Quercy, MNHN, N° QU17160 (l'holotype désigné par ESTES, 1983a est en réalité un lectotype puisque DE STEFANO, 1903, dans sa description originale, avait pris deux syntypes, un dentaire droit et un dentaire gauche, QU17161, fig. 7&12 *in* DE STEFANO, 1903).

Localité type et âge. Phosphorites du Quercy, gisement précis inconnu. Oligocène.

Extension géographique et stratigraphique. Espèce connue uniquement dans les Phosphorites du Quercy, gisements de La Plante 2 et de Mas de Got B, niveau standard de Villebramar (MP22), transition entre l'Oligocène inférieur et l'Oligocène moyen.

Matériel. Anciennes collections du Quercy (gisement précis inconnu): trois dentaires et quatre maxillaires incomplets (QU17183-17189). Nouvelles récoltes du Quercy: La Plante 2 (MP22), un dentaire et un maxillaire très incomplets, deux vertèbres dorsales; Mas de Got B (MP22), un dentaire incomplet.

Diagnose amendée. Le dentaire de *Uromastyx europaeus* se distingue de celui des formes actuelles par son sulcus Meckeli assez large à l'arrière et qui s'ouvre ventro-labialement sur toute sa longueur. Le bord ventral du dentaire ne produit qu'un faible rebord mésial. Au niveau de la symphyse mandibulaire, le dentaire a un bord ventral assez incliné. L'incisure coronoïde, qui échancre la partie postérieure du dentaire, est peu développée. *U. europaeus* diffère aussi de *Pseudotinosaurus asiaticus* par son bord ventral rectiligne et de *Qianshanosaurus* par la forme générale beaucoup moins massive de son dentaire. Les caractères suivants ne sont pas diagnostiques de l'espèce mais ils permettent de séparer le genre *Uromastyx* des autres Agamidae: il n'y a pas de dents pleurodontes à l'avant des mâchoires. Il existe un processus coronoïde bien développé sur le dentaire. Le mode d'usure des dents acrodontes, avec formation d'une vallée allongée antéro-postérieurement sur l'apex, paraît aussi propre au genre *Uromastyx*.

Discussion

Selon FROST & ETHERIDGE (1989) les genres *Uromastyx* et *Leiolepis* Cuvier, 1829 constituent, à eux-deux, une sous-famille à l'intérieur des Agamidae, les Leiolepidinae FITZINGER (1843) (= Uromastycinae de THEOBALD, 1868 ou MOODY, 1980).

Les caractères de *U. europaeus* qui autorisent son affiliation au genre *Uromastyx* ont déjà été discutés ailleurs (MOODY & ROCEK, 1980; AUGE, 1986, 1988). Nous n'y reviendrons pas. Cependant, ALIFANOV (1993) a commenté l'attribution de cette espèce au genre *Uromastyx*. Suivant son opinion, acquise sur la foi des figures publiées pour *U. europaeus*, il existe des différences morphologiques entre la dentition acrodonte de cette forme et celle des espèces d'*Uromastyx* actuels. ALIFANOV (1993) va même plus loin et suggère un rapprochement avec le genre *Qianshanosaurus* Hou, 1974, du Paléocène chinois. Rappelons que HOU (1974) plaçait cette forme dans les Iguanidae et que ESTES (1983a) en fait un Lacertilia *incertae sedis*.

Le mode d'usure de l'ensemble de la rangée dentaire chez le fossile du Quercy, avec une zone antérieure complètement abrasée, le non-remplacement des dents acrodontes usées et l'apparition de nouvelles dents acrodontes à l'arrière de la rangée dentaire, est conforme aux descriptions de COOPER *et al.*, 1970, COOPER & POOLE (1973) et de ROBINSON (1976) concernant les espèces d'*Uromastyx* actuels. De nouvelles dents acrodontes, non usées, sont présentes à l'arrière de la rangée dentaire de quelques-uns des fossiles du Quercy. Ces dents sont petites, leur forme générale est celle d'un cône comprimé linguo-labialement. Leur apex forme un bord arrondi, allongé antéro-postérieurement et sans cuspide ni trace d'épaulement latéral. Les dents suivantes (en allant vers la partie antérieure de la rangée dentaire) sont plus grandes et commencent à montrer des traces d'usure occlusale. Celles-ci débutent sur l'apex de la dent qui se creuse en son centre, déterminant une vallée allongée antéro-postérieurement. Cette vallée s'approfondit de plus en plus sur les dents suivantes, en même temps que son flanc labial tend à disparaître, l'usure étant plus forte du côté labial que du côté lingual. Sur les dents acrodontes antérieures, l'apex redevient simple, tout le flanc labial de la dent ayant été usé, les traces d'occlusion s'étendant alors jusque sur le flanc labial du dentaire, entre les dents. On retrouve ce type d'usure, avec formation d'une vallée apicale chez les espèces d'*Uromastyx* actuels et il paraît unique chez les Agamidae. On pourrait donc avoir affaire à une apomorphie du genre *Uromastyx*, sinon de la sous-famille. L'espèce décrite ici reste dans le genre *Uromastyx*.

Genre *Quercygama* nov. gen.

Espèce type. *Quercygama galliae* (Filhol, 1877)

Diagnose. Quercygama se distingue des autres genres d'Agamidae par la grande extension vers l'avant de l'apophyse antéro-linguale du coronoïde. Celle-ci s'allonge jusqu'au niveau de la sixième dent acrodonte (comptée à partir de l'arrière), c'est-à-dire pas très loin du milieu de la rangée dentaire. Cette apophyse coronoïde dépasse vers l'avant l'extrémité antérieure de l'angulaire. La partie antérieure de ces deux os est en contact sur une courte distance au-dessus du sulcus Meckeli. Le splénial, très réduit, ne s'insinue pas

entre le coronoïde et l'angulaire. Les caractères suivants ne constituent pas à proprement parler des apomorphies, mais ils peuvent être utiles pour distinguer *Quercygama* des autres Agamidae: forme allongée et grêle du dentaire; présence de trois dents pleurodontes à l'avant de la rangée dentaire, dont la dernière est caniniforme tout en restant de dimension modeste; sulcus Meckeli qui s'ouvre ventro-lingualement à l'avant du dentaire.

***Quercygama galliae* (Filhol, 1877)**

(Fig. 2)

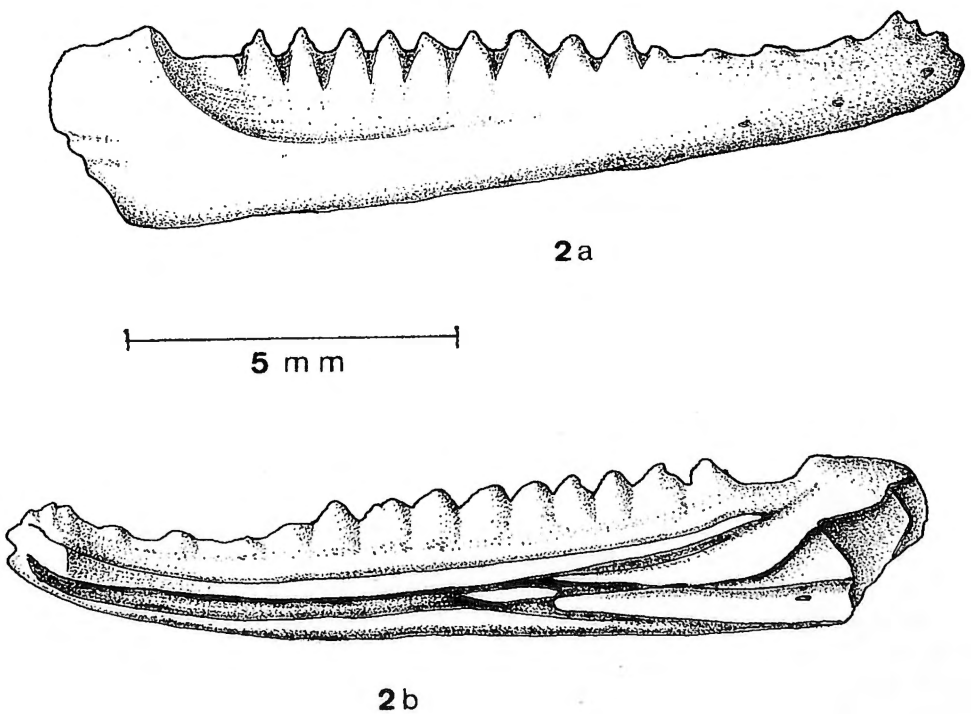


Fig. 2. – *Quercygama galliae*, dentaire, holotype, Oligocène, Phosphorites du Quercy, MNHN, n° QU16560. 2a: vue labiale; 2b: vue linguale.

1877: *Agama galliae;* FILHOL, p. 485, fig. 427.
1877: *Plestiodon galliae;* FILHOL, p. 588, fig. 426 (*errata*: *Agama galliae*, p. 559).
1983a: *Agama? galliae;* ESTES p. 53, fig. 10B.
1986: *Agama galliae;* AUGE, 1986

1993 : *Agama galliae;* RAGE & AUGE, p. 209.
1995 : *Agama galliae;* AUGE & RAGE, p. 16, fig. 3-5.

Holotype. Un dentaire droit légèrement abîmé du côté postérieur. Anciennes collections du Quercy, MNHN, QU16560, fig. 427 *in* FILHOL (1877; fig. 10B, p. 55) *in* ESTES (1983a; fig. 19, p. 44) *in* AUGE (1986; fig. 3-5) *in* AUGE & RAGE (1995).

Localité type et âge. Phosphorites du Quercy, sans précision de gisement. Oligocène.

Extension géographique et stratigraphique. Connue dans les Phosphorites du Quercy uniquement, gisements du Garouillas (MP25) et peut-être de Roqueprune (MP23), Oligocène moyen.

Matériel. Ancien matériel du Quercy; holotype uniquement; Garouillas (MP25); un fragment de dentaire (USTL, GAR2618) et un fragment de maxillaire (USTL, GAR2619), fig. 3-4, p. 16 *in* AUGÉ & RAGE (1995). ?Roqueprune (MP23), un fragment de dentaire (IRScNB).

Diagnose. La même que celle du genre.

Description

Dentaire. Le dentaire holotype de *Quercygama galliae* a déjà été figuré à de nombreuses reprises et le lecteur pourra se reporter aux publications citées plus haut. Il paraît cependant utile d'en reprendre la description, au moins pour les points qui peuvent éclairer la diagnose.

Le dentaire a une forme générale allongée et assez grêle, en cela il évoque celui du genre *Tinosaurus*. Le bord ventral du dentaire est presque rectiligne et il produit un fort repli du côté lingual qui limite ventralement le sulcus Meckeli. Sur la face linguale, le sulcus Meckeli reste étroit sur toute son étendue, son ouverture est d'abord linguale à l'arrière et elle devient linguo-ventrale près de la symphyse. Le splénial (s'il s'agit bien de cet os) n'est que très peu développé et il ne dépasse pas antérieurement le niveau de la dernière dent de la rangée dentaire. Par contre, l'angulaire couvre la partie ventro-postérieure du sulcus Meckeli alors que l'apophyse antérieure du coronoïde couvre sa partie dorso-postérieure jusqu'à un niveau proche du milieu de la rangée dentaire. Il existe une lame horizontale bien marquée au-dessus du sulcus Meckeli, au moins dans la partie antérieure du dentaire. Celle-ci délimite un plateau dentaire assez large à l'avant, où il reçoit la base des dents pleurodontes. Le plateau dentaire tend à s'effacer dans la partie postérieure de l'os. En vue labiale, le bord dorsal du dentaire porte des traces d'usure occlusale assez profondes, entre les dents acrodontes.

La dentition compte trois dents antérieures pleurodontes, la troisième, visible sur un dentaire du Garouillas (fig. 3, p. 16 *in* AUGE & RAGE, 1995) prend même une morphologie caniniforme, bien qu'elle reste de petite taille. Les dents suivantes, au nombre de neuf, sont acrodontes, serrées, en contact étroit par leur base. Un espace qui ne porte que des dents usées les sépare des premières dents. Leur forme générale est triangulaire, légèrement comprimée linguo-labialement, avec un apex plutôt arrondi, sans cuspide ni épaulement, sauf sur la dernière dent qui porte deux petites cuspides antérieure et postérieure. Cette dernière dent ne montre pas de trace d'usure occlusale, contrairement aux dents qui la précèdent et l'on peut penser que les cuspides disparaissent rapidement lorsque les pre-

mières traces d'usure érodent la dent. L'implantation se fait sur l'apex de la crête dentaire, mais avec un léger débordement vers le bord lingual. La base des dents acrodontes n'atteint jamais le plateau dentaire et elle n'est percée par aucun foramen nourricier (foramen de remplacement, selon WHITESIDE, 1986).

Maxillaire. Le seul fossile connu est un fragment antérieur de maxillaire gauche du gisement du Garouillas (fig. 4, p. 16 *in* AUGE & RAGE, 1995). Cet os, de proportions semblables à celle du dentaire, porte au moins une dent pleurodonte, suivie par des dents acrodontes sans cuspide.

Discussion

Les dents pleurodontes, présentes à l'avant des mâchoires de *Quercygama galliae*, montrent que nous avons affaire à un Agamidé. Tous les auteurs qui ont eu à discuter les relations de ce fossile (FILHOL, 1877; DE STEFANO, 1903; HOFFSTETTER, 1955; MOODY, 1980; ESTES, 1983a) l'ont toujours considéré comme un Agamidé. Sa place parmi les genres actuels et fossiles d'Agamidae s'avère plus difficile à établir et MOODY (1980), par exemple, pense que la forme du Quercy appartient, soit à *Agama*, soit à *Stellio*, lui-même ayant revalidé ce dernier genre créé par BOULENGER (1885).

On peut tenir pour certain que *Quercygama galliae* n'appartient pas au genre *Tinosaurus*, malgré une ressemblance superficielle dans la forme générale de leur dentaire. Les espèces appartenant au genre *Tinosaurus* ont des dents triconodontes avec une implantation débordant nettement vers le côté lingual, un sulcus Meckeli élargi à l'arrière et un bord ventral du dentaire ne se repliant que légèrement du côté lingual. Tous ces caractères s'opposent à ce que l'on observe chez les *Quercygama*. spp. Le genre actuel *Agama* n'a pas non plus de dents triconodontes, quoique les nouvelles dents formées à l'arrière de la rangée dentaire puissent comporter une petite cuspide. Précisément, la triconodontie varie avec l'âge de l'individu (SIEBENROCK, 1885) et elle s'atténue chez l'adulte, ce qui jette un doute sur la fiabilité de ce caractère, sauf lorsqu'il est exprimé très nettement, comme chez *Tinosaurus*. *Quercygama galliae* partage l'ouverture ventro-linguale du sulcus-Meckeli à l'avant du dentaire avec les genres actuels *Agama* et *Stellio*, alors que chez les autres Agamidae, le sulcus Meckeli s'ouvre uniquement sur la face linguale (MOODY, 1980). Cependant, la disposition des os post-dentaires (splénial, angulaire, coronoïde), sur la face linguale de la mandibule de *Quercygama galliae* paraît unique à l'intérieur des Agamidae. Elle est, à tous égards, bien différente de celle de *Agama* et *Stellio*, chez qui l'apophyse antéro-linguale du coronoïde ne s'étend pas au-delà de la troisième position dentaire (comptée à partir de l'arrière). Chez tous les Agamidae examinés, l'extrémité antérieure de l'angulaire dépasse l'extrémité antérieure de l'apophyse coronoïde, alors que l'on trouve la disposition inverse chez *Quercygama galliae*. Le splénial, s'il existe, se réduit à un simple triangle qui ne s'insinue pas entre l'angulaire et le coronoïde, si bien que ces deux derniers os se trouvent en contact chez *Q. galliae*, à la différence des autres Agamidae. Ces caractères morphologiques uniques parmi les Agamidae peuvent être considérés comme des apomorphies et nous permettent de proposer un genre nouveau, *Quercygama*, pour recevoir l'espèce créée par FILHOL (1877).

Matériel retiré de l'espèce Q. galliae:

Un frontal des anciennes collections du Quercy (fig. 20, p. 44 *in* AUGE, 1986) avait été attribué à l'espèce *Agama galliae*. Deux raisons s'opposent à une telle appartenance: chez les Agamidae (MOODY, 1980), la terminaison antérieure du frontal a la forme d'une fourchette, avec une apophyse médiane qui s'insinue entre les os nasaux. Cette apophyse n'existe pas sur le fossile du Quercy. D'autre part, un examen attentif de la face dorsale de ce frontal révèle une encoche ovale, située mésialement, près du bord postérieur de l'os. Cette encoche ne peut correspondre qu'à l'emplacement du foramen pariétal, pas totalement ouvert sur cette pièce. Or le foramen pariétal ne s'ouvre pas chez certains Iguanidae et, à l'intérieur des Lacertilia, il ne se trouve sur le frontal que chez une sous-famille des Iguanidae, les Corytophaninae (= Corytophanidae, FROST & ETHERIDGE, 1989).

Agamidae indéterminés

Matériel. Phosphorites du Quercy, gisement de Rigal-Jouet (Oligocène moyen, MP25): trois dentaires incomplets, USTL, N° RIG7021, fig. 21, p. 44 *in* AUGE (1986). Coderet (Oligocène supérieur, MP30): un frontal, UCBL, N° 97692, fig. 22, p. 44 *in* AUGE (1986).

Ces fossiles sont nettement plus petits que ceux attribués à *Uromastyx europaeus* ou *Quercygama galliae*. Les dentaires portent quelques dents acrodontes, alors que le frontal de Coderet a des processus descendants à peine marqués. Ces restes sont de taille trop réduite pour appartenir à l'une des deux espèces d'Agamidé décrites dans l'Oligocène européen, et leurs dimensions relativement homogènes prouvent qu'il ne peut s'agir d'individus jeunes. Ils démontrent que les Agamidae se sont maintenus en Europe de l'Ouest jusqu'à l'Oligocène supérieur au moins.

CONSIDÉRATIONS PALÉOGÉOGRAPHIQUES

Dans l'Eocène inférieur d'Europe occidentale, les Agamidae sont représentés par l'espèce *Tinosaurus europeocaenus*. On connaît d'autres membres du genre *Tinosaurus* dans le Paléogène nord américain (MARSH, 1872; GILMORE, 1928; HECHT, 1959; EMRY, 1973; ESTES, 1983a; HUTCHISON, 1992) et asiatique (CHKHIKVADZE, 1985; LI, 1991a,b; HOU, 1974; DONG, 1965). En outre, GILMORE (1943) a décrit *T. asiaticus* dans l'Eocène moyen de Mongolie, espèce que ALIFANOV (1993) a rapporté à un genre distinct, *Pseudotinosaurus*. En accord avec ESTES (1983b) nous considérons les *Tinosaurus* américains et asiatiques comme le résultat d'une dispersion depuis l'Asie, le détroit de Bering constituant une voie de passage naturelle entre l'Amérique et l'Asie alors que l'Europe reste isolée de l'Asie durant la plus grande partie du Tertiaire inférieur par la mer ouralienne. Néanmoins, les grands épisodes régressifs marquant la fin du Secondaire ou le début du Tertiare ont pu oblitérer provisoirement cette mer (Maastrichien supérieur, Thanétien inférieur, Yprésien supérieur, HAQ *et al.*, 1987; SMITH *et al.*, 1994) et permettre le passage de faunes asiatiques vers l'Europe. D'autre part, la paléogéographie de la Téthys pendant le début du Tertiaire devait autoriser des échanges épisodiques entre l'Asie

et l'Europe, comme ceux pressentis par HEISSIG (1979). De nombreux auteurs, étudiants des groupes taxonomiques divers, ont abouti aux mêmes conclusions (AUGE, 1993 ; VASSE, 1993). Cependant, l'hypothèse d'un relai par l'Amérique du Nord pour expliquer l'arrivée d'Acrodonta d'origine asiatique dans l'Eocène européen ne peut être définitivement écartée. Ajoutons que le Crétacé d'Asie centrale a livré les premiers fossiles d'Acrodonta connus (NESSOV, 1988 ; GAO & HOU, 1995), la nature monophylétique de ce taxon n'ayant jamais été remise en cause (FROST & ETHERIDGE, 1989).

Les Acrodonta disparaissent d'Europe occidentale après l'Eocène inférieur et on ne les retrouve qu'à l'Oligocène moyen, avec *Uromastyx europaeus* et *Quercygama galliae*. Cette disparition temporaire s'explique mal car, pendant ce temps, les autres taxons appartenant aux Lacertilia recensés dans l'Eocène européen prospèrent ou, au moins, font preuve d'une stabilité remarquable jusqu'à la Grande Coupure Eocène/Oligocène (AUGE, 1993). Cependant, à la différence des autres taxons, les Acrodonta de l'Eocène européen ne se sont jamais diversifiés et ils ne sont connus qu'à travers le seul genre *Tinosaurus*.

Les Acrodonta de l'Oligocène européen ne peuvent être issus que d'une dispersion depuis l'Asie ou l'Afrique, puisqu'ils disparaissent d'Amérique dès l'Eocène supérieur. Leurs restes fossiles sont peu abondants, comparés à ceux des autres groupes de lézards (Anguimorpha et Scincomorpha, essentiellement) et SCHLEICH (1988) ne signale pas d' Acrodonta dans les fossiles de Lacertilia livrés par des niveaux de l'Oligocène supérieur et du Miocène inférieur du bassin de Mainz, en Allemagne. Il est difficile de savoir si les Acrodonta d'Europe occidentale franchissent la limite Oligocène/Miocène. Cependant, leur présence est attestée dans l'Oligocène supérieur de Coderet et HOFFSTETTER (1955) fait état de représentants des Agamidae dans des fossiles du Stampien de l'Allier et du Miocène du Lyonnais. Il existe aussi des restes d' Acrodonta dans le Miocène de La Grive (RAGE, communication personnelle). RAGE (1972) rapporte des restes du Villafranchien (Pliocène) de France aux Chamaeleonidae, alors que MOODY (1980) pense avoir affaire à des Agamidae, certainement au genre *Agama*. Des restes d'Agamidae (genre *Agama*) sont décrits par BAILON (1987, 1989, 1991) dans le Pliocène français.

Bien que présents en Europe occidentale pendant une bonne partie du Tertiaire, les Acrodonta n'ont jamais occupé qu'une place marginale dans les herpétofaunes européennes. Il n'y a jamais eu de radiation significative des Acrodonta européens. Un seul genre, représenté par une seule espèce, *Quercygama galliae*, est endémique à l'Europe. Leur histoire sur ce continent paraît faite d'une suite de dispersions depuis des domaines voisins (Asie ou Afrique), suivies par des retraits lorsque les conditions deviennent défavorables. Le dernier de ces retraits date certainement du Pléistocène, puisque les seuls Acrodonta encore présents en Europe forment des populations éparses dans le sud de la Grèce et les îles adjacentes. L'origine de cette réduction de l'aire de répartition des Acrodonta ne peut être que climatique dans ce cas particulier (RAGE & SAINT GIRONS, 1989).

REMERCIEMENTS

Cet article a bénéficié des commentaires et des critiques de J.C. Rage, Z. Rocek, E. Schockaert et O. Rieppel. Les photographies au MEB sont de J. Cillis et de W. Miseur. Nous remercions également D. Delsate qui a mis sa collection à notre disposition.

RÉFÉRENCES

ALIFANOV, V.R. (1989) – New Priscagamids (Lacertilia) from the Upper Cretaceous of Mongolia and their systematic position among Iguania. *Paleontological Journal*, **4**: 68-80.

ALIFANOV, V.R. (1993) – Revision of *Tinosaurus asiaticus* Gilmore (Agamidae). *Paleontological Journal*, **27**: 148-154.

AUGE, M. (1986) – Les Lacertiliens (Reptilia, Squamata) de l'Eocène supérieur et de l'Oligocène ouest-européens. Thèse, Université Pierre et Marie Curie, Paris VI. 218 pp.

AUGE, M. (1988) – Revision du lézard *Uromastix europaeus* (Reptilia, Lacertilia) de l'Oligocène français. *Revue de Paléobiologie*, **7**: 317-325.

AUGE, M. (1990) – La faune de Lézards et d'Amphisbaenes de l'Eocène inférieur de Condé-en-Brie (France). *Bulletin du Museum national d'Histoire naturelle*, Paris, 4e série, section C, **12**: 111-141.

AUGE, M. (1993) – Répartition et dynamisme des faunes de Lacertilia et d'Amphisbaenia dans l'Eocène européen. *Palaeovertebrata*, **22**: 51-71.

AUGE, M. & J.-C., RAGE (1995) – Le Garouillas et les sites contemporains (Oligocène, MP25) des Phosphorites du Quercy (Lot, Tarn-et-Garonne, France) et leurs faunes de vertébrés. Amphibiens et Squamates. *Palaeontographica*, **236**(A): 11-32.

BAILON, S. (1987) – Les plus récents Agamidae fossiles de l'Europe occidentale et centrale (Pliocène supérieur de Seynes, France). *Bulletin de la Société herpétologique de France*, **42**: 1-4.

BAILON, S. (1989) – Les Amphibiens et les Reptiles du Pliocène supérieur de Balaruc II (Herault, France). *Palaeovertebrata*, **19**(1): 7-28.

BAILON, S. (1991) – Amphibiens et Reptiles du Pliocène et du Quaternaire de France et d'Espagne: Mise en place et évolution des faunes. UFR: Biologie et Sciences de la Nature, Thèse, Paris VII, 499 pp.

BORSUK-BIAYNICKA, M. & S.M. MOODY (1984) – Priscagaminae, a new subfamily of the Agamidae (Sauria) from the late Cretaceous of the Gobi desert. *Acta Palaeontologica Polonica*, **29**: 51-81.

BOULENGER, G. (1885) – Catalog of the lizards in the British Museum (Natural History), Second Edition. London, Vol. 2, XIII + 497 pp.

CHKHIKVADZE, V.M. (1985) – Preliminary results of studies on tertiary Amphibians and Squamate Reptiles of the Zaisan Basin. Voprosy gerpetologii, 6th all-soviet herpetological conference, Tashkent: 234-235.

COOPER, J.S. & D.F.G. POOLE (1973) – The dentition and dental tissue of the Agamid lizard *Uromastyx*. *Journal Zoology,* London, **169**: 85-100.

COOPER, J.S., D.F.G. POOLE & R. LAWSON (1970) – The dentition of agamid lizards with special reference to tooth replacement. *Journal Zoology,* London, **162**: 85-98.

DE STEFANO, G. (1903) – I sauri del Quercy appartenenti alla collezione Rossignol. Atti Societa italiana Scienze Naturali Museo civico di storia naturale in Milano, **42**: 382-418.

DONG, Z.M. (1965) – A new species of *Tinosaurus* from Lushih, Honan. *Vertebrata Palasiatica*, **9** (1): 79-82.

EMRY, R.J. (1973) – Stratigraphy and preliminary biostratigraphy of the Flagstaff Rim area, Natrona County, Wyoming. *Smithsonian Contribution to Paleobiology*, **18**: 1-43.

ESTES, R. (1983a) – Sauria terrestria, Amphisbaenia. *In*: O. Kuhn & P. Wellnhofer (editors). Handbuch der Paläoherpetologie, Teil **10**A, G. Fischer Verlag, 249 pp.

ESTES, R. (1983b) – The fossil record and early distribution of lizards. *In*: Advances in Herpetology and evolutionary Biology; Essays in honor of E.E. Williams. Museum of Comparative Zoology, Harvard University: 1-33.

ESTES, R., K. de Queiroz & J. Gauthier (1988) – Phylogenetic relationships within Squamata. *In*: Estes, R. & Pregill, G. (editors.), Phylogenetic relationships of the lizard Families, Stanford University Press: 119-281.

FILHOL, H. (1877) – Recherches sur les Phosphorites du Quercy. Pt. II, *Annales Sciences géologiques*, **8**: 1-338.

FITZINGER, L. (1843) – Systema reptilien, Fasc. 1. Amblyglossae, Vindobonae, Braumüller & Seidel: 1-106.

FROST, D.R. & R. ETHERIDGE (1989) – A phylogenetic analysis and taxonomy of Iguanian lizards (Reptilia: Squamata). University of Kansas, Museum of Natural History, Miscellaneous publication, **81**: 1-62.

GAO, K. & L. HOU (1995) – Late Cretaceous fossil record and paleobiogeography of Iguanian Squamates. *In*: Sun, A. & Wang, Y. (editors.), sixth symposium on mesozoic terrestrial ecosystems and biota, short papers, China Ocean Press, Beijing: 47-50.

GILMORE, C.W. (1928) – Fossil lizards of North America. *Memoirs of the National Academy of Sciences*, **22** (3): 1-201.

GILMORE, C.W. (1943) – Fossil lizards of Mongolia. *Bulletin of the American Museum of Natural History*, **81**: 361-384.

GODINOT, M., F. DE BROIN, E. BUFFETAUT, J.C. RAGE & D. RUSSELL (1978) – Dormaal, une des plus anciennes faunes éocènes d'Europe. *Comptes Rendus de l'Académie des Sciences*, Paris, **287**: 1273-1276.

HAQ, B., J. HARDENBOL & P.R. VAIL (1987) – Chronology of fluctuating sea levels since the Triassic. *Science*, **235**: 1156-1167.

HECHT, M.K. (1959) – Amphibians and reptiles. *In*: P. McGrew *et al.*, The geology and paleontology of the Elk Mt. and Tabernacle Butte area, Wyoming. *Bulletin of the American Museum of Natural History* **117**: 130-146.

HECHT, M.K. & R. HOFFSTETTER (1962) – Note préliminaire sur les Amphibiens et les Squamates du Landénien supérieur et du Tongrien de Belgique. *Bulletin de l'Institut royal des Sciences naturelles de Belgique*. **38** (39): 1-30.

HEISSIG, K. (1979) – Die hypothetische Rolle Südosteuropas bei den Säugertierwanderungen im Eozän und Oligozän. *Neues Jahrbuch für Mineralogie, Geologie und Paläontologie*, Monatshefte **2**: 83-96.

HOFFSTETTER, R. (1955) – Squamates de type moderne. *In*: J. Piveteau, Traité de Paléontologie, **5**: 606-662.

HOFFSTETTER, R. (1962) – Revue des récentes acquisitions concernant l'histoire et la systématique des squamates. *In*: Problèmes actuels de Paléontologie (Evolution des vertébrés). Colloques Internationaux du CNRS, Paris, **104**: 243-279.

HOU, L.H. (1974) – Paleocene lizards from Anhui, China. *Vertebrata Palasiatica*, **12** (3): 193-200.

HUTCHISON, H.J. (1992) – Western North American reptile and amphibia record across the Eocene-Oligocene boundary and its climatic implications. *In*: Prothero, D.R. & Berggren, W.A. (editors), Eocene-Oligocene climatic and biotic evolution, Princeton University Press: 451-463.

LI JINLING (1991a) – Fossil reptiles from Zhaili Member, Hedi Formation, Yuanqu, Shanxi. *Vertebrata Palasiatica*, **29** (4): 276-285.

LI JINLING (1991b) – Fossil reptiles from Hetaoyuan Formation, Xichuan, Henan. *Vertebrata Palasiatica*, **29** (3): 190-203.

MARSH, O. (1872) – Preliminary description of new Tertiary reptiles. Part I. *American Journal of Science*, **4**: 298-309.

MOODY, S.M. (1980) – Phylogenetic and historical biogeographical relationships of the genera in the family Agamidae (Reptilia: Lacertilia). Ph. D. dissertation, University of Michigan, Ann Arbor, 373 pp.

MOODY, S.M. & Z. ROCEK (1980) – *Chamaeleo caroliquarti* (Chamaeleonidae: Sauria): a new species from the lower Miocene of central Europe. *Vestnik Ustredniho Ustavu Geologickeho*, **55** (2): 85-92.

NESSOV, L.A. (1988) – Late mesozoic amphibians and lizards of Soviet Middle Asia. *Acta Zoologica Cracoviense*, **31**: 475-480.

RAGE, J.C. (1972) – Les amphibiens et les reptiles du Würmien II de la grotte de l'Hortus. *Etudes Quaternaires Mémoire*, **1**: 297-298.

RAGE, J.C. & M. AUGE (1993) – Squamates from the Cainozoic of the western part of Europe. A review. *Revue de Paléobiologie,* volume. spécial, **7**: 199-216.

RAGE, J.C. & H. SAINT GIRONS (1989) – Données biogéographiques: mise en place de la faune et facteurs actuels de la répartition. *In*: Atlas des Amphibiens et Reptiles de France, Société herpétologique de France (Edition): 29-32.

ROBINSON, P.L. (1976) – How *Sphenodon* and *Uromastix* grow their teeth and use them. *In*: A. Bellairs & C.B. Cox (editors.), Morphology and biology of reptilia, London: 43-64.

SCHLEICH, H.H. (1988) – Paläoherpetologische Materialien und Faunenspektren aus dem Kalktertiär des Mainzer Beckens (Oberoligozän-Untermiozän). *Geologisches Jahrbuch.*, A**110**: 289-306.

SIEBENROCK, F. (1885) – Das Skelett der Agamidae. *Sitzungberichte der Kaiserlichen Akademie der Wissenschafften in Wien,* **104**: 1089-1196.

SMITH, A.G., D.G. SMITH & B.M. FUNNELL (1994) – Atlas of Mesozoic and Cenozoic coastlines, Cambridge University Press, New York, 99 pp.

SMITH, T. & R. SMITH (1996) – Synthèse des données actuelles sur les vertébrés de la transition Paléocène-Eocène de Dormaal (Belgique). *Bulletin de la Société belge de Géologie*, **104** (1-2): 119-131.

THEOBALD, W. (1868) – Catalogue of the reptiles of British Birma, embracing the provinces of Pegu, Martaban, and Tenasserim; with descriptions of new or little-known species. *The Journal of the Linnean Society of London*, **10**: 4-67.

VASSE, D. (1993) – Les crocodiles du Crétacé supérieur et du Paléogène d'Europe. Aspects paléobiogéographique et paléoécologique. Thèse de Doctorat de l'Université Paris 6, Paléontologie, 173 pp.

WHITESIDE, D.I (1986) – The head skeleton of the Rhaetian Sphenodontid *Diphydontosaurus avonis* gen. et sp. nov. and the modernizing of a living fossil. *Philosophical Transactions of the Royal Society of London, Biological Sciences*, B: **312**: 379-430.

Belg. J. Zool. - Volume 127 (1997) - issue 2 - pages 139-158 - Brussels 1997

Received : 1 July 1997

DIVERGENT PROTONEPHRIDIAL ARCHITECTURE WITHIN THE KALYPTORHYNCHIA (PLATYHELMINTHES) AND IMPLICATIONS FOR THE PHYLOGENY OF THE RHABDOCOELA

NIKKI A. WATSON (1) AND ERNEST R. SCHOCKAERT (2)

(1) Department of Zoology, University of New England, Armidale, NSW, 2351 (Australia)
(2) Researchgroup Zoology, Department SBG, Limburgs Universitair Centrum, B-3590 Diepenbeek (Belgium)
e-mail: nwatson1@metz.une.edu.au

Abstract. Transmission electron microscopy of the protonephridial filtration structures in nine species of platyhelminths from five families in Kalyptorhynchia revealed three different types of architecture. Type A, found in representatives of Schizorhynchia and in two species of Cicerinidae (Eukalyptorhynchia), shows a terminal perikaryon associated with each flame bulb, and the bulb is connected to the proximal canal with a septate junction. Type B, found in two other species of Cicerinidae, also has a junction between the flame bulb and the proximal canal but does not have a nucleus in close association with the flame. Type C, found in the representatives of two other families of Eukalyptorhynchia, has no terminal perikaryon and the flame bulb is continuous with the proximal canal without the intervention of a junction. Type A may be considered the most plesiomorphic arrangement described for any rhabdocoel taxon while Type C is highly derived. Type C resembles that found in other orders of Rhabdocoela, namely Typhloplanida, Dalyelliida and Temnocephalida. The distribution of flame bulb types within Kalyptorhynchia, in conjunction with spermiogenesis data, suggests that the Cicerinidae is paraphyletic and that a subtaxon including the genera *Toia*, *Nannorhynchides* and (probably) *Pocillorhynchus* forms a sister group with the Schizorhynchia, while a subtaxon including at least the genera *Cicerina* and *Ptyalorhynchus* forms a sister group with the remaining Eukalyptorhynchia.

Key words: flame cells, protonephridia, Eukalyptorhynchia, Schizorhynchia, Turbellaria

INTRODUCTION

Ultrastructural studies of the terminal regions of protonephridia have contributed significantly to debate about phylogenetic relationships within the phylum Platyhelminthes (see reviews by ROHDE, 1990, 1991; ROHDE *et al.*, 1995). Species assigned to the large taxon Rhabdocoela (exclusive of the parasitic Neodermata) have in common a flame bulb with a weir or filtration apparatus formed from a single row of longitudinal ribs that are supported by bundles of microtubules. This is clearly a derived condition within the phylum and a synapomorphy for those taxa. Moreover, in contrast to non-rhabdocoels, the first several rhabdocoels studied possess flame bulbs with a further modification – lack of

a terminal perikaryon near the flame, and no separation between the cytoplasmic cylinder around the flame and the proximal canal (REISINGER, 1923; WILLIAMS, 1981; ROHDE, 1987; ROHDE *et al.*, 1987a,b, 1988). With light microscopic studies, REISINGER (1923) demonstrated that in this kind of arrangement, a single nucleus was associated with a number of flames and that this nucleus was some distance along the capillary removed from the flames. More recently, however, several rhabdocoel taxa that do have a terminal perikaryon have been studied: *Syndisyrinx punicea* (Hickman, 1956) and *Pterastericola pellucida* Jondelius, 1989, have several flames per perikaryon (ROHDE *et al.*, 1992), while the kalyptorhynch *Baltoplana magna* Karling, 1949, and possibly *Luriculus australiensis* Faubel, Rohde & Watson, 1994, have a single flame per perikaryon (ROHDE & WATSON, 1994; ROHDE *et al.*, 1993).

Kalyptorhynchia is one of four recognised divisions of the (non-neodermatan) Rhabdocoela, the others being Dalyelliida, Typhloplanida and Temnocephalida (see system in Cannon, 1986). The arrangement found in the kalyptorhynch *B. magna* (one flame per terminal perikaryon) resembles the situation in orders outside the rhabdocoels. It may, therefore, represent the least derived state within the Rhabdocoela. It differs markedly from the arrangement seen in all examined species of Temnocephalida and Typhloplanida, and from most examined species of the Dalyelliida. This study was undertaken to determine protonephridial architecture in a range of kalyptorhynchs from other families in both sub-orders (Eukalyptorhynchia and Schizorhynchia) to contribute to the understanding of relationships within Kalyptorhynchia and within the rhabdocoels in general.

MATERIAL AND METHODS

The following table provides taxonomic and collection data on the species studied.

Species	*Origin*
EUKALYPTORHYNCHIA	
Cicerinidae	
Cicerina remanei Meixner, 1928	Belgian sandy beach (Mariakerke, July 1995)
Ptyalorhynchus coecus Ax, 1951	Belgian sandy beach (Bredene, July 1995)
Toia calceformis Brunet, 1973	Belgian sandy beach (Mariakerke, September, 1987)
Nannorhynchides herdlaensis (Karling, 1956)	Marine algae, 10-12m (Kristineberg Sweden, 1988)
Gnathorhynchidae	
Ancistrorhynchus ischnurus L'Hardy, 1963	French sandy beach (Roscoff, June 1996)
Polycystididae	
Gyratrix hermaphroditus Ehrenberg, 1831	Brackish sands (Stockholm Sweden, August 1995)

SCHIZORHYNCHIA

Schizorhynchidae

Thylacorhynchus conglobatus Meixner, 1928	Belgian sandy beach (Mariakerke, July 1995)
T. pyriferus Karling, 1950	Fjord sandy bottom at 30cms, Kristineberg Sweden, August 1995

Diascorhynchidae

Diascorhynchus rubrus Boaden, 1963	Belgian sandy beach (Bredene, July 1995)

All were fixed in glutaraldehyde, post-fixed in OsO_4, dehydrated in ethanol and embedded in Spurr or Epon/Araldite resin. Ultrathin serial sections were cut through at least half the body of two individuals of the species *C. remanei*, *D. rubrus* and *T. calceformis*, and of one individual of each of the remaining species. Sections were collected on Formvar-coated parallel-bar grids (75p), stained with uranyl acetate and lead citrate and examined with a JEOL 1200EX transmission electron microscope (TEM). In most cases, strategic photographs were taken through at least two longitudinally and two transversely oriented flame regions for each species, with many additional (but often oblique) flames examined for confirmation of character consistency.

RESULTS

Schizorhynchia

The three species examined in this division are from different families (Schizorhynchidae and Diascorhynchidae), but all showed essentially the same organisation of the terminal regions. Features of the congeners *T. conglobatus* and *T. pyriferus* were identical, so only the former is illustrated. Flame cells were nucleated, and the nucleus was located close to the flame bulb (Figs 1, 3, 5, 50A), although its exact position varied between basal and baso-lateral, even within each individual. Cilia of the flame had rootlets (Figs 1, 5, 7) with cross striations (Fig. 1). The filtration apparatus consisted of a single row of longitudinal ribs containing bundles of microtubules, with extracellular material in slits between the ribs (Fig. 11). Near the top of the flame, the ribs fused into a distal cylinder (Fig. 10), and this cylinder was joined to the proximal canal cell which overlapped it, by a septate junction adjacent to the lumen (Figs 1, 2, 4, 6, 9). Tips of cilia projected a short distance into the capillary (Figs 6, 8). No nucleus of the proximal canal cell was located nearby the flames, and capillaries were formed by an entire cellular cylinder. In *T. conglobatus*, microtubules of the ribs were surrounded with dense material (Figs 7, 10, 11), and dense material also lay beneath the cell membrane along the distal tube (Figs 6, 9). A compact ring of similar material surrounded the most distal part of the tube in *D. rubrus* (Fig. 2), and the proximal canal regions were also lined with a layer of dense cytoplasm that formed microvillar projections (Fig. 4). Such dense cytoplasm and projections were absent from the capillaries of the two *Thylacorhynchus* spp.

Eukalyptorhynchia

Cicerinidae: Four species were examined from this family *Cicerina remanei* and *Ptyalorhynchus coecus* had very similar protonephridial organisation. The perikaryon of the flame bulb was adjacent to the flame (Figs 12, 16) and the filtration apparatus was formed by a single row of ribs supported by bundles of microtubules (Figs 12, 13, 16) with extracellular matrix between the ribs (Figs 13, 16). At the distal end the ribs fused into a tube in which the microtubules continued (Figs 14, 17), and there was a septate junction between this distal tube and the proximal canal (Fig. 50A). They differed in that the proximal canal cell enwrapped the lumen, with a junction between the two adjacent edges, in *P. coecus* but not in *C. remanei*. In *C. remanei*, therefore, the lumen was surrounded by an unbroken cylinder. More distal capillaries of *C. remanei* were similarly without junctions (Fig. 15). Dense material lining the distal tube of the terminal cell was more pronounced in *P. coecus* (Figs 16-18) than in *C. remanei* (Fig. 14). In both species the tips of the flame cilia projected slightly into the proximal canal lumen (Figs 14, 19).

The other two species studied, *Toia calceformis* and *Nannorhynchides herdlaensis,* resembled one another closely but differed from the two cicerinids described above. In *T. calceformis* and *N. herdlaensis* no terminal perikaryon was found despite examination of several sets of serial sections through transverse and longitudinally positioned flames. This was unexpected, since the cytoplasm of the terminal bulb surrounding the flame was clearly separated from the proximal canal by a septate junction (Figs 29, 31, 35, 37, Fig. 50B). In both species, the proximal canal also had a long junction to itself (Figs 29, 30, 35, 36), and the cilia of the flame projected a considerable distance into this lumen (Figs 29, 31, 35). Ribs supported by microtubules were much less regular than in the other cicerinids (Figs 23-28). In *N. herdlaensis* there were also a few internal leptotriches (Fig. 33) and the slits between ribs appeared to be formed both by finger-like interdigitations of the column surrounding the flame and as discrete longitudinal slits (Fig. 34). Cilia of the flames had cross-striated rootlets (Figs 20-22, 32). Lateral flames were present in some capillaries of both species (see Fig. 30 for *T. calceformis),* and there were junctions in capillary walls. No nearby nuclei were identified.

Gnathorhynchidae and Polycystididae: The representatives of these two families, *A. ischnurus* and *G. hermaphroditus* had the same basic arrangement of protonephridial terminal structures as one another, but the arrangement differed from those described above. The filtration apparatus was formed by a single row of longitudinal ribs supported by microtubules (Figs 38-41, 46-48) and these ribs fused into a tube in the distal region of the flame (Figs 42-44, 49, 50C). The slits between the ribs were covered with extracellular material (Figs 40, 47). The arrangement of slits was more regular in *G. hermaphroditus* than in *A. ischnurus*. There was no terminal nucleus and no junction between the flame region and the proximal canal (Figs 42-44, 46); that is the cytoplasmic tube surrounding the top of the flame was continuous with the proximal canal region. In *G. hermaphroditus* but not in *A. ischnurus* dense material lined the inner plasma membrane of the cytoplasmic tube between the end of the ribs and the beginning of the canal region where the cilia terminated (Fig. 44). There was no junction within the wall of the proximal canal (Fig. 43).

DISCUSSION

The protonephridial flame bulbs and capillaries of four other kalyptorhynch species have previously been examined in varying degrees of detail: *Baltoplana magna* (ROHDE & WATSON, 1994), *Gyratrix* sp. and *Odontorhynchus* sp. (ROHDE *et al.*, 1987a) and *Rhinolasius* sp. (ROHDE *et al.*, 1988). All, as well as those reported in the present study, have a filtration apparatus of longitudinal slits between a single row of cytoplasmic ribs that are supported by bundles of microtubules. This architecture is characteristic of all (non-neodermatan) rhabdocoels that have been examined from all subtaxa (see review by ROHDE, 1991 and earlier references therein; LUMBSCH *et al.*, 1995; ROHDE *et al.*, 1992; WILLIAMS, 1994). It has not been found outside of the Rhabdocoela with the single exception of the prolecithophoran *Archimonotresis limophila* (see EHLERS, 1989), but other prolecithophorans studied do not share this arrangement (EHLERS & SOPOTT-EHLERS, 1997, WATSON & ROHDE personal observations).

The results presented here together with the previous studies of other kalyptorhynchs, reveal three different arrangements of protonephridial components within Kalyptorhynchia, illustrated in Fig. 50. Type A: two species of Cicerinidae (*C. remanei* and *P. coecus*) and all examined schizorhynchs (*B. magna*, *Thylacorhynchus* spp and *D. rubrus*) from three different families have a terminal perikaryon and a septate junction between the terminal cell which forms the filter region, and the adjacent proximal canal. The canal is an entire cylinder in the schizorhynchs and in *C. remanei*, but a junction is present in *P. coecus*. Type B: *T. calceformis* and *N. herdlaensis* resemble Type A but no nucleus of the terminal «cell» could be located. Serial sections were followed completely through six individual flame cells of *T. calceformis* and one of *N. herdlaensis* but no nucleus was found. We cannot, however, rule out the possibility that a perikaryon is located at a considerable distance from the flame bulb, possibly connected to it by a very thin cytoplasmic strand, since it is hard to imagine how such an active cell could function without a nucleus. Both species have an extensive junction along the proximal canal, joining the edges of the enwrapping cell, as well as the pronounced septate junction between the flame/filter region and the proximal canal. Type C: species examined from the eukalyptorhynch families Gnathorhynchidae, Polycystididae and Koinocystidae have protonephridia without a terminal perikaryon, no junction separating the filter region from the proximal canal and no junction within the wall of the proximal canal. This corresponds to the arrangement found in all other rhabdocoels that have been studied with the exceptions of the dalyelliids *Syndisyrinx punicea*, *Pterastericola pellucida* and *Luriculus australiensis* (ROHDE *et al.*, 1992; ROHDE *et al.*, 1993). Type C may be considered the most derived state since it lacks the terminal perikaryon found in all other flatworm groups as well as all outgroups with protonephridia (see BARTOLOMAEUS & AX, 1992). Type A represents the most plesiomorphic condition within Rhabdocoela, having major elements in common with outgroups within the Platyhelminthes. Light microscopy has shown that in the Type C state in typhloplanids, a number of flame bulbs that lack nuclei, together with their proximal canal regions, join smaller branches and then the main longitudinal protonephridial ducts, where the few nuclei are located (REISINGER, 1923).

The two species with Type B protonephridia, placed by conventional classification into the family Cicerinidae (see CANNON, 1986), nevertheless have synapomorphies which separate them from other cicerinids (DE VOCHT, 1992), such as eyes with lenses and sperm without two incorporated axonemes. DE VOCHT (1992) linked a third genus, *Pocillorhynchus,* with *Toia* and *Nannorhynchides* because it has lenses in the eyes (2 lens elements in *Pocillorhynchus,* 1 in *T. calceformis* and 3 in *N. herdlaensis*) as well as other synapomorphies of the proboscis bulb musculature and glands. The four cicerinids studied here are all clearly distinguished from other eukalyptorhynchs in possessing the less derived type of protonephridia with a distinct junction between terminal and canal cells, and in this regard they closely resemble the schizorhynchs. However, *T. calceformis* and *N. herdlaensis* also share with schizorhynch taxa a reduction in the number of incorporated axonemes in the sperm brought about by a comparable process during spermiogenesis (WATSON in press). This contrasts with the presence of two fully incorporated axonemes in the sperm of *C. remanei, P. coecus* and all other examined eukalyptorhynchs (see L'HARDY, 1988; DE VOCHT, 1992; personal observations). It therefore appears likely that Cicerinidae in its conventional composition is paraphyletic, and that a subset of that family including the genera *Toia*, *Nannorhynchides* and probably *Pocillorhynchus* forms a sister group to the schizorhynch kalyptorhynchs. A further subset of species probably forms a sister group with the remainder of the Eukalyptorhynchia.

The species that have been examined from three other eukalyptorhynch families have a protonephridial architecture resembling that found in typhloplanids, temnocephalids and the majority of dalyelliids, *i.e.* a highly derived arrangement. However, if the Kalyptorhynchia is a monophyletic taxon with the apomorphies of a muscular proboscis and fused axonemes in the spermatozoon, then Type C protonephridia may have evolved at least twice (separately within the eukalyptorhynchs and in other rhabdocoel taxa). An alternative scenario homologising the derived protonephridia of these eukalyptorhynchs with those found in other rhabdocoels would require the assumption of loss of the proboscis in the other rhabdocoel taxa, or the development of a proboscis in three separate lineages.

Clearly, ultrastructural studies of protonephridia can make an important contribution to elucidation of phylogenetic relationships within the Platyhelminthes and especially within the Rhabdocoela. There are still many families within the four main rhabdocoel taxa (Kalyptorhynchia, Typhloplanida, Dalyelliida and Temnocephalida) where no species have been studied in this regard. Such further studies are needed to clarify the distribution of protonephridial types and the likely relationships between them.

ACKNOWLEDGEMENTS

The work was supported by an Australian Research Council Grant # A19602750 to K. Rohde, University of New England (UNE), Armidale, Australia; by a visiting academic grant provided by Limburgs Universitair Centrum, Belgium to NW; and by the Fund for Scientific Research Flanders (Belgium) (Researchproject G.0086.96 to ES). We thank Dr. Alain de Vocht for provision of embedded material of *T. calceformis*, *N. herdlaensis* and *P. coecus*, Dr. Ulf Jondelius (Swedish Museum of Natural History, Stockholm) for help in collecting the *Gyratrix* specimens, Professor Jean-Pierre

L'Hardy (Université du Maine, France) for collecting and fixing the *A. ischnurus* specimens, Zhixian Xin (UNE) for sectioning and staining some of the material, Rick Porter and Zoltan Enoch (UNE) respectively for developing and printing the electronmicrographs.

REFERENCES

BARTOLOMAEUS, T. & P. AX (1992) – Protonephridia and Metanephridia – their relation within the Bilateria. *Zeitschrift für zoologische Systematik und Evolutionsforschung*, **30**: 21-45.

CANNON, L.R.G. (1986) – Turbellaria of the World. A Guide to Families and Genera. Queensland Museum, Brisbane: 131pp.

DE VOCHT (1992) – Anatomy and ultrastructure of the proboscis in Eukalyptorhynchia (Platyhelminthes, Rhabdocoela). PhD Thesis, Limburgs Universitair Centrum, Belgium. 260 pages.

EHLERS, U. (1989) – The protonephridium of *Archimonotresis limophila* Meixner (Plathelminthes, Prolecithophora). *Microfauna Marina*, **5**: 261-275.

EHLERS, U. & B. SOPOTT-EHLERS (1997) – Ultrastructure of protonephridial structures within the Prolecithophora (Plathelminthes). *Microfauna Marina*, **11**: 291-315.

L'HARDY, J.-P. (1988) – Sperm morphology in Kalyptorhynchia (Platyhelminthes, Rhabdocoela). *Fortschritte der Zoologie*, **36**: 303-307.

LUMBSCH, M., U. EHLERS & B. SOPOTT-EHLERS (1995) – Proximal regions of the protonephridial system in *Pseudograffilla arenicola* (Plathelminthes, Rhabdocoela): ultrastructural observations. *Microfauna Marina*, **10**: 67-78.

REISINGER, E. (1923) – Untersuchungen über Bau und Funktion des Excretionsapparates bei rhabdocölen Turbellarien. 2. Über die Terminalorgane und das Kanalsystem einiger bekannter Typhloplaniden. *Zoologischer Anzeiger*, **56**: 205-224.

ROHDE, K. (1987) – Ultrastructure of the flame cells and protonephridial capillaries of *Craspedella* and *Didymorchis* (Platyhelminthes, Rhabdocoela). *Zoomorphology*, **106**: 346-351.

ROHDE, K. (1990) – Phylogeny of Platyhelminthes, with special reference to parasitic groups. *International Journal for Parasitology*, **20**: 979-1007.

ROHDE, K. (1991) – The evolution of protonephridia of the Platyhelminthes. *Hydrobiologia*, **227**: 315-321.

ROHDE, K., L.R.G. CANNON & N. WATSON (1987a) – Ultrastructure of epidermis, spermatozoa and flame cells of *Gyratrix* and *Odontorhynchus* (Rhabdocoela, Kalyptorhynchia). *Journal of Submicroscopic Cytology*, **19**: 585-594.

ROHDE, K., L.R.G. CANNON & N. WATSON (1988) – Ultrastructure of the flame bulbs and protonephridial capillaries of *Gieysztoria* sp. (Rhabdocoela Dalyelliida), *Rhinolasius* sp. (Rhabdocoela Kalyptorhynchia) and *Actinodactylella blanchardi* (Rhabdocoela Temnocephalida). *Journal of Submicroscopic Cytology and Pathology*, **20**: 605-612.

ROHDE, K., A.M. JOHNSON, P.R. BAVERSTOCK & N.A. WATSON (1995) – Aspects of the phylogeny of Platyhelminthes based on 18S ribosomal DNA and protonephridial ultrastructure. *Hydrobiologia*, **305**: 27-35.

ROHDE, K. & N.A. WATSON (1994) – Ultrastructure of the terminal parts of the protonephridial system of *Baltoplana magna* (Platyhelminthes, Kalyptorhynchia, Schizorhynchia, Karkinorhynchidae). *Malaysian Journal of Science*, **15A**: 13-18.

ROHDE, K., N. WATSON & L.R.G. CANNON (1987b) – Ultrastructure of the protonephridia of *Mesostoma* sp. (Rhabdocoela, 'Typhloplanoida'). *Journal of Submicroscopic Cytology*, **19**: 107-112.

ROHDE, K., N.A. WATSON & A. FAUBEL (1993) – Ultrastructure of the epidermis and protonephridium of an undescribed species of Luridae (Platyhelminthes: Rhabdocoela). *Australian Journal of Zoology*, **41**: 415-421.

ROHDE, K., N.A. WATSON & U. JONDELIUS (1992) – Ultrastructure of the protonephridia of *Syndisyrinx punicea* (Hickman, 1956) (Rhadocoela, Umagillidae) and *Pterastericola pellucida* (Jondelius, 1989) (Rhabdocoela, Pterastericolidae). *Australian Journal of Zoology*, **40**: 385-399.

WATSON, N.A. (in press) – Spermiogenesis and eyes with lenses in two kalyptorhynch flatworm species, *Toia calceformis* and *Nannorhynchides herdlaensis* (Eukalyptorhynchia, Platyhelminthes). *Invertebrate Biology.*

WILLIAMS, J.B. (1981) – The protonephridial system of *Temnocephala novaezealandiae*: structure of the flame cells and main vessels. *Australian Journal of Zoology*, **29**: 131-146.

WILLIAMS, J.B. (1994) – Ultrastructural observations on *Temnocephala minor* (platyhelminthes, Temnocephaloidea), including notes on endocytosis. *New Zealand Journal of Zoology*, **21**: 195-208.

Legends to the figures (see pages 149-158)

Figs 1-4. – TEM of flame cells of *Diascorhynchus rubrus*. C, canal cell; F, cilia of the flame; J, junction; TCN, terminal cell nucleus. Fig. 1. – Longitudinal section showing basal nucleus, long roots of the cilia and microtubules extending deeply below the ribs. Fig. 2. – Distal region of the flame showing microtubules in the ribs (arrowhead), septate junction between the terminal cell and the canal cell, and dense region at the tip of the terminal cell (large arrow). Fig. 3. – Two close flame cells, each with a somewhat lateral nucleus. Fig. 4. – Oblique transverse section at the distal tip of a flame cell. Note microtubules (arrowhead) in the terminal cell, junction with the canal cell, dense region at the tip of the terminal cell (large arrow) and canal lumen lined with dense cytoplasm with microvillus-like projections into the lumen. Scales 0.5μm (Figs 2, 4), 1μm (Fig. 1), 2μm (Fig. 3)

Figs 5-11. – TEM of 2 flame cells of *Thylacorhynchus conglobatus*. Figs 5-7. – Longitudinal section of one cell, Figs 8-11 Transverse section of second cell. C, canal cell; E, extracellular matrix; F, cilia of the flame; J, junction; TCN, terminal cell nucleus; R, rib. Fig. 5. – Longitudinal section showing lateral nucleus, rootlets of the cilia (arrow) and dense material in the ribs (arrowhead). Fig. 6. – Distal region of the flame showing microtubules in the ribs surrounded by dense material (arrowhead), continuation of dense material beyond the tips of the microtubules (double arrowhead), septate junction between terminal and canal cells and cilia projecting into the lumen of the canal cell. Fig. 7. – Basal end of the flame bulb showing basal bodies and rootlets (arrow) together with microtubules that are continuous with the ribs (arrowhead). Fig. 8. – Distal tip of the flame where cilia project into the lumen of the proximal canal cell. Fig. 9. – Less distal region where mainly only dense material lines the terminal cell, and a continuous septate junction joins terminal and canal cells. Fig. 10. – Still less distal. All but one of the slits has terminated leaving a cytoplasmic cylinder containing microtubules in dense material (arrowhead) surrounding the cilia of the flame. Fig. 11. – Basal level of the flame showing basal bodies of some cilia, termi-

nal plates of other cilia, ribs supported by microtubules surrounded by dense material (arrowhead) and joined by extracellular matrix in the slits. Scales 0.2μm (Figs 6-11), 1μm (Fig. 5).

Figs 12-15. – TEM of flame cells and capillaries of *Cicerina remanei*. C, canal cell; E, extracellular matrix; F, cilia of the flame; J, junction; TCN, terminal cell nucleus; R, rib. Fig. 12. – Mid-level transverse section showing the nucleus and ribs supported by microtubules (arrowhead) surrounding the flame. Fig. 13. – Ribs supported by microtubules (arrowhead) are joined by extracellular matrix across the intervening slits. Fig. 14. – Two flames, the top one at its tip where cilia project into the lumen of the canal cell. Note septate junction joining terminal and canal cells. Fig. 15. – Extensive region of expanded protonephridial capillaries (arrows) just beneath the sub-epidermal muscle layers. Scales 0.5μm

Figs 16-19. – TEM of the one flame bulb of *Ptyalorhynchus coecus*. C, canal cell; E, extracellular matrix; F, cilia of the flame; J, junction; R, rib; TCN, terminal cell nucleus. Fig. 16. – Mid-flame level showing terminal cell nucleus, ribs and extracellular matrix, and microtubules in ribs (bottom arrowhead) and in the cellular column above the termination of ribs (top arrowhead). Fig. 17. – Higher up the flame where some cilia have terminated, the flame is surrounded by a cellular column still containing bundles of microtubules, and the canal cell partly surrounds the terminal cell and is joined to it by a septate junction. Fig. 18. – Few cilia remain (double arrowheads) at the tip of the flame and the terminal cytoplasmic column (TC) with microtubules still visible (arrowhead) is no longer closed. The canal cell surrounds the tip of the terminal cell and shows a septate junction to itself. Fig. 19. – Only one cilium from the flame remains (double arrowhead), surrounded by the canal cytoplasm which is joined to itself by a septate junction. Scales 0.5μm.

Figs 20-29. – TEM showing part of a series through the one flame bulb of *Toia calceformis* from base (Fig. 20) to capillary (Fig. 29). At first rootlets only are visible (arrows), followed by basal bodies (BB) and microtubules (arrowheads). Ribs (R) with extracellular matrix (E) between them are few and rather irregular along the cytoplasmic column of the terminal cell (TC). Fig. 29 shows that the canal cell, with a junction to itself, enwraps the higher parts of the flame cell column and is joined to it by a septate junction. There is no nucleus closely associated with the flame nor with any nearby region of the capillary. Scales 0.2μm.

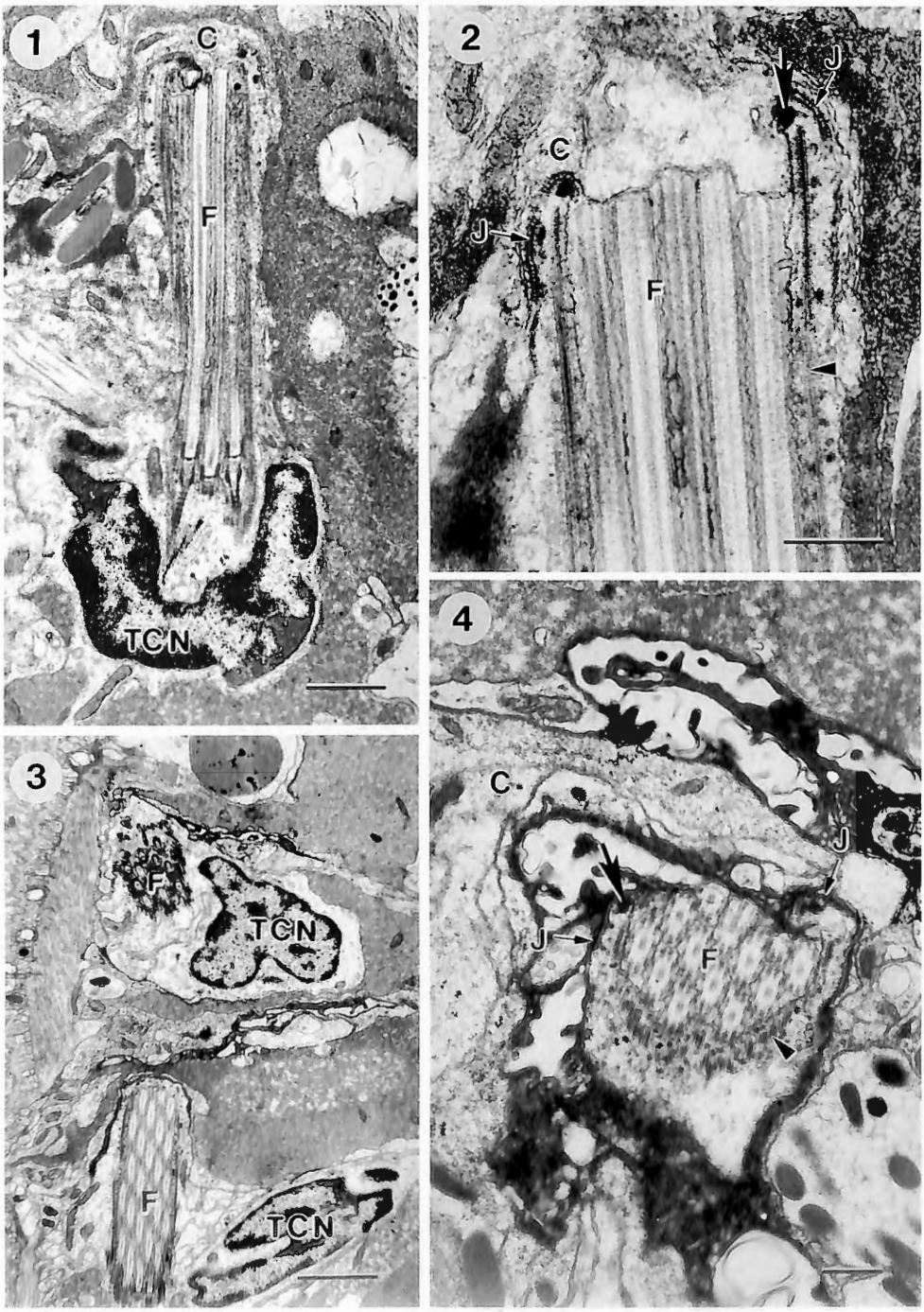
Figs 30-31. – TEM of parts of flame cells and capillaries of *Toia calceformis*. Fig. 30. – A single cilium in the narrow lumen of a capillary cell (C) which has a long junction (J) from the lumen to the outer cell membrane. Note long rootlets (arrows) of cilia of a lateral flame, and microtubules in capillary cell cytoplasm (arrowhead). Fig. 31. – The top of a flame cell where the terminal region is joined by a junction (J) to the capillary (C). Ribs (R) and microtubules (arrowhead) are still visible in the terminal cytoplasm. No nucleus was found associated with the flame nor with any nearby region of the capillary. Scales 0.5μm (Fig. 30), 0.2μm (Fig. 31).

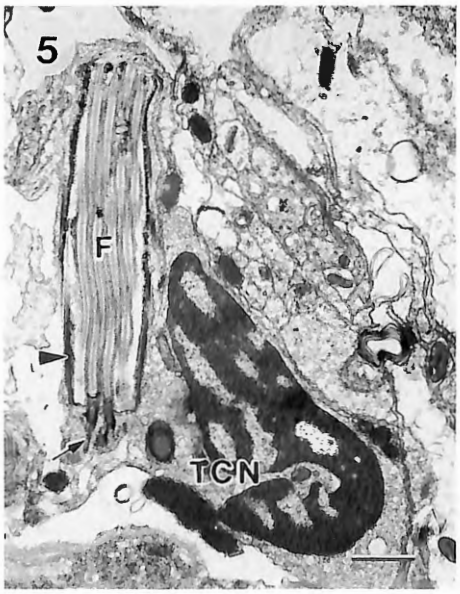
Figs. 32-37. – TEM showing part of a series through a single flame bulb and capillary of *Nannorhynchides herdlaensis*. Fig. 32. – Long rootlets (arrows) in the basal cytoplasm accompanied by microtubules (arrowheads). Fig. 33. – Oblique view through the flame showing cilia, microtubules in the wall (arrowhead), ribs (R) with extracellular matrix (E) between them, and one of several internal leptotriches (L). Fig. 34. – Oblique section through the flame wall showing ribs (R) and extracellular matrix (E). Note that in one case (on the left) the extracellular material goes completely around a rib tip, suggesting that this «rib» is an interdigitation with the distal part of the column. Fig. 35. – Cilia of the flame (F) extend beyond the ribs into the adjacent capillary. There is a junction between the terminal and capillary regions (J on right) and another within the capillary itself (J on left). Fig. 36. – Enlargement from Fig. 35 of the junction (J) within the capillary cell (C). Fig. – 37. A more tangential section through the junction region to the right in Fig. 35. Note cilia of the flame (F), capillary cytoplasm (C), junction (J) and terminal cell

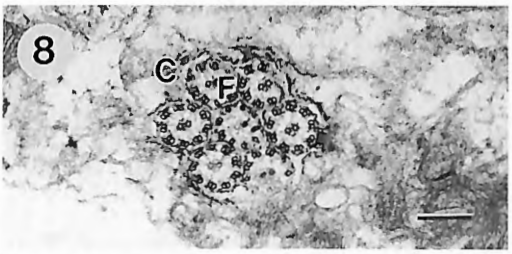
cytoplasm (TC). No nucleus was found associated with the flame nor with any nearby region of the capillary. Scales 0.5μm.

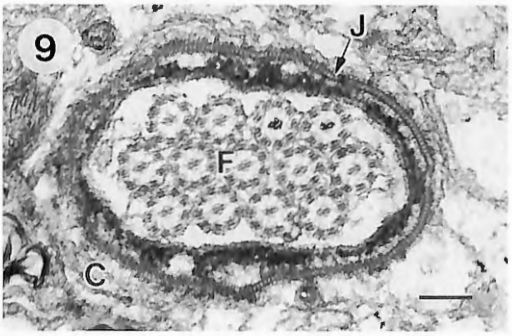
Figs 38-43. – Part of a series of serial sections through two adjacent flame cells of *Ancistrorhynchus ischnurus*. BB, basal bodies; F, flame; C, capillary cytoplasm; R, ribs; arrow, rootlets; arrowhead, microtubules. The top cell of the two is depicted from the basal cytoplasm with rootlets in Fig. 38 through to the diminished flame surrounded by a cytoplasmic column without ribs in Fig. 43. The lower cell is depicted from a level of the flame just above basal bodies (surrounding ribs contain bundles of microtubules with extracellular matrix between them), to the level of the disappearance of the cilia and a collapsed lumen (arrow) in the capillary in Fig. 43. There is no nucleus associated with the flame nor with any nearby region of the capillary. Scales 0.5μm.

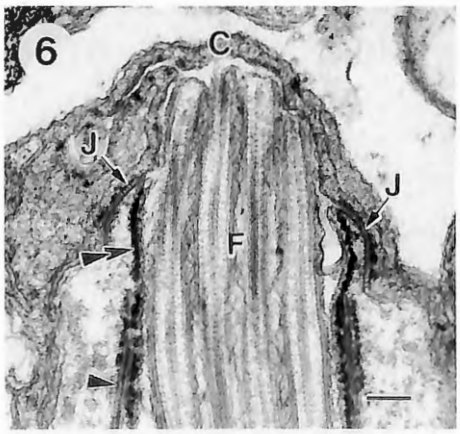
Figs 44-49. – TEM of flame cells of *Gyratrix hermaphroditus*. Figs 44-46 show longitudinal sections of the one cell. C, capillary cytoplasm; F, cilia of the flame; R, ribs; T, cytoplasmic tube. In this species the cytoplasm is continuous from below the cilial rootlets (arrow in Fig. 45) through the ribs with bundles of microtubules (arrowheads) (Figs 46-48), to the cytoplasmic tube still with microtubules (in Figs 44 and 49), to the capillary beyond the tip of the flame. There is no nucleus associated with the flame nor with any nearby region of the capillary. Scales 0.2μm (Fig. 44), 0.5μm (Figs 45-49).

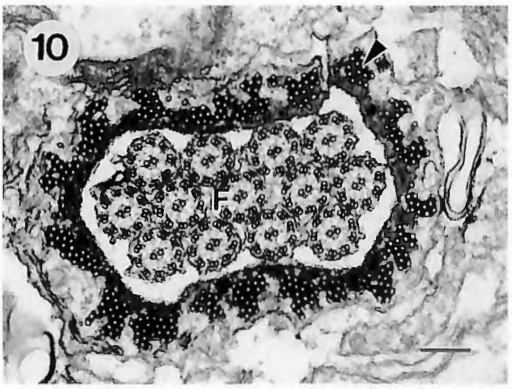

1
C
F
TCN
2
C
J
J
F
3
F
TCN
F
TCN
4
C
J
J
F

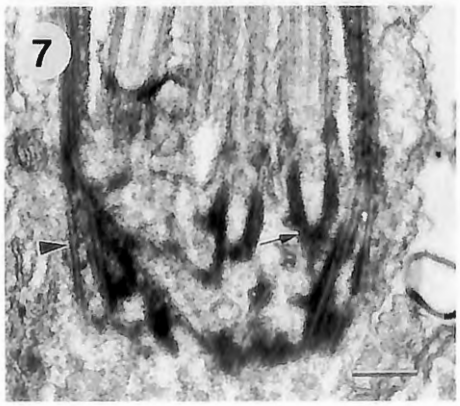

5
F
TCN
C

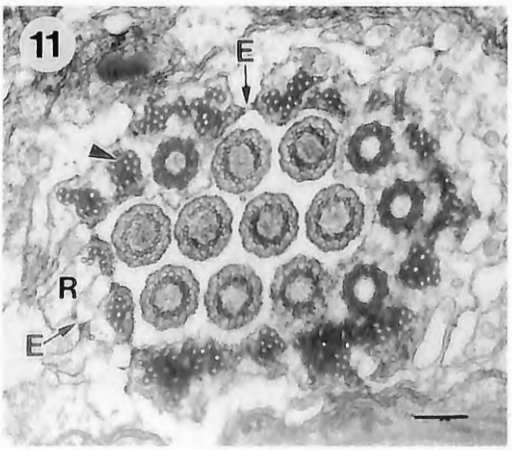

8
C
F

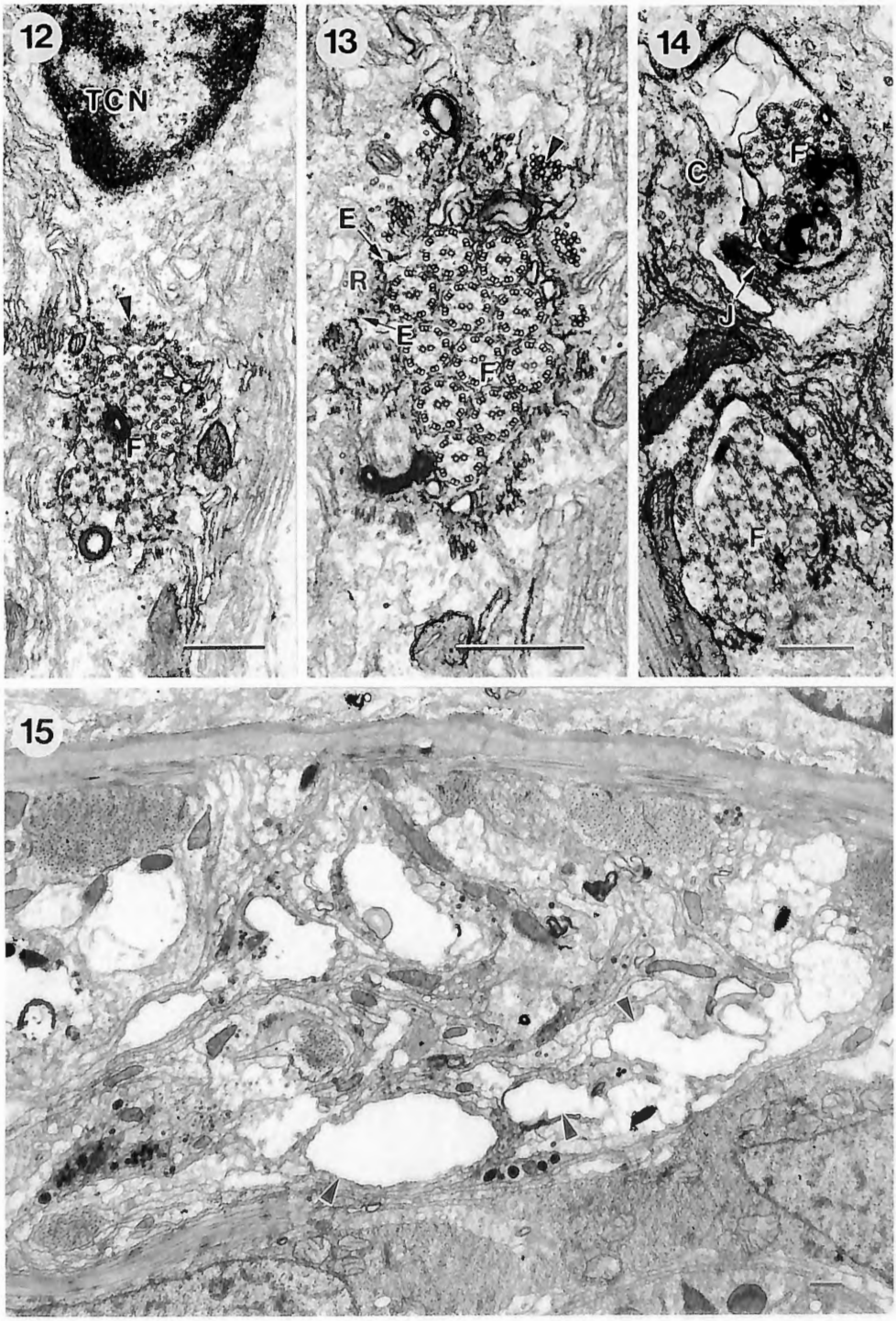

9
J
F
C

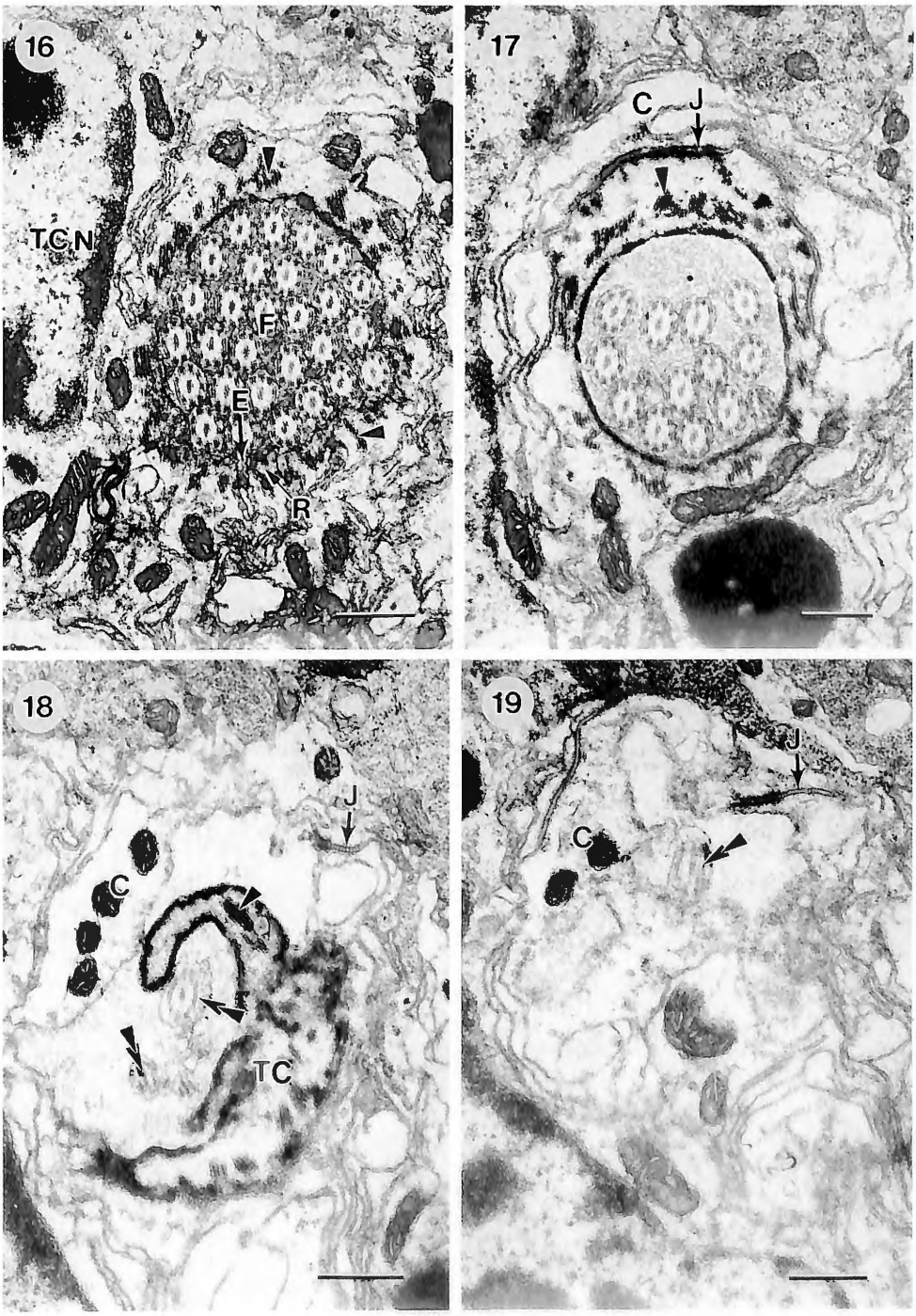

6
C
J
J
F

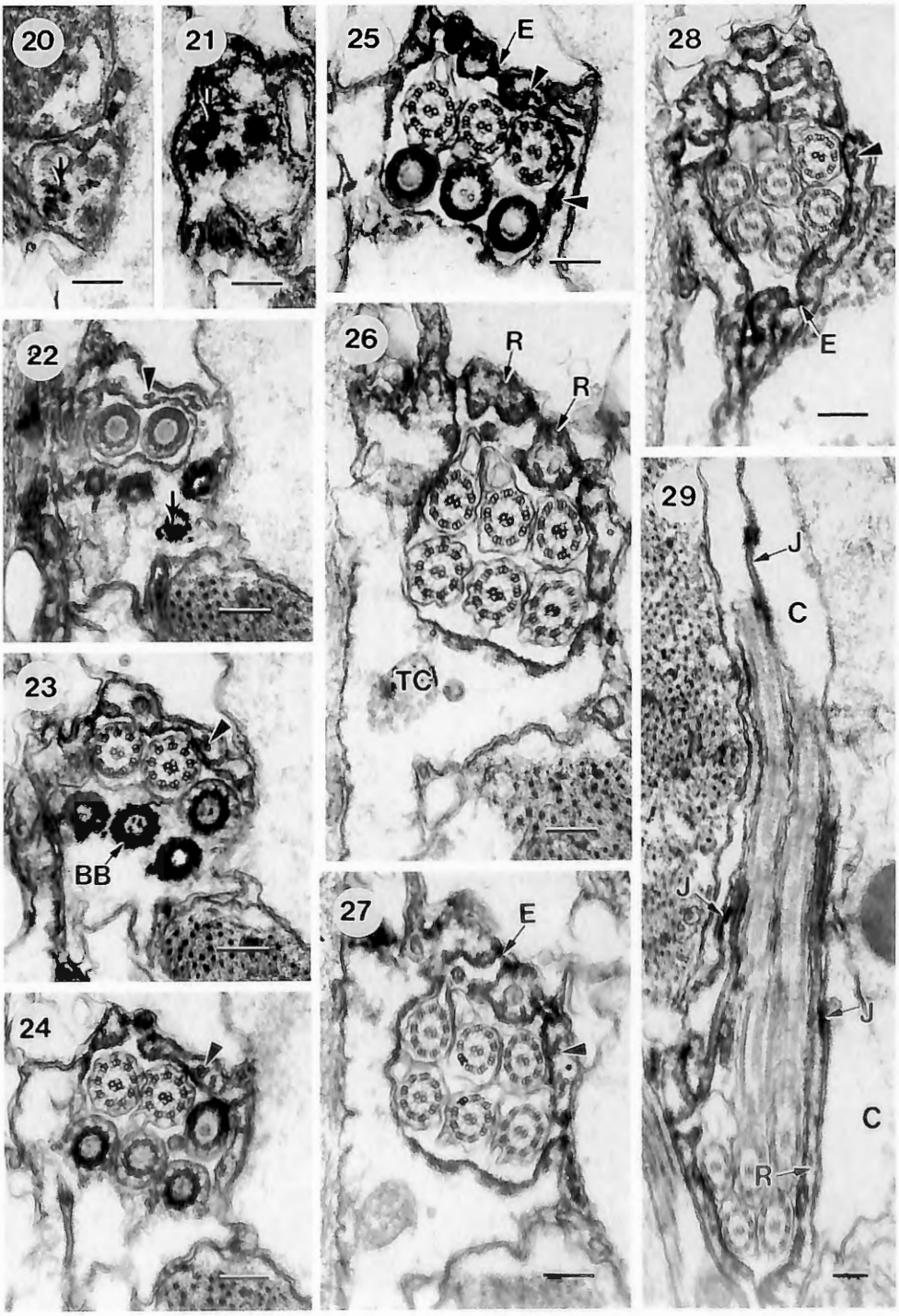

10
F

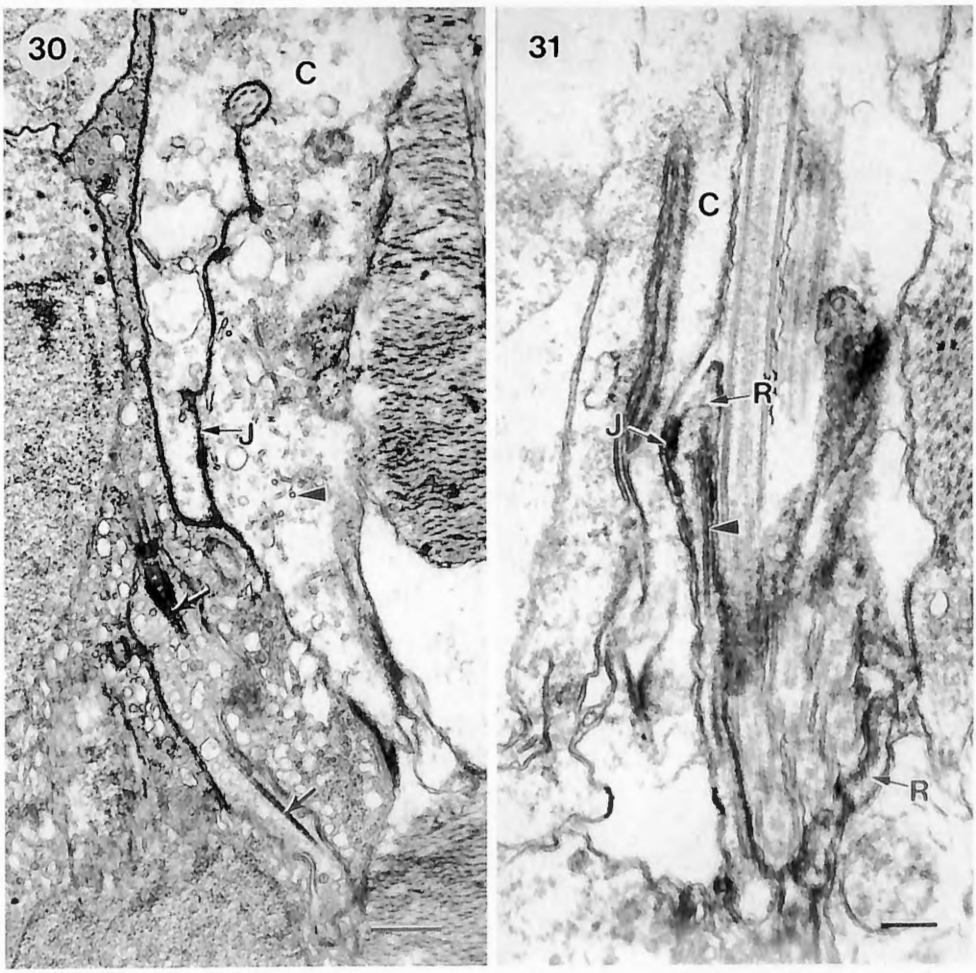

7

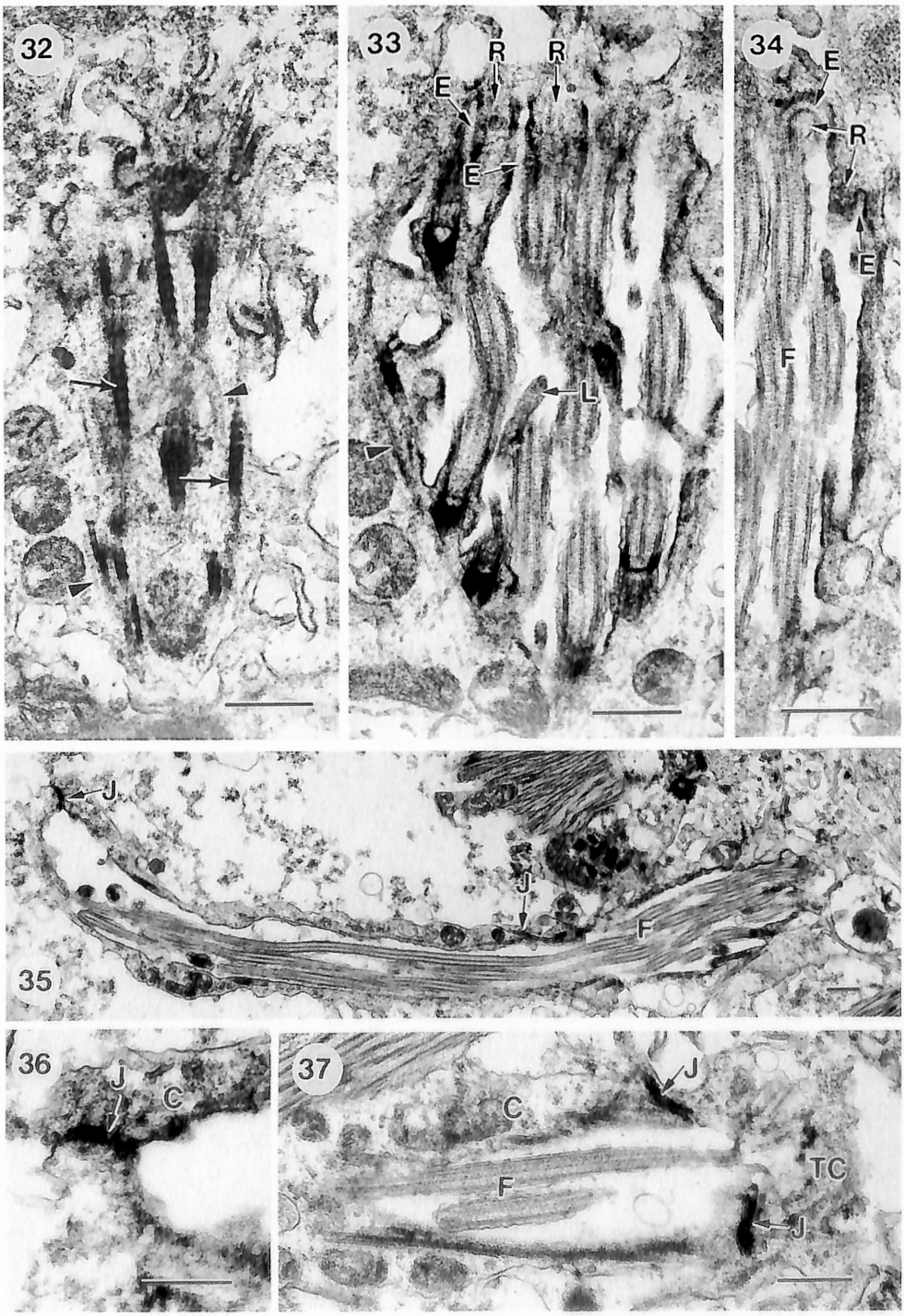

11
E
R
E

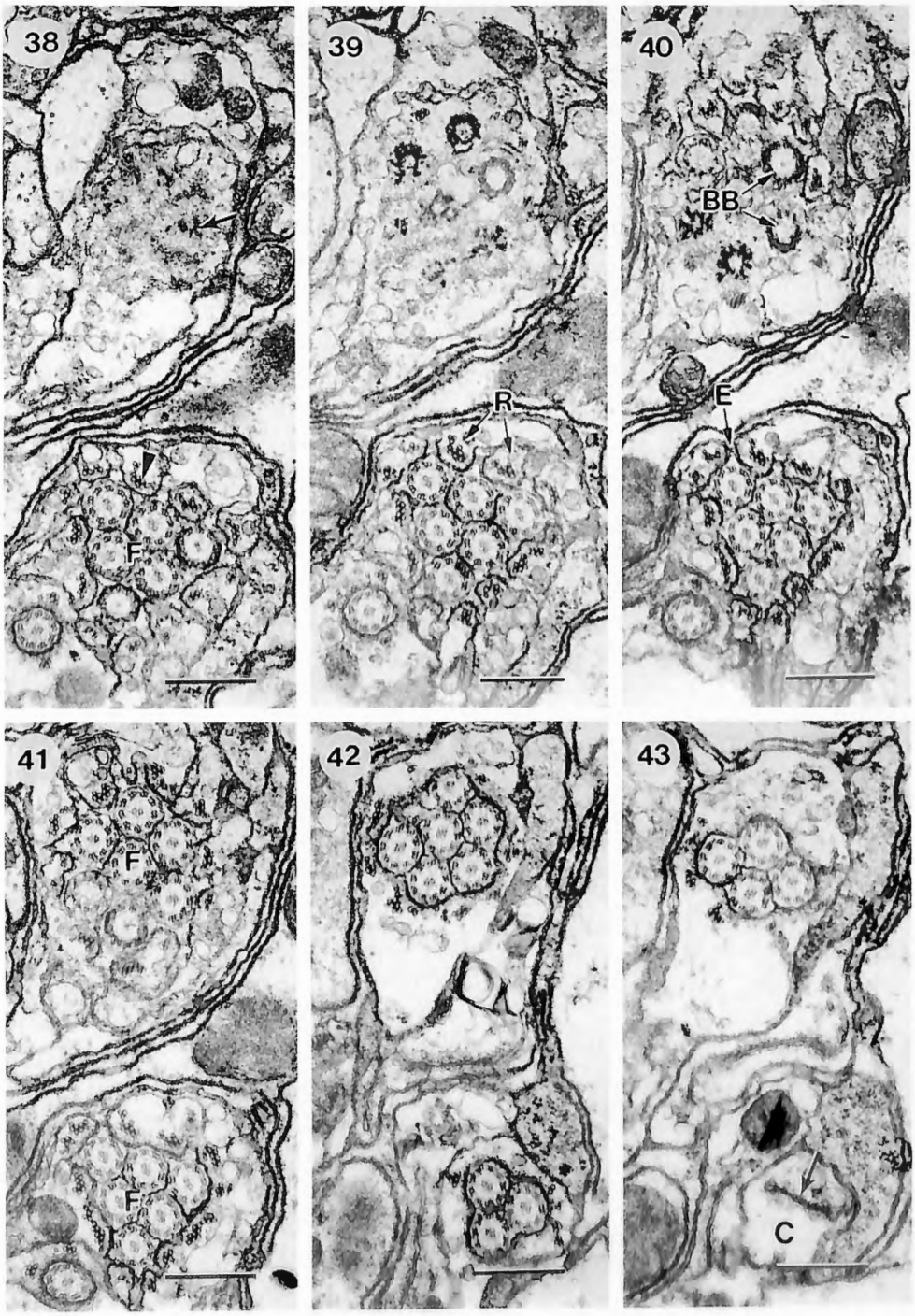

12
TCN
F
13
E
R
E
F
14
C
F
J
F
15

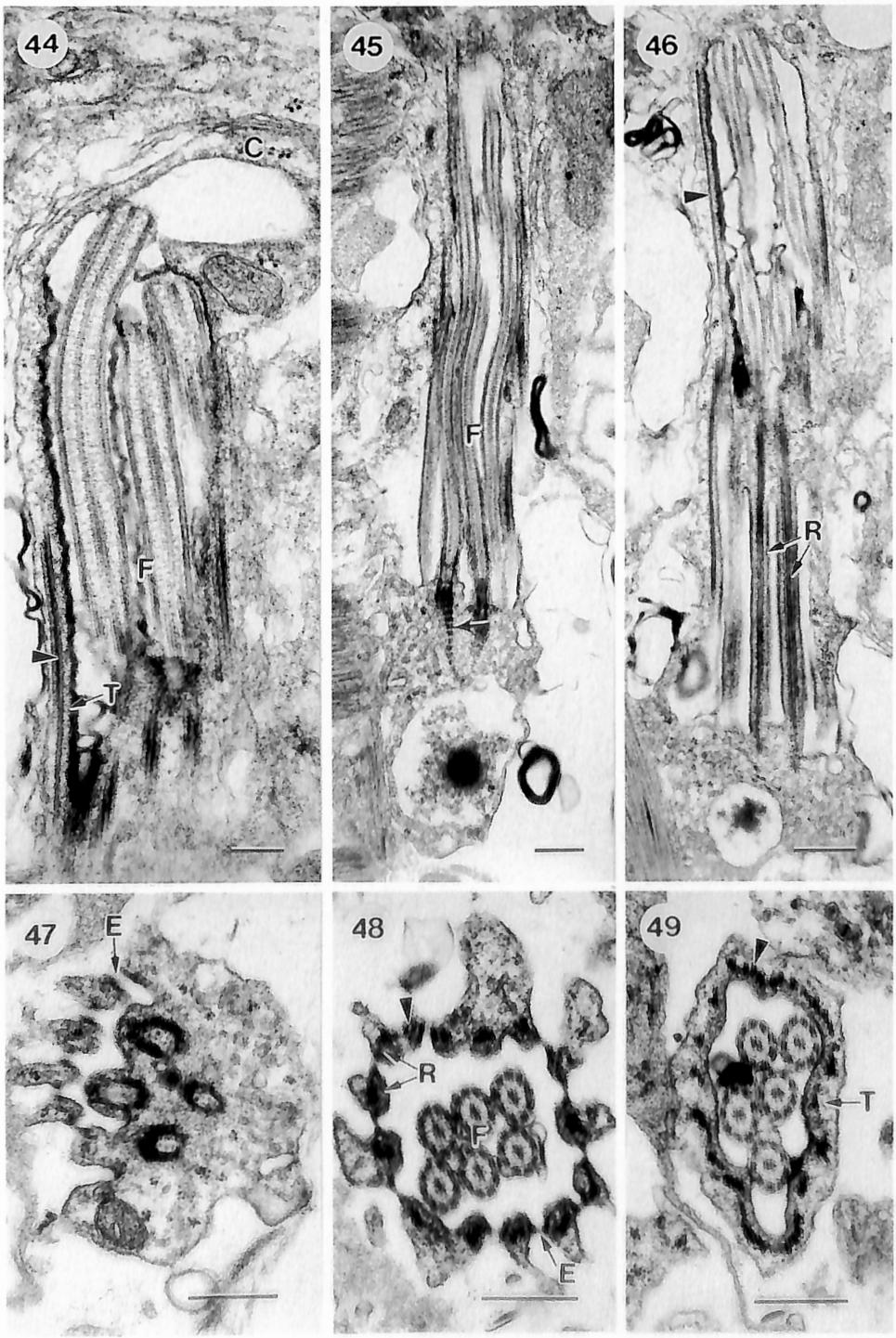

16
T.C.N
F
E
R
17
C
J
18
J
C
TC
19
J
C


20
21
25
E
28
E
22
26
R
R
TC
23
BB
29
J
C
J
J
C
R
24
27
E


30
C
J
31
C
R
J
R


32
33
R
R
E
E
L
34
E
R
E
F
J
J
F
35
36
J
C
37
J
C
F
TC
J


38
F
39
R
40
BB
E
41
F
F
42
43
C


44
C
F
T
45
F
46
R
47
E
48
R
F
E
49
T

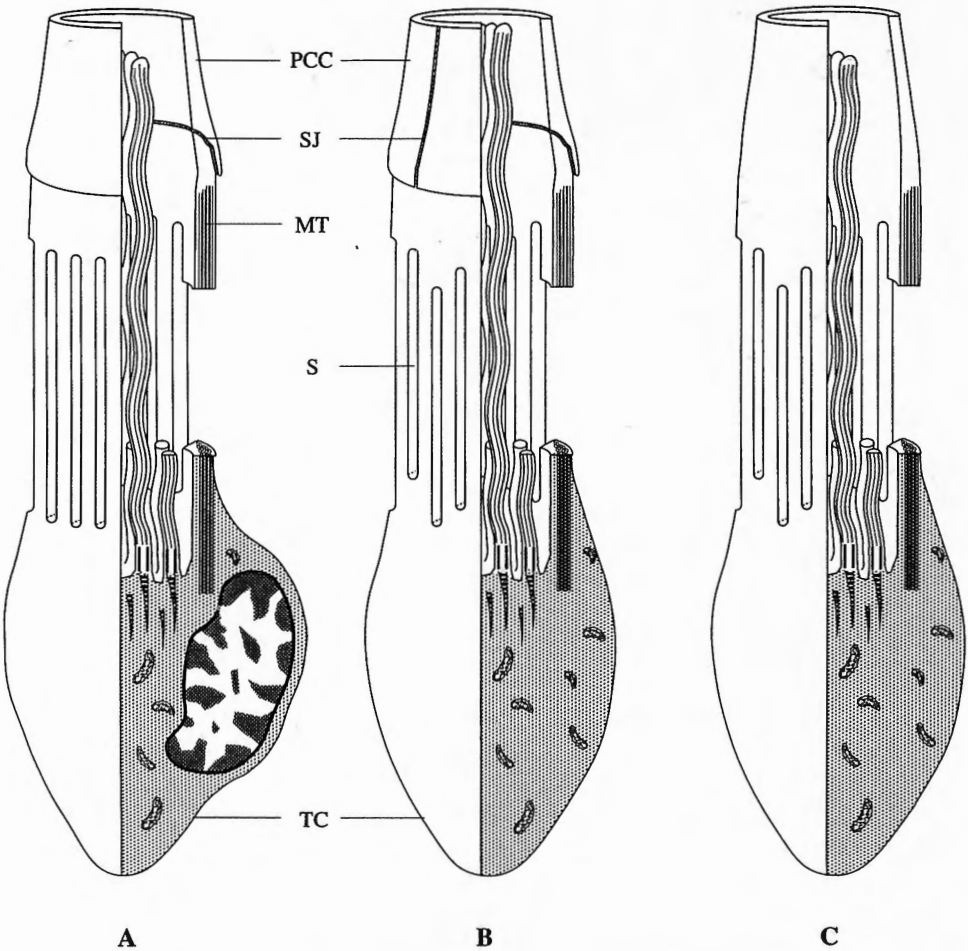


Fig. 50. – Diagrammatic representation of the terminal protonephridial filtration regions in kalyptorhynch turbellarians. Type A found in *Ptyalorhynchus coecus, Cicerina remanei, Thylacorhynchus* spp. and *Diascorhynchus rubrus*. In *P. coecus*, the canal cell enwrapped the lumen and a junction was thus formed between the adjacent edges. Type B found in the two cicerinids *Toia calceformis* and *Nannorhynchides herdlaensis*. No nucleus could be located for the «terminal cell». Type C found in the gnathorhynchid *Ancistrorhynchus ischnurus* and the polycystid *Gyratrix hermaphroditus*. In Type C the filtration region is continuous with the proximal canal and no nuclei are found in the vicinity.

MT, microtubules, PCC, proximal canal cell; S, filtration slits; SJ, septate junction; TC, terminal cell.

Belg. J. Zool. - Volume 127 (1997) - issue 2 - pages 159-166 - Brussels 1997

Received : 7 July 1997

MORPHOLOGY AND ULTRASTRUCTURE OF THE METATIBIAL GLAND IN THE ARMY ANT *DORYLUS MOLESTUS* (HYMENOPTERA, FORMICIDAE)[1]

JOHAN BILLEN

Zoological Institute, University of Leuven,
Naamsestraat 59, B-3000 Leuven (Belgium)
e-mail: Johan.Billen@bio.kuleuven.ac.be

Abstract: Beneath the cuticle of the ventral side of the distal fourth of the hindleg tibia of workers of the army ant *Dorylus molestus,* there is a conspicuous glandular epithelium with a thickness of approx. 35 µm. The columnar secretory cells are characterized by the presence of a well developed smooth endoplasmic reticulum and numerous secretory inclusions. Their basal cell membrane shows numerous invaginations, while an extensive but irregular microvillar differentiation occurs apically. The overlying cuticle is traversed by pores that guide the glandular secretion to the outside. The function of the gland, that is probably absent in queens and males, is unknown. In each of the three pairs of legs, there is an additional cluster of so far unknown glandular cells with accompanying duct cells in the most distal part of the tibia, as well as a glandular epithelium dorsally underneath the proximal part of the basitarsus.

Key words: ants, Dorylinae, exocrine glands, scanning microscopy.

INTRODUCTION

Social insects are well known for their elaborate exocrine system, in which 63 different glands have been distinguished so far (BILLEN, 1994). Although the majority of these are located in the head, thorax and abdomen, the appendages also contain exocrine glands. Recent research revealed that the legs of ants may show various glandular structures that can be located in the coxa (SCHOETERS and BILLEN, 1993), tibia (LEUTHOLD, 1968; PASTEELS *et al.*, 1970; BILLEN, 1984; HÖLLDOBLER *et al.*, 1996), basitarsus (HÖLLDOBLER and PALMER, 1989a; HÖLLDOBLER *et al.*, 1992; SCHÖNITZER *et al.*, 1996) and pretarsus (HÖLLDOBLER and PALMER, 1989b; BILLEN, 1993). Among these, various types of tibial and basitarsal glands can be distinguished, both in terms of their general occurrence and cellular organization.

Two kinds of tibial glands have been reported so far, both occurring in the hindlegs. An internalized epithelial gland in the metatibia is only known for species of the genus *Crematogaster,* where it is the source of the trail pheromone (LEUTHOLD, 1968). The exis-

[1] Dedicated to the memory of the late Professor J. VAN BOVEN.

tence of a metatibial gland underneath the tegumental cuticle was first mentioned by Bolton (1990) as a synapomorphic character of ants of the doryline section. Its distribution and general organization was recently described in a comparative study by Hölldobler *et al.* (1996). Since no ultrastructural data on this gland are available, we examined the well developed metatibial gland of the army ant *Dorylus molestus,* and report on our findings.

MATERIAL AND METHODS

Foraging workers of *Dorylus (Anomma) molestus* Gerstaecker, 1858 were collected from a natural raiding column in Nairobi, Kenya. Legs of minor, medium and major (soldier) workers were cut off near the proximal side of the tibia and fixed in 2% glutaraldehyde, buffered at pH 7.3 with 50 mM sodium cacodylate and 150 mM saccharose. Postfixation in a buffered osmium tetroxide solution was followed by dehydration in a graded acetone series and embedding in Araldite. Semithin sections (1 µm thickness) for light microscopy were stained with methylene blue and thionin. Thin sections for ultrastructural examination (70 nm thickness) were double stained with uranyl acetate and lead citrate in an LKB 2168 Ultrostainer and were examined in a Zeiss EM 900 microscope. Tissues for scanning microscopy were dehydrated in an ethanol series after postfixation and were critical point dried. They were coated with gold and viewed in a Philips SEM 515 microscope.

RESULTS

The ventral side of the distal fourth of the hindlegs of workers of *Dorylus molestus* shows an obvious elongated and flattened area which is devoid of the hairs and sculpture found elsewhere on the legs (Fig. 1). The same region on the fore- and midlegs, in contrast, clearly lacks this modification (Figs 2 and 3, respectively). This flattened area on the metatibia at first appears as a smooth zone, but at high magnification shows numerous very small pores with a diameter of approx. 50 nm that open through the cuticle at various angles (Fig. 4). Histological sections through the distal part of the metatibia reveal the presence of a conspicuous glandular epithelium with high columnar cells underneath the flattened area (Figs. 5, 6). This epithelium is a continuation of the squamous tegumental epidermis, and reaches a constant thickness of approx. 35 µm (range among individuals 28-44 µm). There is no correlation between epithelial thickness and worker size. When checking preserved queens and males, the hindleg tibia appears to lack the specialized distal area described for workers, which probably indicates the absence of the gland in the reproductive castes.

Ultrastructural examination of the gland reveals the modified appearance of the cuticle overlying the glandular epithelium, with conspicuous and irregular pores that traverse the cuticle from the apical side of the secretory cells towards the surface, where they open as holes with a diameter of approx. 50 nm (Figs 6,7). This porous appearance is only found in the cuticle overlying the glandular epithelium; elsewhere the usual horizontally layered

cuticle occurs (Fig. 7). The cuticle has a constant thickness of 13 μm. The apical region of the glandular cells is characterized by an extensive though irregular microvillar differentiation (Figs 6, 8). The cytoplasm is occupied by a well developed tubular smooth endoplasmic reticulum, and, especially in the apical part of the cell, numerous electron-dense secretory vesicles with a diameter of approx. 0.15 μm (Figs 8, 9). Granular endoplasmic reticulum does not occur, while free ribosomes are scattered through the cytoplasm (Fig. 9). The basal cell membrane displays obvious invaginations. Elongated mitochondria also occur in this region (Fig. 10). The cells are characterized by rounded to ovoid nuclei with a diameter around 5 μm (Figs 6, 10).

Examination of the leg sections revealed, in addition to the metatibial gland, the existence of another gland in the very distal part of the tibia, as well as the presence of a glandular epithelium in the proximal part of the basitarsus. The additional tibial gland is formed by a cluster of 5-10 rounded secretory cells with a diameter around 25 μm with accompanying duct cells that open through the tibio/basitarsal articulation membrane (Fig. 11). The glandular epithelium in the basitarsus is a differentiation of the tegumental epidermis. It occurs at the dorsal side, and reaches a thickness of approx. 15 μm (Fig. 11). Both glands appear in the three pairs of legs, and represent hitherto unknown exocrine structures.

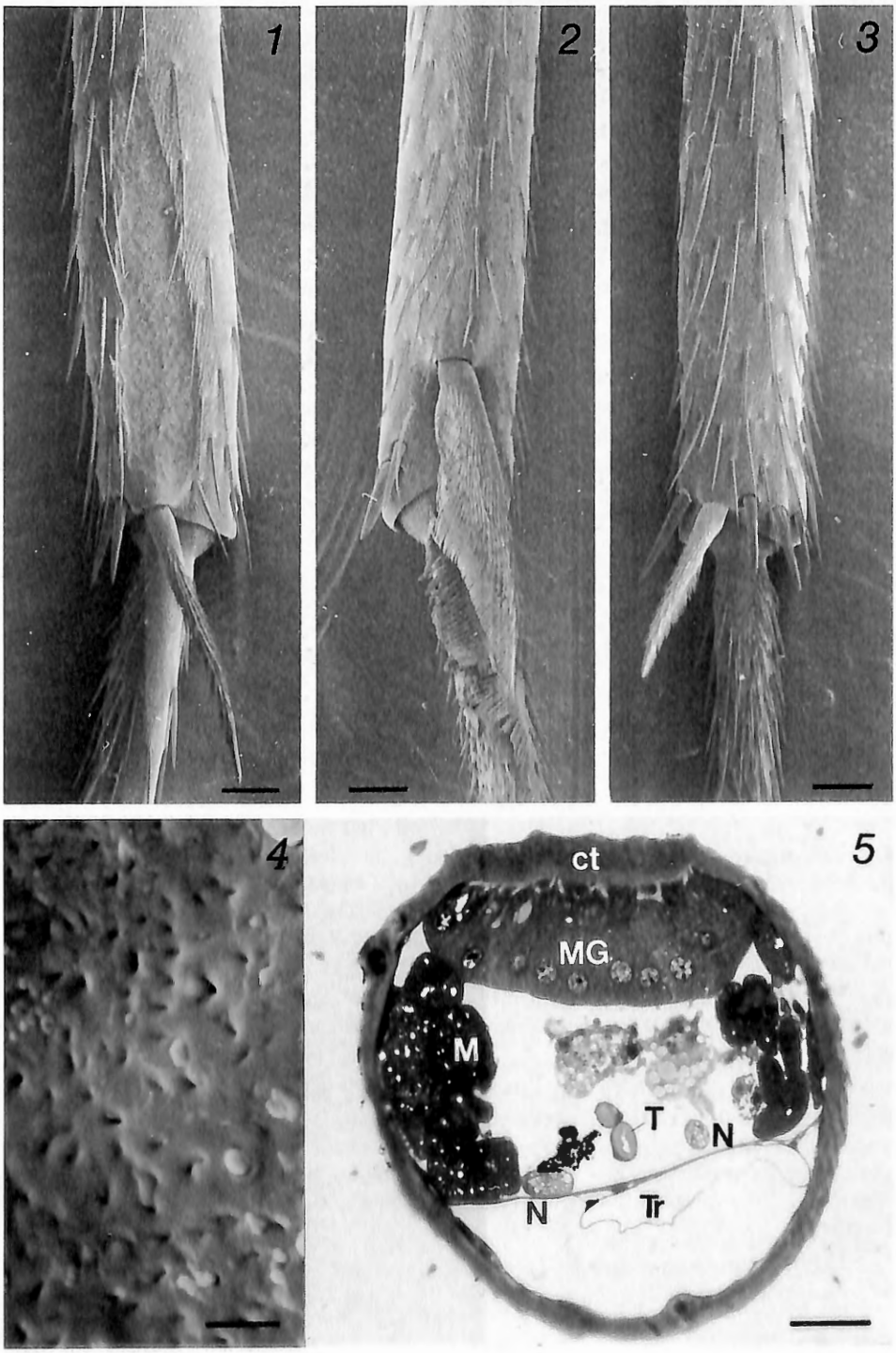
Figs 1-4. – Scanning micrographs showing the ventral side of the tibiae near the articulation with the basitarsus in major workers of *Dorylus molestus*. Fig. 1. – hindleg with flattened area overlaying the metatibial gland (bar 100 μm). Fig. 2. – foreleg (with antennal cleaning apparatus, bar 100 μm). Fig. 3. – midleg (bar 100 μm). Fig. 4. – detail of the cuticle of the flattened area in the hindleg with numerous small pores (bar 1 μm).

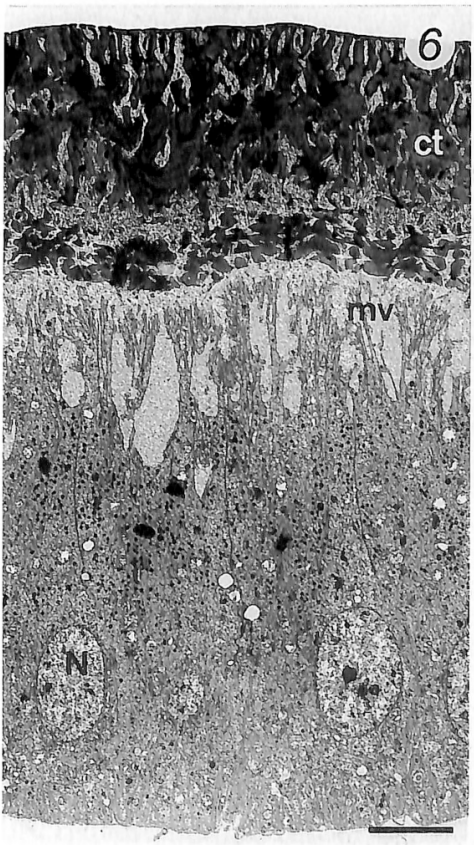
Fig. 5. – Semi-thin cross section through the hindleg tibia of a minor worker showing the metatibial gland (MG). ct: cuticle, M: muscles, N: nerves, T: tibial tendon, Tr: trachea (bar 20 μm).

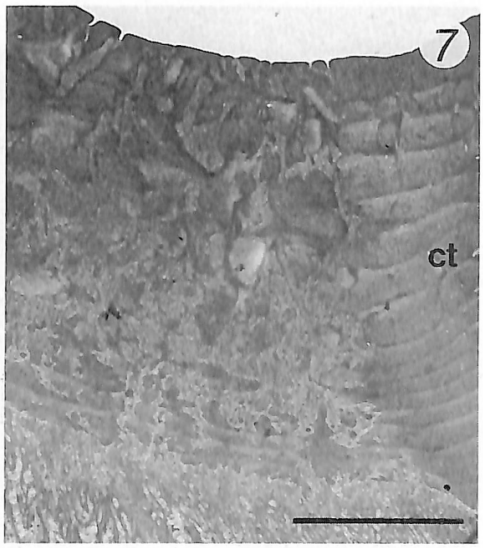
Figs 6-10. – Electron micrographs of the metatibial gland. Fig. 6. – survey of the glandular epithelium and overlying cuticle (major worker, bar 5 μm). Fig. 7. – porous cuticle overlying the glandular epithelium, normal cuticle at right (medium worker, bar 5 μm). Fig. 8. – apical cytoplasm with microvillar differentiation and secretory vesicles (major worker, bar 1 μm). Fig. 9. – detail of cytoplasm showing abundant tubules of smooth endoplasmic reticulum (medium worker, bar 1 μm). Fig. 10. – basal cytoplasm showing conspicuous invaginations of cell membrane (medium worker, bar 5 μm).

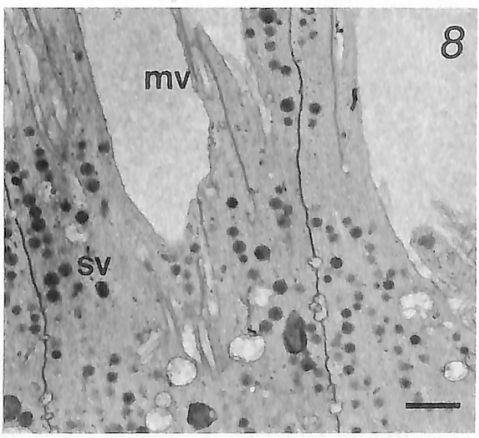
bi: basal invaginations, ct: cuticle, mv: microvilli, N: nucleus, SER: smooth endoplasmic reticulum, sv: secretory vesicles.

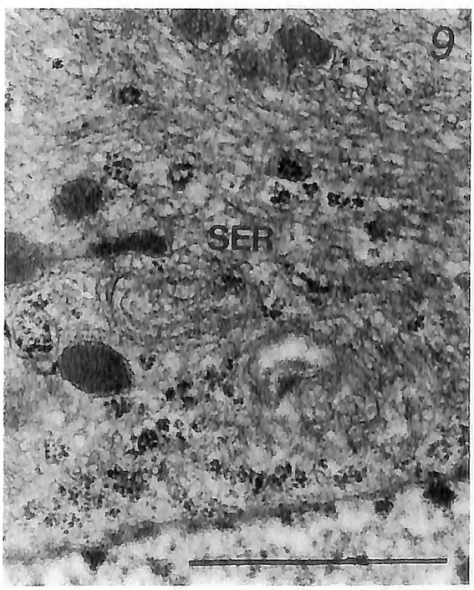
[See figures pages 162 and 163]

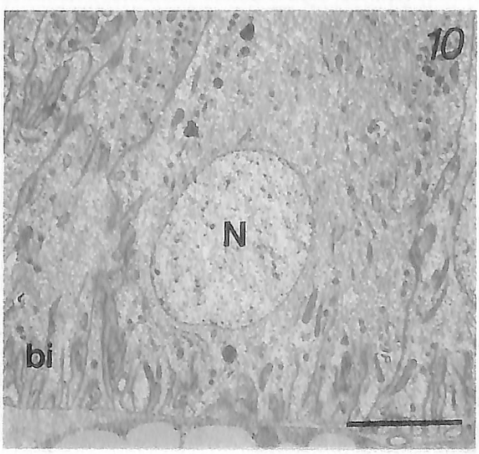

1
2
3
4
5
ct
MG
M
T
N
N
Tr


6
ct
mv
N


7
ct


8
mv
sv


9
SER


10
N
bi

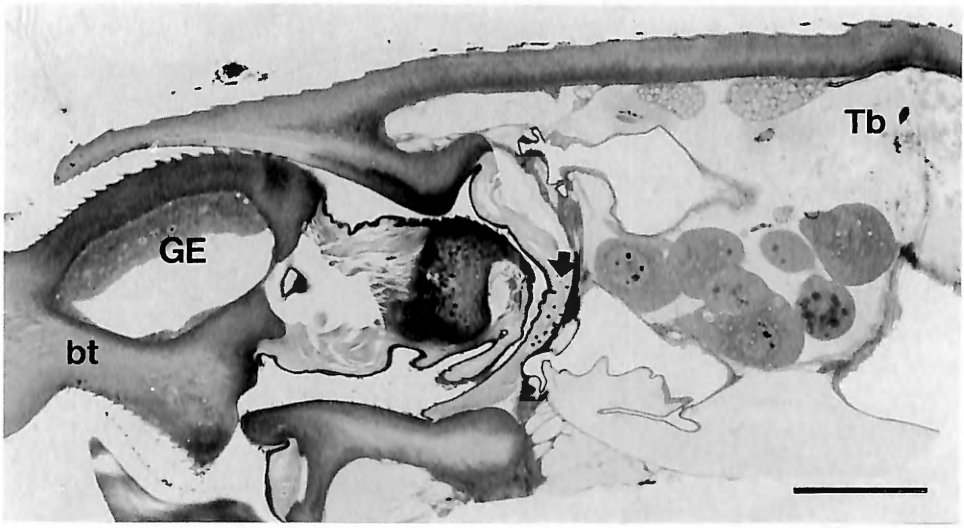


Fig. 11. – Semi-thin longitudinal section through the articulation between the foreleg tibia (Tb) and basitarsus (bt) of a major worker, showing cluster of metatibial glandular cells of which corresponding ducts open through the articulation membrane (arrow), as well as glandular epithelium (GE) in proximal part of basitarsus (bar 50 µm).

DISCUSSION

The metatibial gland was recently described in a comparative survey study by Hölldobler *et al.* (1996) for ants of the poneroid group. Its presence was thus also reported for the Old World army ant *Dorylus nigricans,* for which it was briefly described as similar to that of the New World army ant *Eciton hamatum,* where it occurs as a «relatively thin glandular epithelium extending more than three-quarters of the length of the tibia». In all specimens of *Dorylus molestus* we examined, regardless of worker size, the gland is less extended in length (it occurs in the distal quarter of the hindleg tibia only) and is considerably more pronounced in thickness.

The ultrastructural characteristics of the gland as reported here for *Dorylus molestus* are indicative for an active transport of substances through the epithelium and overlaying cuticle. Precursor molecules from the haemolymph are probably taken up through the numerous basal invaginations, and undergo further metabolic processes in the well developed smooth endoplasmic reticulum. The secretory products appear as small rounded electron-dense vesicles. The extensive apical microvilli and the conspicuous cuticular pores form an easy pathway for secretion to the outside. The cytoplasmic organization with an extensive smooth endoplasmic reticulum is in agreement with that of pheromonal glands (Noirot and Quennedey, 1974; Billen, 1991). The function of the metatibial gland, however, is still unknown (Hölldobler *et al.*, 1996). For several queenless *Diacamma* species, the gland appears to be involved in sexual calling by the dominant worker

(HÖLLDOBLER *et al.*, 1996), but this is not applicable for army ants workers. The apparent absence of the metatibial gland in the army ant queen and male moreover excludes a role in reproductive regulation. An eventual function in trail laying, as has been demonstrated for leg glands of other ants (LEUTHOLD [1968] for the tibial gland in *Crematogaster,* HÖLLDOBLER and PALMER [1989a] and HÖLLDOBLER *et al.* [1992] for the basitarsal glands in *Onychomyrmex* and *Prionopelta,* respectively, and the pretarsal gland in *Amblyopone* (HÖLLDOBLER and PALMER [1989b]), does not seem to be the case for *Dorylus,* as these army ants rely on venom gland secretions as the source of their trail pheromone (BILLEN and GOBIN, 1996). The intense and obvious inter-individual contacts in the extremely large army ant colonies (GOTWALD, 1995) may indicate the distribution of chemical signals, although this remains purely speculative.

Our discovery of additional glandular structures in the legs is a clear illustration of the overwhelming extent of the exocrine apparatus of social insects in general and ants in particular. Their functions remain to be discovered, but they confirm the description of ants as walking glandular batteries (HÖLLDOBLER and WILSON, 1990).

ACKNOWLEDGEMENTS

I am grateful to D. Corstjens for assistance in ultramicrotomy, to J. Cillis and Dr. E. Schoeters for their help in tissue preparation for scanning microscopy, and to Prof. W.H. Gotwald, Jr. for critical reading of this manuscript. This research was supported through grant N° G.0254.96 from the Belgian Fund for Scientific Research.

REFERENCES

BILLEN, J. (1984) – Morphology of the tibial gland in the ant *Crematogaster scutellaris*. *Naturwissenschaften,* **71**: 324-325.

BILLEN, J. (1991) – Ultrastructural organization of the exocrine glands in ants. *Ethol. Ecol. Evol., special issue,* **1**: 67-73.

BILLEN, J. (1993) – Morphology of the exocrine system in ants. Proc. Coll. Soc. Insects, V.E. Kipyatkov (Ed.), St. Petersburg, pp. 1-15.

BILLEN, J. (1994) – Morphology of exocrine glands in social insects: an up-date 100 years after Ch. Janet. *In*: *«Les Insectes Sociaux»* (A. Lenoir, G. Arnold and M. Lepage, Eds.), Publications Université Paris Nord, p. 214.

BILLEN, J. and B. GOBIN (1996) – Trail following in army ants (Hymenoptera, Formicidae). *Neth. J. Zool.,* **46**: 272-280.

BOLTON, B. (1990) – Army ants reassessed: the phylogeny and classification of the doryline section (Hymenoptera, Formicidae). *J. Nat. Hist.,* **24**: 1339-1364.

GOTWALD, W.H. Jr. (1995) – Army Ants. The Biology of Social Predation. *Comstock Publishing Associates, Ithaca - London,* pp. 302.

HÖLLDOBLER, B. and J.M. PALMER (1989a) – A new tarsal gland in ants and the possible function in chemical communication. *Naturwissenschaften,* 76: 385-386.

HÖLLDOBLER, B. and J.M. PALMER (1989b) – Footprint glands in *Amblyopone australis* (Formicidae, Ponerinae). *Psyche,* 96: 111-121.

HÖLLDOBLER, B., M. OBERMAYER and E.O. WILSON (1992) – Communication in the primitive cryptobiotic ant *Prionopelta amabilis* (Hymenoptera: Formicidae). *J. Comp. Physiol.,* **170A**: 9-16.

HÖLLDOBLER, B., M. OBERMAYER and C. PEETERS (1996) – Comparative study of the metatibial gland in ants (Hymenoptera, Formicidae). *Zoomorphology,* **116**: 157-167.

HÖLLDOBLER, B. and E.O. WILSON (1990) – The Ants. *Harvard University Press, Cambridge Mass.,* 732 pp.

LEUTHOLD, R.H. (1968) – A tibial gland scent-trail and trail-laying behavior in the ant *Crematogaster ashmeadi* Mayr. *Psyche,* **75**: 233-248.

NOIROT, C. and A. QUENNEDEY (1974) – Fine structure of insect epidermal glands. *Ann. Rev. Entomol.,* **19**: 61-80.

PASTEELS, J.M., R.M. CREWE and M.S. BLUM (1970) – Etude histologique et examen au microscope électronique à balayage de la glande sécrétant la phéromone de piste chez deux *Crematogaster* nord-américains (Formicidae, Myrmicinae). *C.R. Acad. Sc. Paris,* **271D**: 835-838.

SCHOETERS, E. and J. BILLEN (1993) – Glandes coxales de *Pachycondyla obscuricornis* (Formicidae, Ponerinae). *Actes Coll. Insectes Soc.*, **8**: 183-186.

SCHÖNITZER, K., H. DOTT and R.R. MELZER (1996) – The antenna cleaner gland in *Messor rufitarsis* (Hymenoptera, Formicidae). *Tissue & Cell,* **28**: 107-113.

Belg. J. Zool. - Volume 127 (1997) - issue 2 - pages 167-178 - Brussels 1997

SEASONAL PHENOTYPIC VARIATION IN THE SPECKLED WOOD BUTTERFLY (PARARGE AEGERIA L.): PATTERNS IN AND RELATIONSHIPS BETWEEN WING CHARACTERS

HANS VAN DYCK ([1]), ERIK MATTHYSEN ([1]), JACK J. WINDIG ([1]) ([*])
AND ANDRÉ A. DHONDT ([1,2])

([1]) Department of Biology (U.I.A.), University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium.
([2]) Cornell Laboratory of Ornithology, Sapsucker Woods Road 159, Ithaca, NY 14850, U.S.A.
([*]) Present address: Department of Zoology, University of Stockholm, S-106 91 Stockholm, Sweden.
e-mail: hvdyck@uia.ua.ac.be

Abstract. We studied the variation in dorsal wing colour, dorsal hindwing spotting and wing length within a population of the speckled wood butterfly *Pararge aegeria* (L.) in two successive years. Over time in the first generation, successively emerging butterflies were darker, larger and of higher spot type (a clearer fourth submarginal spot). In the second generation they remained on average rather dark and of median size, but variation in spotting repeated the pattern of the first generation. We interpret these patterns of variation in colour and size in relation to seasonal changes in the irradiance conditions within a temperate forest. We suggest that there is a larger genetic control of spotting than of wing colour and wing length. Females were darker, larger and of higher spot type than males. Darker males were on average larger and of higher spot type than pale males. These relationships can be interpreted in relation to the males' mate-locating strategies.

Key words: *Pararge aegeria*, butterflies, phenotypic variation, seasonal variation, wing colour, wing spots, mate-locating strategies.

INTRODUCTION

The reproductive success or fitness of an individual largely depends on how well its phenotype is adapted to the abiotic and biotic environment it experiences. Variation in phenotype may help individuals to better match the environment in time (seasonal) and/or space (geographic) (ENDLER, 1986). Phenotypes of co-occurring individuals may vary adaptively in relation to for instance their behaviour (VAN DYCK *et al.*, 1997a, 1997b). In butterflies, phenotypic variation in wing features (colour patterns, wing size and shape, etc.) is a popular object of study since the wings are essential for flight activity (*e.g.*, DUDLEY, 1990) and play significant roles in predator escape (BRAKEFIELD *et al.*, 1992), thermoregulation (WASSERTHAL, 1975; DENNIS, 1993), sexual communication and mate choice (SILBERGLIED, 1984). Here we report on the phenotypic variation in a population of

a butterfly which emerge over a long period during the year. In order to interpret the relationships between phenotypic variation, environment and behaviour, we need to know how the phenotypes vary with time of emergence and how the different phenotypic features are linked to one another.

Alternative phenotypes (WEST-EBERHARD, 1989) may exist as genetic polymorphisms caused by different genes (FORD, 1975) or polyphenisms induced by the environment (SHAPIRO, 1976). Examples in butterflies are white-yellow polymorphism in *Colias* (WATT, 1968), dry-wet seasonal polyphenism in tropical Satyrinae (BRAKEFIELD & LARSEN, 1984; WINDIG *et al.*, 1994) and spring-summer polyphenism in the European map butterfly *Araschnia levana* (MÜLLER, 1955). Polyphenism does not exclude genetic variation (HAZEL *et al.*, 1987) and can be itself under genetic control (BRADSHAW, 1965; WINDIG, 1994). Sometimes what looks like a genetic polymorphism turns out to be under environmental control (OWEN & GOULSON, 1994).

In the speckled wood butterfly *Pararge aegeria* (L.) there is geographic variation in wing length (size), wing shape and the pattern of dorsal pale patches (BRAKEFIELD & SHREEVE, 1992). Since in northwestern Europe adults of this satyrine butterfly emerge over an extended period from April to September (BINK, 1992), different individuals experience very different larval conditions throughout the season which may influence their adult phenotype. The adaptive value of the phenotype probably also varies over the season. When reared outdoors, British speckled woods vary seasonally in forewing length and in the size of the dorsal pale patches (ROBERTSON, 1980), as well as in the number of submarginal spots on the hindwings and the brown dorsal wing colour (pale or dark) (PACKER, 1984).

We studied the variation in dorsal wing colour, dorsal hindwing spotting and wing size (forewing length) amongst individuals within a Belgian population of the speckled wood in two successive years. We analysed (1) the temporal pattern of these wing features in both generations, and (2) the relations between the features. Of the two biometric studies on (British) speckled woods one did not consider the variation in brown dorsal wing colour (ROBERTSON, 1980) and the other one (on collection specimens) did not consider size (PACKER, 1984).

MATERIAL AND METHODS

The speckled wood occurs primarly in different types of woodland and the larvae feed on grasses (SHREEVE, 1986a). We studied the speckled wood (North-European subspecies *P. aegeria tircis* Butler 1867) in a set of deciduous woodland fragments («Lankem») in Herentals, northern Belgium (51°08' N, 4°49' E). As in the rest of Belgium, *P. aegeria* is a common species and has become even more abundant in recent decades (MAES & VAN DYCK, 1996). The study area consisted of five fragments of the same habitat type (<1 ha to >9 ha) which were close to one another and separated by maize fields and meadows. In all sites the dominant tree species was common oak, *Quercus robur*, with a mixture of other trees and shrubs. A large part of the forest floor was covered by brambles, *Rubus* sp. Larval hostplants (*e.g. Agropyron repens*, *Dactylis glomerata* and *Poa annua*) were abundant in and around the study areas.

We collected data on sex, dorsal wing colour, dorsal submarginal spot pattern and forewing length in two successive years: between 20 April and 18 September 1993 and 27 April and 25 August 1994. These periods covered the first and the second generation of each year, also called spring and summer generation respectively. In both years the first generation lasted until the end of June and the second generation started at the beginning of July. The end of the first generation was marked by a strong decrease in abundance and absence of newly emerged, *i.e.* undamaged and unmarked, butterflies. We captured butterflies with a hand net and marked them to avoid double measurements by writing a small number with a non-toxic black pen on the hindwings. We took all measurements at the first capture. We assigned the dorsal wing colour to one of four categories from pale brown (1) to dark brown (4). The dorsal pattern of black submarginal spots on the hindwings was scored in four categories: (1) three spots, (2) three spots and a vestigial fourth spot, (3) four spots but the fourth is small, and (4) four clear spots. Both indices have a high repeatability (VAN DYCK *et al.*, 1997a). Forewing length was measured from the wing joint to the wing tip and read to the nearest 0.05 mm with calipers. Date (April 20 being 1), time and exact location of the capture were also noted.

First, we compared the overall mean values of the different wing features between males and females. Further analyses were performed on the males only since we had insufficient data for the females. Second, we analysed the temporal variation of wing colour, wing length and spot pattern in relation to date, generation and year. In the graphs (Fig. 2) we expressed the mean values by four equally long periods in the first and second generation each, for convenience. In the second generation of 1993 we have data of a fifth period. Third, we analysed the relations between the wing features by calculating correlations and performing a principal component analysis. Smoothed values (moving averages) were used to plot the first principal component over time (cf. WINDIG *et al.*, 1994). These moving averages were calculated over a period extending immediately before and after each date so as to include a total of at least eleven individuals (five before and five after). Temporal variation in the wing features and the principal component was analysed by general linear regression models (GLM-procedure in SAS, with type III Sum of Squares). GLM handles discrete variables (*e.g.*, colour classes) and continuous variables (*e.g.*, wing length) (SAS, 1990). The full model for each wing feature or principal component started with the seasonal parameters (date, generation and year) and all interaction terms. Minimum adequate model selection was done by backward elimination of the least significant factors of the full model. We used the SAS-package for all the statistical analyses (SAS, 1990). Means are given ± SE.

RESULTS

Differences between sexes

In both years, males were the most commonly observed sex: 87.8% in 1993 (N=213) and 91.1% in 1994 (N=136). This is mainly caused by differences in activity, and thus apparency, of the sexes (cf. DAVIES, 1978). Considering all data, we found females on average to be larger ($F_{1,314}$=35.81, P=0.0001), darker ($F_{1,349}$=5.09, P=0.024) and of a high-

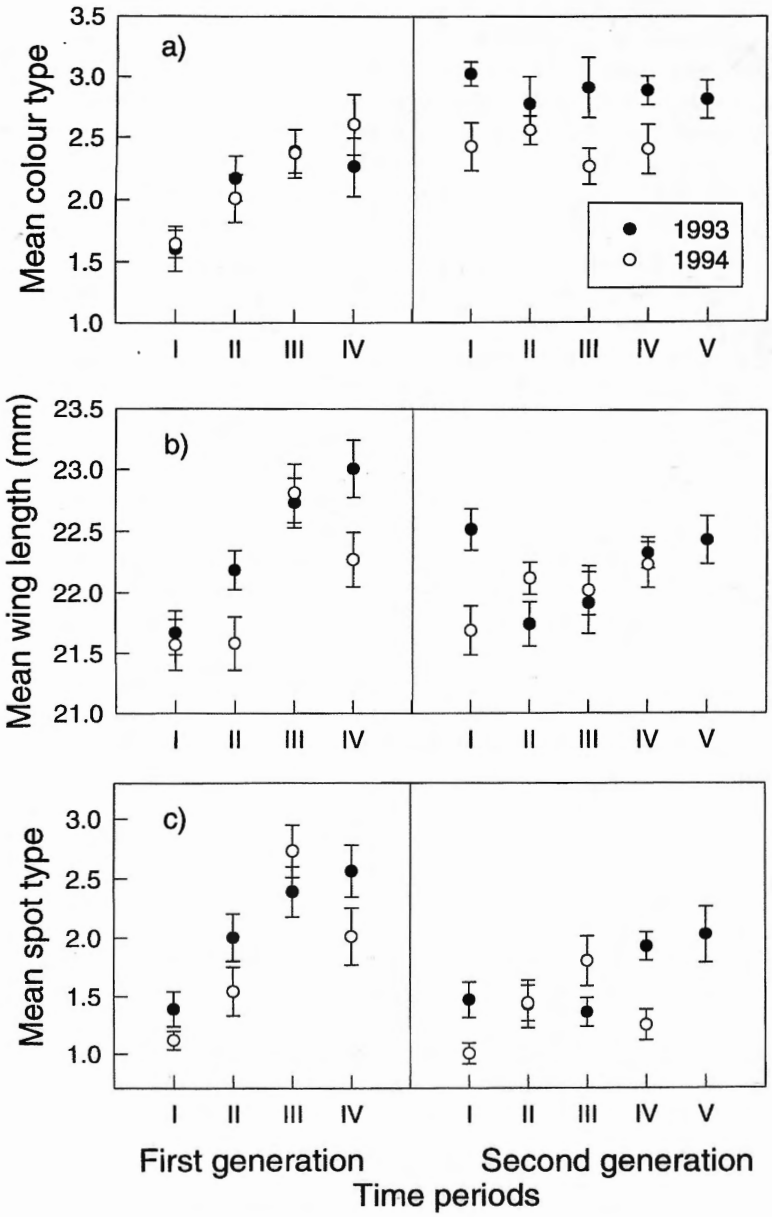


Fig. 2. – Seasonal pattern for (a) dorsal wing colour, (b) forewing length and (c) dorsal hindwing spot type in the males for 1993 and 1994. The mean values (±SE) are given for 4 equally long time periods of the first and second generation of 1993 and 1994. For the second generation of 1993 there is a fifth period. Generation 1: four periods between 20/4 - 25/6 and generation 2: five periods between 5/7-18/9.

er spot type ($F_{1,349}$=10.86, P=0.0011) than males. Male wing length varied between 19.4 and 24.8 mm with a mean of 22.17±0.01 mm, and female wing length between 21.5 and 25.4 mm with a mean of 23.41±0.04 mm. Fig. 1. shows the frequencies of the colour and spot types for both sexes.

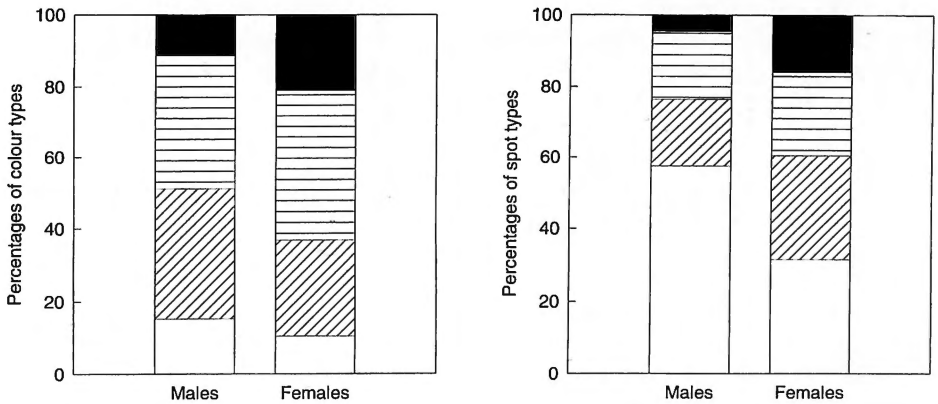


Fig. 1. – Total frequencies of (left) the different colour types (white=colour 1 or pale, shaded= colour 2, horizontal lines=colour 3 and black=colour 4 or dark) and (right) the different spot types (white=spot type 1, shaded=2, horizontal lines=3 and black=4) for males (N=311) and females (N=38) in the combined data set from 1993-94.

Temporal variation in wing colour, wing length and hindwing spotting

Earlier emerging first-generation males were paler than late emerging first-generation and second-generation males (Fig. 2). The analysis of variation in the brown background colour in relation to date, generation and year, showed a significant generation-year (P= 0.021) and generation-date (P=0.0007) interaction. When analysing the generations separately, we found a strong effect of date and not of year in the first generation (F1,129= 13.55, P=0.0003) and a strong effect of year but not date in the second generation (F1,182=19.04, P=0.0001). The dorsal wing colour became darker with successive emergence within the first generation and remained dark during the second generation, but at a lower level in 1994 compared to 1993 (Fig. 2).

The largest individuals were found in the second half of the first generation (Fig. 2). The analysis of wing length variation showed a significant generation-date interaction (P= 0.0003). In the separate analysis by generation, we found date as well as year to be significant in the first generation ($F_{2,109}$=11.53, P=0.0001: date F=20.13, P=0.0001 and year F=4.57, P=0.034) but we found no significant relation with any variable in the second generation ($F_{2,109}$=1.15, P=0.31). Successively emerging individuals were larger within the first generation of both years, but were smaller in 1994, which explains the year effect. In the second generation mean size was comparable to the first generation but with no seasonal trend.

Wing spotting increased with later emergence during the first generation, reaching the highest values in the late first-generation individuals (Fig. 2). The pattern of the second generation was more complicated and differed between years. The regression model kept date, year and the interaction between generation and date as significant terms ($F_{4,310}$= 11.76, P=0.0001: date F=28.84, P=0.0001, year F=6.60, P=0.0107, generation F=1.44, P=0.23, and generation x date F=5.98, P=0.015). Since there was a significant generation x date interaction, we split the further analysis for the first and second generation. In both cases, the only significant term retained by the final model was date (first generation: $F_{1,129}$=21.60, P=0.0001 and second generation: $F_{1,181}$=5.15, P=0.024). In contrast to seasonal variation of wing colour and wing length, we also found a significant, though weak, date effect in the second generation. The results suggest, as shown by fig. 2, that the temporal variation of spotting from the first generation is repeated in the second, although the values for the second generation were lower.

Relations between the wing features

Darker individuals were on average larger and of higher spot type (Fig. 3). The correlations between the wing features are: wing colour – spotting: r=0.41, P=0.0001; wing colour – wing length: r=0.35, P=0.0001 and spotting – wing length: r=0.32, P=0.0001. The first principal component (PC1) accounted for nearly 60 % of the total variation of the three features together. PC1 was equally loaded by wing colour, spotting and wing length (Table I). The second and third principal component (PC2 and PC3) had smaller eigenvalues (about 20 % each) and thus represented less variation than one trait separately. PC2 mainly contrasted a large size with a low spot type (or vice versa) and PC3 a dark wing colour with low spot type (or vice versa) (Table I).

TABLE I

Weightings and eigenvalues from principal component analysis of the three studied wing features (dorsal wing colour, dorsal hindwing spotting and wing length) for the 285 males (all data from 1993 and 1994)

	PC1	*PC2*	*PC3*
WING COLOUR	0.599	-0.236	-0.764
SPOTTING	0.581	-0.526	0.619
WING LENGTH	0.549	0.816	0.178
Eigenvalues	57.3%	23.1%	19.6%

PC1 values increased with date in the first generation in both years and in the second generation the values of 1994 were lower than those of 1993 (Fig. 4). The general seasonal pattern of the PC1 was similar between the years (Fig. 4). In the analysis of the PC1 with the temporal variables we found a significant date-generation interaction (P=0.0001). When splitting the further analysis for generation, there was a significant date effect in the

first generation ($F_{1,110}$=30.55, P=0.0001) and a significant year effect in the second ($F_{1,173}$= 13.90, P=0.0003).

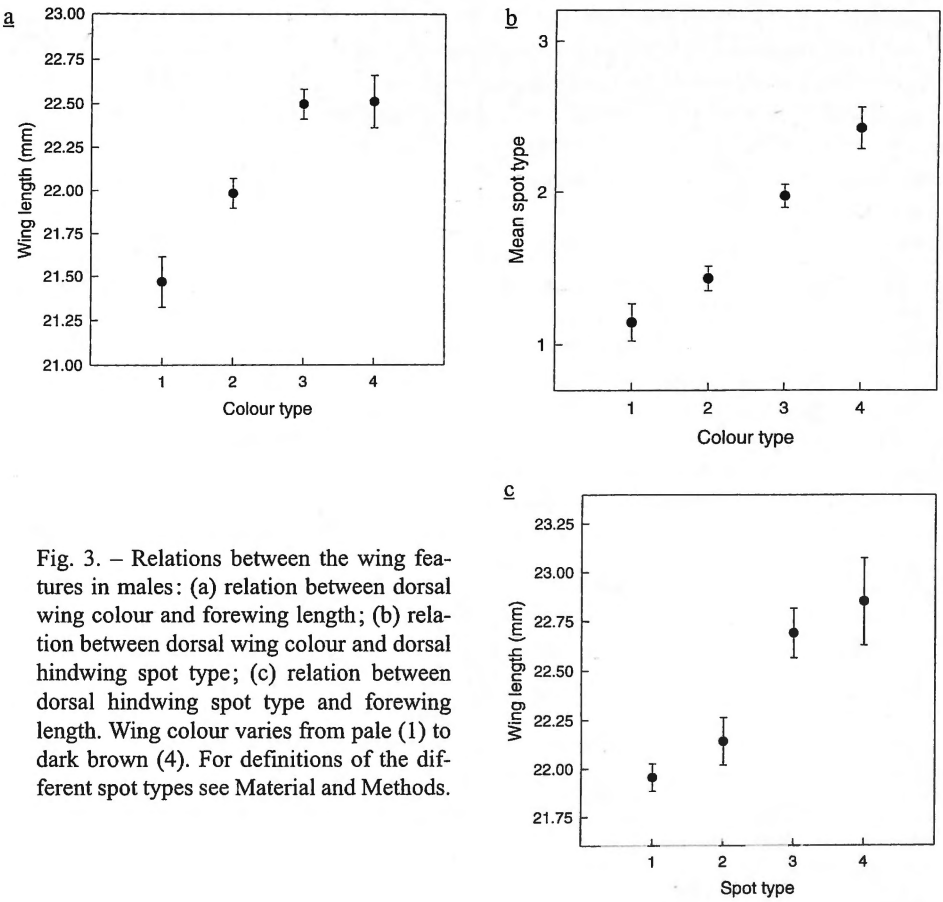


Fig. 3. – Relations between the wing features in males: (a) relation between dorsal wing colour and forewing length; (b) relation between dorsal wing colour and dorsal hindwing spot type; (c) relation between dorsal hindwing spot type and forewing length. Wing colour varies from pale (1) to dark brown (4). For definitions of the different spot types see Material and Methods.

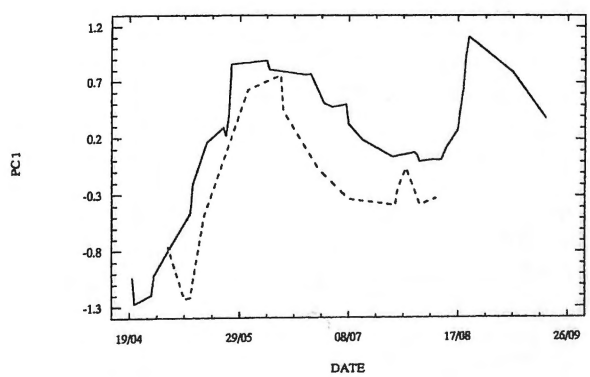


Fig. 4. – Seasonal variation of the PC1 of the principal component analysis in 1993 (black line) and 1994 (stippled line) expressed as moving averages (see Material and Methods).

DISCUSSION

We found that dorsal wing colour, spotting and wing length in the males of the speckled wood butterfly were interrelated and that these wing features varied seasonally but not necessarily in parallel. Darker males were on average larger and of higher spot type. In the first generation successively emerging males were darker, larger and of higher spot type. In the second generation they remained on average rather dark and of medium size, but the variation in spotting repeated the pattern of the first generation though less markedly. From the total dataset we showed that females were on average darker, larger and of higher spot type than the males.

The phenotypic composition of the population changed gradually over time, which implies that there were no discrete seasonal forms. The observed patterns of size and wing colour agree with the results on British speckled woods of ROBERTSON (1980) and PACKER (1984), respectively. The seasonal pattern of submarginal wingspots appeared to be different from the one of wing length and colour, since the trend of the first generation was more or less repeated in the second generation. PACKER (1984) also found a repetition of the seasonal pattern for wing spotting in the two generations. However, this was only the case when he analysed the specimens from a single location. In his entire dataset, with collected specimens from different localities, this trend was not detected and geographical differences for spot type within Great-Britain were found.

Is there an adaptive explanation for being small and pale in early spring? In several insects, including butterflies, it has been shown that spring individuals were more melanized than summer individuals for thermoregulatory reasons (e.g., SHAPIRO, 1976; KINGSOLVER & WIERNASZ, 1991; HOLLOWAY, 1993), which is contrary to the pattern described here. In temperate regions ambient temperature and particularly irradiance is the highest in summer (June-July-August). However, irradiance conditions vary in a different way at the floor of a temperate deciduous forest since there is interference with the degree of foliation: irradiance at the forest floor peaks in the period April-May (LARCHER, 1995). Later in the season increasing fraction of the forest floor becomes shaded and other parts receive less radiation. In these conditions an individual may benefit from a larger size, which implies less influence of convective cooling when flying through the forest (HEINRICH, 1993), and from a darker dorsal colour, which means an increased heating rate (VAN DYCK & MATTHYSEN, in press). Speckled wood males emerging in early spring (April-May) could afford a smaller size and a paler dorsal surface. From the beginning of July radiation levels on the forest floor remain at a low constant level (LARCHER, 1995), which could explain why we did not find significant differences with date for either wing colour and wing length in the second generation.

Submarginal wing spotting is often considered as a predator deflecting device (BLEST, 1957), hence our results might suggest seasonal variation in predation pressure. Alternatively, predation pressure may vary with the activity of the butterfly (BRAKEFIELD, 1984). Brakefield showed that males of the meadow brown butterfly, *Maniola jurtina*, which are more active and therefore more visible for predators than females, had more wingspots. Frequencies of behavioural strategies (probably with different predation risks) vary seasonally in the speckled wood (WICKMAN & WIKLUND, 1983; VAN DYCK *et al.*,

1997a) and may vary geographically as well. DENNIS *et al.* (1986) showed that spotting differences were correlated with activity levels in *Coenonympha tullia* on a geographic scale (*i.e.* Britain).

In our population three-spot-individuals (classes 1 and 2) were more abundant (76.5 % from the total dataset of males) than four-spot-individuals (classes 3 and 4). This is in contrast with the results of British studies with 35.1 % and 33.8 % three-spot males only (PACKER, 1984 and SHREEVE, 1987, respectively). Our study population seems to be representative for the Belgian population since three-spots were also more abundant in a large collection of Belgian speckled woods (Royal Belgian Institute for Natural Sciences, Brussels): from 313 male specimens 73.2 % were three-spot-butterflies (VAN DYCK, unpublished). This suggests a spotting difference on a geographic scale.

The correlations between the studied wing features in the male speckled woods can be interpreted in relation to their mate-locating and possibly dispersal behaviour (VAN DYCK *et al.*, 1997a): darker, larger males mainly patrol, while paler ones mainly perch in a territorial way. A higher spot type (i.e. four in stead of three wing spots) may be more beneficial to a patroller and disperser since the fourth wing spot is visible when flying but is rarely shown when sitting (VAN DYCK *et al.*, 1997a). However, it remains unclear whether having a small, fourth wing spot has an adaptive value for predator deflection, and whether there is selection on that feature in our study population.

The results for the females (which were on average darker, larger and with more spots than the males) suggest that they have the wing features of a «patroller». Females, which are single egg-layers, spend a lot of time searching for suitable hostgrasses (within a range of 24-30 °C) and they often have to cross shaded parts of the forest floor or even deposit eggs in shaded locations (SHREEVE, 1986a). The adaptive value of the female's phenotype needs however further investigation since not only mobility matters for the females but other aspects such optimal thermoregulation in relation to egg maturation and crypsis may play significant roles as well.

The role of genes in the production of the studied wing features cannot be estimated directly from the results of this study. Nevertheless, it is likely that wing length and wing colour are examples of phenotypic plasticity. The second generation, descendents of the first generation, had a different average phenotype compared to their parental generation. Both wing length and colour are probably influenced by the duration or timing of the larval stage (development time) which varies at least seasonally. As in Britain (SHREEVE, 1986b) and southern Sweden (NYLIN *et al.*, 1989), *P. aegeria* is able to overwinter in Belgium in the pupal stage and in the larval stage as well (VAN DYCK, personal observation). As a result the early spring individuals overwinter as pupae, the late spring butterflies as larvae. The late-spring or larva-overwintering individuals were darker and larger than early spring or pupa-overwintering butterflies. The former live for a very long period as larvae (from autumn to early spring) and continue feeding when the ambient temperature is up to only a few degrees Celsius (6.5°C, LEES, 1962; 3-4°C, WIKLUND *et al.*, 1983). The adults of the second generation all develop and pupate in late spring or early summer conditions which vary less, and we found wing length and wing colour also to be less variable in the summer generation of both years. The temporal trends were similar in both years, but we found lower mean wing colour values in the second generation of 1993

compared to 1994. It is worth remarking that the spring of 1994 (when the summer butterflies were larvae) was wet and cold by Belgian standards. Nevertheless the wing features can be influenced by the same environmental variables (suggested by their conjuction expressed in the PC1). Wing colour and spotting are known to be developmentally independent since different physiological pathways are used (NIJHOUT, 1991). Therefore it is unlikely that the correlation between pigmentation and spotting is causally linked. Moreover, we did find individuals with a dark wing colour and low spotting (or vice versa) as indicated by the weightings of PC 3 of the principal component analysis.

Knowing that (1) the first individuals of the summer generation are the offspring of the first of the spring generation (although there may be some overlap between the two emergences of the first generation), (2) the pattern of spot type of the spring generation was repeated in the summer generation, while this was not the case for wing colour and wing length, we suggest that the genetic control for spotting is considerably larger than for colouration and size. This suggests that there would be seasonal variation in the genetic composition of the population. In other satyrine butterflies, spot pattern characters have been shown to have a considerable heritability (BRAKEFIELD, 1984; BRAKEFIELD & VAN NOORDWIJK, 1985; WINDIG, 1994). In the tropical satyrine *Bicyclus anynana* heritabilities of wing patterns vary with ambient temperature (WINDIG, 1994) and within temperature regimes development time was related to the wing phenotype (BRAKEFIELD & REITSMA, 1991; WINDIG, 1992). In the speckled wood, complex variation in development rates and even developmental pathways have been documented (e.g., NYLIN *et al.*, 1989), but possible associations with phenotype production remain obscure. Experiments are now underway rearing several families under controlled climatic conditons in order to examine some of these interactions.

ACKNOWLEDGEMENTS

HVD was supported by a grant of the Flemish Institute for the Promotion of Scientific and Technological Research (IWT). EM is research associate with the National Fund for Scientific Research (NFWO).

REFERENCES

BINK, F.A. (1992) – *Ecologische atlas van de Dagvlinders van Noordwest-Europa*. Schuyt & Co Uitgevers, Haarlem.

BLEST, A.D. (1957) – The function of eyespot patterns in the Lepidoptera. *Behaviour*, **11**: 209-255.

BRADSHAW, A.D. (1965) – Evolutionary significance of phenotypic plasticity in plants. *Advantures in Genetics*, **13**: 115-155.

BRAKEFIELD, P.M. (1984) – The ecological genetics of quantitative characters in *Maniola jurtina* and other butterflies. In: R.I. VANE-WRIGHT & P.R. ACKERY (Eds), *The biology of butterflies*. Princeton University Press, Princeton: 167-190.

BRAKEFIELD, P.M. & T.B. LARSEN (1984) – The evolutionary significance of wet and dry season forms in some tropical butterflies. *Biological Journal of the Linnean Society*, **22**: 1-12.

BRAKEFIELD, P.M. & T.G. SHREEVE (1992) – Case studies in evolution. In: DENNIS, R.L.H. (Ed), *The ecology of butterflies in Britain*. Oxford University Press, Oxford: 197-216.

BRAKEFIELD, P.M., T.G. SHREEVE & J.A. Thomas (1992) – Avoidance, concealment, and defence. In: DENNIS, R.L.H. (Ed), *The ecology of butterflies in Britain*. Oxford University Press, Oxford: 93-119.

BRAKEFIELD, P.M., & A.J. VAN NOORDWIJK (1985) – The genetics of spot pattern characters in the meadow brown butterfly *Maniola jurtina* (Lepidoptera: Satyrinae). *Heredity*, **54**: 275-284.

DAVIES, N.B. (1978) – Territorial defence in the speckled wood butterfly (*Pararge aegeria*): the resident always wins. *Animal Behaviour*, **26**: 138-147.

DENNIS, R.L.H. (1993) – *Butterflies and climate change*. Manchester University Press, Manchester.

DENNIS, R.L.H., K. PORTER & W.R. WILLIAMS (1986) – Ocellation in *Coenonympha tullia*. (Müller) (Lep., Satyridae). II. Population differentiation and clinal variation in the context of climatically-induced anti-predator defence strategies. *Entomologist's Gazette*, **37**: 133-172.

DUDLEY, R. (1990) – Biomechanics of flight in Neotropical butterflies: morphometrics and kinematics. *Journal of experimental Biology*, **150**: 37-53.

ENDLER, J.A. (1986) – *Natural selection in the wild*. Princeton University Press, Princeton.

FORD, E.B. (1975) – *Ecological genetics*, 4th ed. Methuen, London.

HAZEL, W.N., R. BRANDT & T. GRANTHAM (1987) – Genetic variability and phenotypic plasticity in pupal colour and its adaptive significance in the swallowtail butterfly *Papilio polyxenes*. *Heredity*, **59**: 449-455.

HEINRICH, B. 1993. – *The hot-blooded insects: strategies and mechanisms of thermoregulation*. Springer-Verlag, Berlin Heidelberg.

HOLLOWAY, G.J. (1993) – Phenotypic variation in colour pattern and seasonal plasticity in *Eristalis* hoverflies (Diptera: Syrphidae). *Ecological Entomology*, **18**: 209-217.

KINGSOLVER, J.G. & D.C. WIERNASZ (1991) – Seasonal polyphenism in wing-melanin pattern and thermoregulatory adaptation in *Pieris* butterflies. *The American Naturalist*, **137**: 816-830.

LARCHER, W. (1995) – *Physiological plant ecology* (third edition). Springer-Verlag, Berlin Heidelberg.

LEES, E. (1962) – Factors limiting the distribution of the speckled wood butterfly (*Pararge aegeria* L.) in Great Britain. *Entomologist's Gazette*, **13**: 101-103.

MAES, D. & H. VAN DYCK (1996) – A documented Red list of the butterflies of Flanders (in Dutch with English summary). *Communications of the Institute of Nature Conservation*, 1996(1), 1-154.

MÜLLER, H.J. (1955) – Die Saisonenformenbildung von *Araschnia levana*. *Naturwissenschaften*, **42**: 134-135.

NIJHOUT, H.F. (1991) – *The Development and Evolution of Butterfly Wing Patterns*. Smithsonian Institution Press, Washington.

NYLIN, S., P.-O. WICKMAN & C. WIKLUND (1989) – Seasonal plasticity in growth and development of the speckled wood butterfly, *Pararge aegeria* (Satyrinae). *Biological Journal of the Linnean Society*, **38**: 155-171.

OWEN, D.F. & D. GOULSON (1994) – Effect of temperature on the expression of the *medionigra* phenotype of the moth *Panaxia dominula* (Lepidoptera: Arctiidae). *Oikos*, **71**: 107-110.

PACKER, L. (1984) – The ecological genetics of the speckled wood butterfly, *Pararge aegeria* L.: a preliminary study. *Heredity*, **52**: 179-188.

ROBERTSON, T.S. (1980) – Seasonal variation in *Pararge aegeria* (Linnaeus) (Lepidoptera: Satyridae): a biometrical study. *Entomologist's Gazette*, **31**: 151-156.

SAS (1990) – *SAS/STAT User's guide*, version 6, fourth edition. SAS Institute Inc., Cary, NC.

SHAPIRO, A.M. (1976) – Seasonal polyphenism. *Evolutionary Biology*, **9**: 259-333.

SHREEVE, T.G. (1986a) – Egg-laying by the speckled wood (*Pararge aegeria*): the role of female behaviour, host plant abundance and temperature. *Ecological Entomology*, **11**: 229-236.

SHREEVE, T.G. (1986b) – The effect of weather on the life cycle of the speckled wood butterfly *Pararge aegeria*. *Ecological Entomology*, **11**: 325-332.

SHREEVE, T.G. (1987) – The mate location behaviour of the male speckled wood butterfly, *Pararge aegeria*, and the effect of phenotypic differences in hind-wing spotting. *Animal Behaviour*, **35**: 682-690.

SILBERGLIED, R.E. (1984) – Visual communication and sexual selection among butterflies. In: VANE-WRIGHT, R.I. & P.R. ACKERY (Eds), *The biology of butterflies*. Princeton University Press, Princeton: 207-223.

VAN DYCK, H. & E. MATTHYSEN (in press) – Thermoregulatory differences between phenotypes in the speckled wood butterfly: hot perchers and cold patrollers ? *Oecologia*.

VAN DYCK, H., E. MATTHYSEN & A.A. DHONDT (1997a) – The effect of wing colour on male behavioural strategies in the speckled wood butterfly. *Animal Behaviour*, **53**: 39- 51.

VAN DYCK, H., E. MATTHYSEN & A.A. DHONDT (1997b) – Mate-locating strategies are related to relative body length and wing colour in the speckled wood butterfly *Pararge aegeria*. *Ecological Entomology*, **22**: 116-120.

WASSERTHAL, L.T. (1975) – The role of butterfly wings in regulation of body temperature. *Journal of Insect Physiology*, **21**: 1921-1930.

WATT, W.B. (1968) – Adaptive significance of pigment polymorphisms in *Colias* butterflies. I. Variation in melanin pigment in relation to thermoregulation. *Evolution*, **22**: 437-458.

WEST-EBERHARD, M.J. (1989) – Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics*, **20**: 249-278.

WICKMAN, P-O. & C. WIKLUND (1983) – Territorial defence and its seasonal decline in the speckled wood butterfly (*Pararge aegeria*). *Animal Behaviour*, **31**: 1206-1216.

WIKLUND, C., A. PERSSON & P.-O. WICKMAN (1983) – Larval aestivation and direct development as alternative strategies in the speckled wood butterfly, *Pararge aegeria*, in Sweden. *Ecological Entomology*, **8**: 233-238.

WINDIG, J.J. (1992) – Seasonal polyphenism in *Bicyclus safitza*: a continuous reaction norm. *Netherlands Journal of Zoology*, **42**: 583-594.

WINDIG, J.J. (1994) – Reaction norms and the genetic basis of phenotypic plasticity in the wing pattern of the butterfly *Bicyclus anynana*. *Journal of evolutionary Biology*, **7**: 665-695.

WINDIG, J.J., P.M. BRAKEFIELD, N. REITSMA & J.G.M. WILSON (1994) – Seasonal polyphenism in the wild: survey of wing patterns in five species of *Bicyclus* butterflies in Malawi. *Ecological Entomology*, **19**: 285-298.

Belg. J. Zool. - Volume 127 (1997) - issue 2 - pages 179-189 - Brussels 1997

Received : 14 August 1997

DIFFERENTIATION AMONG BLUE TIT (*PARUS CAERULEUS*) POPULATIONS MEASURED WITH FIVE MINISATELLITE SINGLE LOCUS PROBES

GEERT R. VERHEYEN([1*2]), ERIK MATTHYSEN([1]), FRANK ADRIAENSEN([1]), CHRISTINE VAN BROECKHOVEN([2]) AND ANDRE A. DHONDT([1*3])

([1])Laboratory of Animal Ecology, Department of Biology, University of Antwerp (UIA), Universiteitsplein 1, B-2610 Antwerpen, Belgium.
([2])Laboratory of Neurogenetics, University of Antwerp (UIA), Department of Biochemistry, Universiteitsplein 1, B-2610 Antwerpen, Belgium.
([3])Laboratory of Ornithology, Cornell University, Ithaca, New York 14850, USA.
e-mail: vheyen@hgins.uia.ac.be

Abstract. Five hypervariable minisatellite loci were scored by Southern hybridisation to study the genetic differentiation among eight blue tit (*Parus caeruleus*) populations. All loci display extreme levels of polymorphism in each of the populations. Deviations from Hardy-Weinberg equilibrium are detected for loci in some populations. There does not seem to be an influence of the current degree of habitat fragmentation on genetic variation. However, despite high levels of gene flow (low Fst-values), significant differentiation is found among some populations at these loci. The populations differentiate according to random drift patterns, but, as suggested by cluster analysis, there might still be a small influence of gene flow.

Keywords: minisatellites, single locus probe, genetic structure, random drift

INTRODUCTION

The application of DNA fingerprinting (GILBERT *et al.*, 1990), RAPDs (HADRYS *et al.*, 1993) and single locus profiling with minisatellite (HANOTTE *et al.*, 1991) or microsatellite (ESTOUP *et al.*, 1995) markers in population genetic studies is increasing. However, to date, most population genetic studies in birds employ allozyme polymorphisms (JOHNSON & MARTEN, 1989) or mitochondrial DNA (WENINK *et al.*, 1993), and rarely use the more recently developed techniques (HAIG *et al.*, 1993).

As part of our study on the reproductive success of the blue tit (*Parus caeruleus*, KEMPENAERS *et al.*, 1992), several hypervariable minisatellite single locus probes (SLPs) were developed (VERHEYEN *et al.*, 1994). These markers were subsequently applied in a preliminary study to describe the genetic population structure of blue tits in three populations around Antwerp, North Belgium (VERHEYEN *et al.*, 1995). Here, using 5 minisatellite loci, we study the genetic structure of 8 blue tit populations over a wider geographic

range in more depth. Our goal is to determine if the genetic variation and population differentiation follow specific geographical patterns and if habitat fragmentation has an effect on genetic variation within blue tit populations. In this respect, this study is part of a larger program in which the effects of habitat fragmentation on several animal species (birds, mammals and insects) are evaluated (MATTHYSEN *et al.*, 1995).

MATERIAL AND METHODS

Isolation and characterisation of minisatellite markers

Five minisatellite loci detected by three single locus probes (SLPs; cPcaMS1, cPcaMS3 and cPcaMS14) and one double locus probe (DLP; cPcaMS8, the two detected loci have well separated allele sizes), were used. The alleles of these markers range from 1.8 kb up to 14 kb. The isolation and initial characterisation of these blue tit specific markers is described in VERHEYEN *et al.* (1994). Details concerning the collection of blood samples and the techniques used during the processing of blood samples to single locus profiles can be found in VERHEYEN *et al.* (1994 and 1995). In short: high molecular weight DNA was digested with *Hinf*I restriction enzyme. Fragments were separated overnight by electrophoresis in 0.6% agarose gels. The DNA was subsequently transferred onto nylon membranes by Southern blotting. SLPs are [α-^{32}P]dCTP labelled and hybridised overnight to the nylon membranes at 68°C, followed by several high stringency washes. After autoradiography (overnight at -70°C) the patterns were analysed.

Defining alleles

The limited resolution of the agarose gel electrophoresis technique, combined with the hypervariability of the minisatellite loci, make it impossible to score the alleles discretely, resulting in quasi-continuous allele distributions (BUDOWLE *et al.*, 1991). Therefore, allele classes have to be constructed before any analysis can take place. In a previous analysis (VERHEYEN *et al.*, 1995), we estimated the allele sizes by comparing the migration distance of the fragments to the migration distances of length markers (DUGGLEBY *et al.*, 1981). The alleles were then grouped in 100 bp classes. The drawback of this method is that the 100 bp classes are too wide for the low molecular weight alleles (<5 kb) and too narrow for the larger molecular weight alleles. Therefore, the resulting distributions do not accurately reflect the genetic variation observed on the gels. In this study another approach to classify the alleles was used. By comparing the genotypes of individuals that were analysed more than once on separate gels, we observed that alleles differed by an average of 0.75% from their mean size. Allele size is not correlated with the procentual deviation ($r=-0.0003$, details not shown). For each of the markers, all alleles from all populations were sorted by size. Starting from the smallest allele, the next significantly differing allele was determined and "tagged". Significance was determined using a t-test and by determining if the difference in size of the two alleles was larger than zero (5% significance level; the standard deviation was based on the mean size of both alleles). If not, the smallest allele was compared to the next (larger) allele, until a significant difference was found.

Starting from the larger of the 2 significantly differing alleles, the process was repeated until the whole allele range of the locus was covered. Subsequently, all alleles of a locus were grouped to the closest "tagged" allele. The mean size of each of the resulting groups was determined and was considered the midpoint of that allele class. For each separate population all alleles were grouped according to these classes and the resulting allele distributions were used in the analyses.

Populations sampled

During spring 1994, blue tits from 8 populations were sampled. Five populations are situated near Antwerp: plot PB (plot B in DHONDT 1989, 12.5 ha) is part of the large "mainland" population Peerdsbos (total > 200 ha); Calixbergen (hereafter called CX, plot C in DHONDT 1989, 17 ha) is an isolated estate situated 2 km south of PB; the Boshoek populations (three plots were sampled: ZZ, KB and LL, all 7 to 11 ha) are a set of small and isolated woodlots to the south of Antwerp, 17 km from the northern populations CX and PB. One population near Ghent (Hutsepot, approximately 50 km west of Antwerp, hereafter called GE) and two populations near Kortrijk (called KA and SA, separated by 2 km; situated 90 km west of Antwerp) were also sampled. The number of individuals sampled from each population is shown in table 1. All study sites are optimal habitat for blue tits with perhaps the exception of the Ghent population (DHONDT & EYCKERMAN, 1980).

TABLE 1

Characteristics of the genetic variation displayed by the minisatellite markers in the populations surveyed. Nind= number of individuals sampled. Nall= observed number of different alleles in the population. Hexp= (unbiased) heterozygosity expected under Hardy-Weinberg (HW) equilibrium. Hobs= observed heterozygosity. P= exact probability with standard error (SE) for detection of deviations from HW-equilibrium. Fis= inbreeding coefficient. M= mean for all markers. Significant deviations (P<0.05) from HW-equilibrium are indicated in bold.

Marker	*Nind*	*Nall*	*Hexp*	*Hobs*	*P*	*SE*	*Fis*
			KA	Kortrijk			
MS1	18	21	0.961	0.889	0.332	0.004	0.076
MS3	15	16	0.911	0.800	0.216	0.004	**0.156**
MS8a	17	21	0.966	0.941	0.489	0.001	0.027
MS8b	17	21	0.972	0.941	0.650	0.006	0.032
MS14	16	16	0.935	0.875	0.125	0.004	0.067
M			0.955	0.889			
			SA	Kortrijk			
MS1	43	21	0.945	0.860	**0.024**	0.004	**0.085**
MS3	41	31	0.966	0.902	**0.036**	0.003	**0.067***
MS8a	42	37	0.976	0.976	0.417	0.012	-0.003

MS8b	43	35	0.975	0.977	0.960	0.002	-0.001
MS14	44	28	0.935	0.864	**0.009**	0.001	**0.097**
M			0.964	0.916			
			GE	Ghent			
MS1	28	20	0.937	0.821	**0.005**	0.001	0.125
MS3	27	24	0.958	0.815	**0.0004@**	0.000	**0.151***
MS8a	28	30	0.974	1.000	0.588	0.015	-0.026
MS8b	26	30	0.972	0.846	**0.037**	0.008	**0.132***
MS14	28	24	0.962	0.893	**0.0004@**	0.000	0.073
M			0.961	0.875			
			PB	Antwerp			
MS1	34	20	0.947	0.824	**0.017**	0.003	**0.132**
MS3	33	29	0.959	0.788	**0.002**	0.000	**0.180***
MS8a	33	31	0.966	1.000	0.707	0.011	-0.036
MS8b	33	31	0.974	0.909	0.305	0.006	0.067
MS14	34	27	0.963	0.882	**0.032**	0.004	**0.089**
M			0.962	0.881			
			ZZ	Antwerp			
MS1	47	25	0.943	0.851	**0.002**	0.001	**0.098**
MS3	44	34	0.961	0.932	0.516	0.008	0.034
MS8a	48	35	0.962	0.979	0.658	0.012	-0.017
MS8b	49	37	0.966	0.898	**0.021**	0.003	0.071
MS14	50	32	0.968	0.960	0.615	0.017	0.008
M			0.960	0.924			
			KB	Antwerp			
MS1	23	19	0.944	0.913	0.688	0.003	0.033
MS3	24	23	0.960	0.875	0.246	0.005	**0.090***
MS8a	23	25	0.964	0.870	0.118	0.007	**0.105**
MS8b	23	26	0.968	0.957	0.642	0.006	0.012
MS14	23	23	0.957	1.000	0.772	0.008	-0.052
M			0.959	0.923			
			LL	Antwerp			
MS1	30	24	0.956	0.933	0.442	0.004	0.023
MS3	29	29	0.954	0.793	**0.020**	0.005	**0.178***
MS8a	29	27	0.956	0.931	0.181	0.009	0.027

MS8b	29	28	0.970	0.862	**0.007**	0.001	**0.113**
MS14	30	29	0.966	0.933	0.541	0.010	0.034
M			0.960	0.891			
			CX	Antwerp			
MS1	49	21	0.945	0.980	0.970	0.001	-0.038
MS3	48	35	0.971	0.960	0.587	0.006	0.012
MS8a	50	34	0.962	0.878	**0.011**	0.002	**0.088***
MS8b	49	35	0.964	0.938	0.703	0.004	0.028
MS14	50	33	0.968	0.941	0.360	0.014	0.027
M			0.962	0.939			

* P<0.005; @ significant after correcting for multiple testing

Analysis

Exact tests on Hardy-Weinberg (HW)-equilibrium and population differentiation were performed using the Genepop version 2 software (RAYMOND ROUSSET, 1995). Wright's Fst was also estimated using Genepop version 2. Fst is a measure of the amount of genetic differentiation between subpopulations. Significance of Fst-values was estimated by resampling over genotypes (RAYMOND & ROUSSET, 1995). The inbreeding coefficient Fis is a measure of the deviation from HW-proportions within populations and was also estimated with Genepop. PHYLIP 3.5c (FELSENSTEIN, 1989) was used to perform clusteranalyses (using the CONTML option). CONTML assumes that the loci evolve under the influence of random genetic drift. Significance of the nodes in the dendrograms was tested by bootstrapping over loci.

RESULTS

Genetic variation, heterozygosity and Hardy-Weinberg equilibrium

The main characteristics of the minisatellite loci in each of the populations are given in table 1. All loci displayed extreme polymorphism in each of the populations, which was illustrated by the large number of alleles and resulting high observed and expected heterozygosities. The mean number of alleles (observed over all populations) per locus equalled 50. This number might even be an underestimate because of the limited resolution of the agarose gel electrophoresis technique and the possible variation within the repeat sequence of the minisatellites (JEFFREYS *et al.*, 1991).

The mean observed heterozygosities were always lower than their expected values, and some of the Fis-values were significantly larger than zero. These values might at first appear to suggest that inbreeding occurs within the populations. However, there was no consistent deficit of heterozygotes for all loci within a specific population as could be expected with inbreeding. More probably, given the technical limitations in the resolution

of the alleles, the deviations were mainly caused by pseudohomozygosity (DEVLIN *et al.*, 1990) and the occurrence of null-alleles (alleles that only have a limited amount of repeats and therefore fail to hybridise efficiently; CHAKRABORTY *et al.*, 1992).

We tested the differences between mean heterozygosities among pairs of populations as described by NEI (1987; details not shown). No significant differences were found. However, single locus heterozygosities (especially the observed values) fluctuated more between populations and several significant differences ($P<0.05$, details not shown) were found.

According to the exact test, in 15 (out of 40) cases, loci had genotype frequencies that did not conform to their expected HW-proportions. However, when Bonferroni-corrections were applied, only 2 significant deviations remained ($P<0.00125$).

Genetic differentiation between populations

An exact test over all populations indicated that there were significant differences among the populations (table 2). Four of the 5 loci showed significant differentiation, the fifth was just off significance at the 5% level.

TABLE 2

Exact test (Raymond and Rousset 1995) of differentiation over all 8 populations. Significant P-values (S.E. = standard error) are indicated in bold.

Locus	P	S.E.
MS1	0.0645	0.0025
MS3	**0.0006**	0.0002
MS8a	**0.0031**	0.0005
MS8b	**0.0003**	0.0001
MS14	**0.0320**	0.0035
Total	**<0.0001**	

Table 3 gives the Fst-values and the results of the exact test of population differentiation between all combinations of 2 populations. Significant differentiation was found among many of the populations. Because 2 loci showed significant deviations from HW-equilibrium, resampling procedures over genotypes were also performed (Fst-based test). The level of significance is indicated by a (not significant), b ($0.05>P>0.01$), c ($0.005>P>0.002$) or d ($P<0.002$). Most Fst-values are relatively low, indicating high levels of gene flow between the populations. The Fst-values are not correlated with sample size ($r=0.007$; $df=26$; ns) and were used to search for correlations with geographic distance. The expected positive correlation (under the hypothesis of isolation-by-distance) was not found (Mantel test; $r=-0.22$; $df=26$; ns), suggesting random differentiation between the populations. Finally, a cluster analysis was performed on the data. CONTML assumes that the loci evolve under the influence of pure random drift and might therefore provide the most appropriate model. The results are shown in figs. 1a and 1b. The first

dendrogram shows that nearby populations are preferentially clustered together. The result of the bootstrap option is shown as well but indicates that several nodes are not significant.

TABLE 3

Exact P-values for differentiation among pairs of populations (below the diagonal) and Fst-values above the diagonal. a, b, c, d: levels of exact P-values among the populations according to the (genotype based) exact test of Raymond and Rousset (1995). (a: P> 0.05; b: 0.05> P> 0.01; c: 0.005> P> 0.002; d: P< 0.002).

	KA	SA	GE	PB	CX	ZZ	KB	LL
KA	/	0.000a	0.003a	0.004a	0.003a	0.010d	0.004a	0.008b
SA	0.413	/	0.001a	0.001a	0.001a	0.005d	0.003a	0.002b
GE	0.010	0.117	/	0.002a	0.003a	0.005b	0.004a	0.004b
PB	0.025	0.052	0.009	/	0.001a	0.005d	0.003a	0.006c
CX	0.107	0.005	0.030	0.099	/	0.005d	0.004b	0.007d
ZZ	0.00005	0.00003	0.002	0.0002	0.00002	/	0.004b	0.006d
KB	0.036	0.039	0.016	0.025	0.003	0.027	/	0.001a
LL	0.012	0.004	0.006	0.0005	0.000005	0.00008	0.256	/

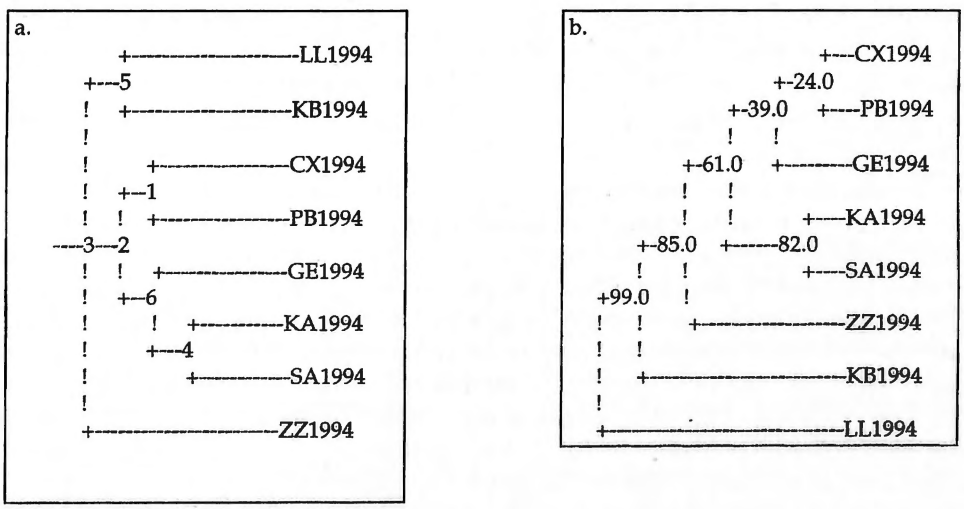


Fig. 1. – Dendrograms clustering the 8 populations. Figure 1.a. results of the CONTML-option; figure 1.b. results of the bootstrap option. Numbers at the nodes indicate the number of times this node was formed in 100 dendrograms.

DISCUSSION

Apart from its frequent use in paternity studies (KEMPENAERS *et al.*, 1992), multilocus DNA fingerprinting has only been used in some population genetic studies in natural populations, for example to find the phylogenetic relationships between small, isolated populations (GILBERT *et al.*, 1990). WAUTERS *et al.*, (1994) have also shown that genetic variation is reduced in isolated populations of the red squirrel (*Sciurus vulgaris*) due to reduced immigration. However, multilocus profiles are not easy to score and cannot be used in studies where the characterisation of single loci is desirable. Most population genetic studies that use minisatellite SLPs are performed in human populations (FLINT *et al.*, 1989; DEKA *et al.*, 1991). This is due to the species-specificity of the SLPs. However, the difficulties in the characterisation of the alleles at these loci is probably also an important reason, because studies using species-specific microsatellite markers are increasing (ESTOUP *et al.*, 1995). The allele scoring method used here is specific for this study, making direct comparisons among different studies impossible (even if they make use of the same markers). New studies, making use of the same markers used in this study, should preferentially reanalyse all data in order to construct allele classes that can be used to compare directly the allele distributions of new populations with the populations studied here. However, this should not be necessary if one wishes to compare the general results obtained over studies. The inability to score all alleles discretely is a serious drawback of minisatellites, which limits the use of these markers. However, the markers are capable of detecting subtle differences among populations (as shown in this study) and are therefore very helpful in testing specific hypotheses (as has been documented in Dias *et al.*, 1996). Microsatellite markers might also be useful in these kinds of studies, because they are highly polymorphic (although they are less polymorphic than the minisatellite markers described here). The alleles of microsatellites can be scored without error, making direct comparisons among different studies possible. Analysing microsatellite markers is much faster and requires less DNA than Southern blot based methods. Moreover, using fluorescence techniques, several markers can be analysed simultaneously. Unfortunately, there are no microsatellite markers available for the blue tit.

In this study, all loci were extremely polymorphic in each of the populations. This high heterozygosity is found in human minisatellite loci as well (JEFFREYS *et al.*, 1988). The heterozygosities reported here are much larger than those found for minisatellite loci in the Indian peafowl *Pavo cristatus* (22-78%, HANOTTE *et al.*, 1991) or in the chicken (BRUFORD BURKE, 1991). However in those studies the birds belonged to semi-captive or inbred populations, which might explain the reduced heterozygosities. Here, the observed genetic variation of the separate loci was highly variable and fluctuated strongly between populations. However, the mean heterozygosity was comparable in each of the populations. It therefore appears that, despite the highly fragmented nature of some of these populations, there is no loss of genetic variation within the populations. This result is not surprising, considering the dispersal capacities of blue tits. Nevertheless, KEMPENAERS *et al.*, (1996) found that in population CX the proportion of bands shared between parents on multilocus fingerprints was correlated with the number of eggs which failed to hatch from their nests, providing evidence of a deleterious effect of possible inbreeding.

Apart from technical influences, the observed deviations from HW-equilibrium (table 1) can be caused by several population processes. However, because the alleles are grouped in classes, the results can only be considered as possible indications of deviations from equilibrium. Minisatellites seem to behave neutrally (CLARK, 1987; JEFFREYS *et al.*, 1988), excluding selection as a possible cause for the deviations. The high mutation rates at these loci are probably negligible compared to other processes that might influence HW-equilibrium. Rather, the deviations are influenced by the combination of dispersal (which is high in the blue tit; BERNDT & STERNBERG, 1969), variation in reproductive success (KEMPENAERS *et al.*, 1992) and random genetic drift.

As also observed in an earlier study (VERHEYEN *et al.*, 1995), significant differentiation between populations sometimes occurs, despite high gene flow levels. Interestingly and unlike in the previous study, only a proportion of the populations appear to be strongly differentiated from the other populations (if the Fst-based test is used, which is based on resampling over genotypes): ZZ, KB and LL, which all belong to the BO-population. In our previous study, data from 2 breeding seasons were pooled. Perhaps this stressed the differences among the populations due to the conservation of related alleles over breeding seasons. If this is so, the significant results of the BO subpopulations might be explained by a smaller genetic turnover in these populations, which could be due to the high level of fragmentation from these populations. However, as already mentioned, no significant effects of fragmentation on genetic diversity were seen.

Despite the use of a different type of marker, the Fst-values are comparable with estimates in other bird species (ROCKWELL BARROWCLOUGH, 1987). The previously reported isolation-by-distance effect (VERHEYEN *et al.*, 1995) was not observed in this study over a wider geographical area, and there is no correlation between Fst and geographic distance. We also estimated Nei's genetic distance, but Nei's distance appears to be negatively correlated with sample size ($r=-0.73$; $df=26$; $P<0.001$), and was therefore not suitable for further analyses (details not shown). The Fst-values rather suggest a random drift pattern of differentiation. This pattern is supported by the fluctuations in heterozygosities. Nevertheless, the results of a clusteranalysis could indicate that gene flow between close-by populations might influence the random drift effects. However, several nodes were not significant, making alternative dendrograms possible as well.

In conclusion, we can say that the present high level of habitat fragmentation does not seem to have an effect of loss of genetic variation within blue tit populations as measured with minisatellite SLPs. Differentiation between populations follows a pattern resembling random drift. Random drift and migration probably cause the observed deviations from HW-equilibrium (apart from technical reasons) and the fluctuations in heterozygosities. Gene flow and/or the resolution of the markers are not high enough to induce an isolation-by-distance effect. The correlations between the genetic distance measures (Nei) and the sample size are very troublesome and we urge caution in interpreting minisatellite data. Fortunately, Fst-values were not correlated with sample size. Nevertheless, when using VNTR markers in population genetic studies, all the drawbacks have to be kept in mind and appropriate sampling designs worked out whenever possible.

ACKNOWLEDGEMENTS

We would like to thank Mr. De Baere and his collegues from Kortrijk for collecting the blue tit bloodsamples of KA and SA populations. In this respect we also thank Dr. J. De Laet for taking bloodsamples from the population near Ghent. We wish to thank Dr. Michel Raymond for providing the Genepop software. We thank the Bracht family for allowing us to work on their estate. GRV was supported by a grant from the IWONL (Instituut voor Wetenschappelijk Onderzoek in Nijverheid en Landbouw). EM is Research Associate with the Belgian National Fund for Scientific Research.

REFERENCES

BERNDT, R. & H. STERNBERG (1969) – Terms, studies and experiments on the problems of bird dispersion. *ibis*, **110**: 256-269.

BRUFORD, M.W. & T. BURKE (1991) – Hypervariable DNA markers and their applications in the chicken. In: BURKE, T., DOLF, G., JEFFREYS, A.J. & WOLFF, R. (Eds) *DNA fingerprinting: approaches and applications.* Birkhä user Verlag, Basel, Boston, Berlin: 230-242.

BUDOWLE, B., A.M. GIUSTI, J.S. WAYE, F.S. BAECHTEL, R.M. FOURNEY, D.E. ADAMS, L.A. PRESLEY, H.A. DEADMAN & K.L. MONSON (1991) – Fixed-bin analysis for statistical evaluation of continuous distributions of allelic data from VNTR loci, for use in forensic comparisons. *Am. J. Hum. Genet.*, **48**: 841-855.

CHAKRABORTY, R., M. DE ANDRADE, S.P. DAIGER & B. BUDOWLE (1992) – Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. *Ann. Hum. Genet.*, **56**: 45-57.

A.G. CLARK (1987) – Neutrality tests of highly polymorphic restriction fragment length polymorphisms. *Am. J. Hum. Genet.*, **41**: 948-956.

DEKA, R., R. CHAKRABORTY, & R.E. FERRELL (1991) – A population genetic study of six VNTR loci in three ethnically defined populations. *Genomics*, **11**: 83-92.

DEVLIN, B., N. RISCH & K. ROEDER (1990) – No Excess of Homozygosity at Loci Used for DNA Fingerprinting. *Science*, **249**: 1416-1420.

A.A. DHONDT (1989) – Blue Tit. In: NEWTON, I.A. (ed) *Lifetime Reproduction in Birds.* Academic Press, London: 15-33.

DHONDT, A.A. & R. EYCKERMAN (1980) – Competition and the regulation of numbers in Great and Blue Tit. *Ardea*, **68**: 121-132.

DIAS, P.C., G.R. VERHEYEN & M. RAYMOND (1996) – Source-Sink populations in Mediterranean blue tits: evidence using single-locus minisatellite probes. *J. Evol. Biol.*, **9**: 965-978.

DUGGLEBY, R.G., H. KINNS & J.L. ROOD (1981) – A computer Program for Determining the Size of DNA Restriction Fragments. *Analyt. Biochem.*, **110**: 723-725.

ESTOUP, A., L. GARNERY, M. SOLIGNAC & J.-M. CORNUET (1995) – Microsatellite variation in Honey Bee (*Apis Mellifera* L.) populations: hierarchical genetic structure and test of the infinite allele and stepwise mutation models. *Genetics*, **140**: 679-695.

J. FELSENSTEIN (1989) – PHYLIP—Phylogeny Inference Package (version 3.2). *Cladistics*, **5**: 164-166.

FLINT, J., A.J. BOYCE, J.J. MARTINSON & J.B. CLEGG (1989) – Population bottlenecks in Polynesia revealed by minisatellites. *Hum. Genet.*, **83**: 257-263.

GILBERT, D.A., N. LEHMAN, S.J. O'BRIEN & R.K. WAYNE (1990) – Genetic fingerprinting reflects population differentiation in the California channel island fox. *Nature*, **344**: 764-766.

HADRYS, H., B. SCHIERWATER, S.L. DELLAPORTA & L.W. BUSS (1993) –Determination of paternity in dragonflies by Random Amplified Polymorphic DNA fingerprinting. *Molec. Ecol.,* **2**: 79-87.

HAIG, S.M., J.R. BELTHOFF & D.H. ALLEN (1993) – Examination of population structure in red-cockaded woodpeckers using DNA profiles. *Evolution,* **47**: 185-194.

HANOTTE, O., T. BURKE, J.A.L. ARMOUR & A.J. JEFFREYS (1991) – Hypervariable minisatellite DNA sequences in the Indian Peafowl Pavo cristatus. *Genomics,* **9**: 587-597.

HILLIS, D.M. & C. MORITZ (eds) (1990) – *Molecular Systematics.* Sinauer Ass. Inc., ... pp.

JEFFREYS, A.J., N.J. ROYLE, V. WILSON & Z. WONG (1988) – Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. *Nature,* **332**: 278-281.

JEFFREYS, A.J., A. MACLEOD, K. TAMAKI, D.L. NEIL & D.G. MONCKTON (1991) – Minisatellite repeat coding as a digital approach to DNA typing. *Nature,* **354**: 204-209.

JOHNSON, N.K. & J.A. MARTEN (1991) – Evolutionary genetics of flycatchers. III. Variation in *Empidonax hammondii* (Aves: *Tyrannidae*). *Can. J. Zool.* **69**: 232-238.

KEMPENAERS, B., G.R. VERHEYEN, M. VAN DEN BROECK, T. BURKE, C. VAN BROECKHOVEN & A.A. DHONDT (1992) – Extra-pair paternity results from female preference for high-quality males in the blue tit. *Nature,* **357**: 494-496.

KEMPENAERS, B., F. ADRIAENSEN, A.J. VAN NOORDWIJK & A.A. DHONDT (1996) – Genetic similarity, inbreeding and hatching failure in blue tits: are unhatched eggs infertile? *Proc. R. Soc. London,* in press.

MATTHYSEN, E., L. LENS, S. VAN DONGEN, G.R. VERHEYEN, L.A. WAUTERS, F. ADRIAENSEN & A.A. DHONDT (1995) – Diverse effects of forest fragmentation on a number of animal species. *Belg. J. Zool.,* **125**: 175-183.

M. NEI (1987) – *Molecular Evolutionary Genetics.* Columbia University Press, New York, ... pp.

RAYMOND, M. & F. ROUSSET (1995) – Genepop (ver. 1.2.): a population genetics software for exact test and ecuniscism. *J. Hered.,* **95**: 248-249.

ROCKWELL, R.F. & G.F. BARROWCLOUGH (1987) – Gene flow and the genetic structure of populations. In: F. COOKE & P.A. BUCKLEY (Eds), *Avian Genetics: a population and ecological approach.* Acad. Press Inc. London: 223-255.

VERHEYEN, G.R., B. KEMPENAERS, T. BURKE, M. VAN DEN BROECK, C. VAN BROECKHOVEN & A.A. DHONDT (1994) – Identification of hypervariable single locus minisatellite DNA probes for the blue tit (*Parus caeruleus*). *Molec. Ecol.,* **3**: 137-143.

VERHEYEN, G.R., B. KEMPENAERS, F. ADRIAENSEN, M. VAN DEN BROECK, E. MATTHYSEN, C. VAN BROECKHOVEN & A.A. DHONDT (1995) The genetic structure of blue tit (*Parus caeruleus*) populations as revealed by minisatellite single locus probes. *Heredity,* **75**: 571-577.

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

WENINK, P.W., A.J. BAKER & M.G.J. TILANUS (1993) – Hypervariable-control-region-sequences reveal global population structuring in a long-distance migrant shorebird, the dunlin (*Caledris alpina*). *Proc. Natl. Acad. Sci. USA,* **90**: 94-98.

Belg. J. Zool. - Volume 127 (1997) - issue 2 - pages 191-195 - Brussels 1997

SHORT NOTE

EFFECTS OF SOIL ACIDIFICATION ON REPRODUCTIVE SUCCESS IN GREAT TITS BREEDING IN FORESTS ON NUTRIENT-POOR SOILS IN FLANDERS, BELGIUM

RIANNE PINXTEN AND MARCEL EENS

Department of Biology, University of Antwerp, U.I.A., Universiteitsplein 1, B-2610 Wilrijk, Belgium.
e-mail: eens@uia.ua.ac.be

Key words: Acid rain, soil acidification, eggshell, *Parus major*.

It has been recently shown that on nutrient-poor, acidified soils in the Netherlands an increasing number of great tits, *Parus major* (LINNAEUS, 1758), and other forest passerines produce eggs with thin and porous shells and have low reproductive success as a result of calcium deficiency (1, 2). In a mixed deciduous-coniferous forest on nutrient-poor sandy soil, representative of 80% of the Dutch forests, the proportion of great tits laying eggs with defective shells increased from 10% in 1983-1984 to 40% in 1987-1988 (15). A similar increase in eggshell defects and associated laying irregularities have been reported for tits and other species in Europe and in North America (3, 4, 5, 6, 7, 8, 9). The causes of this phenomenon have been studied in detail in great tits in the Netherlands (1, 2, 10, 11).

Female granivorous and insectivorous birds, including great tits, apparently depend on a high level of dietary calcium during the laying phase, which they normally obtain through the uptake of snail shells or other calcium-rich items (2, 10, 12, 13). Atmospheric deposition of acidifying compounds («acid rain») has caused a decline in snail populations on poorly buffered sandy soils, because calcium needed for shell formation becomes unavailable. This decrease in snail abundance on nutrient-poor soils caused by man-made acid rain has been demonstrated to be responsible for the decline in eggshell quality in great tits breeding in forests on nutrient-poor soils (11). In forests with a scarcity of snails and high rates of eggshell defects, some of the birds try to compensate for the lack of snail shells by using anthropogenic calcium sources such as chicken eggshells and mortar that they obtain outside the forest at farms and picnic sites.

Although the detailed studies of Graveland in the Netherlands (1, 2, 10, 11) provide evidence that calcium deficiency can be a widespread phenomenon in many acidified areas, there are very few data to support this claim. This is due to several reasons (see 11), an important one being that eggshell defects are hard to recognize for the untrained eye and also may be overlooked because eggs with defective shells are often removed by the parents. Also, in most countries study sites are usually situated close to human settlements, where calcium deficiency is unlikely to occur due to the presence of anthropogenic calcium sources. Determining the extent of calcium deficiency in other countries, and especially in remote areas where anthropogenic calcium sources are not available, should be a first priority (11).

To our knowledge, the occurrence of this phenomenon has not been investigated or reported in Belgium. Here, we report the results of a preliminary study on the effects of soil acidification on the eggshell quality and reproductive success in great tits breeding in a forest on nutrient-poor sandy soil in a relatively remote area in Flanders, Belgium.

The study was conducted in 1995 in a coniferous forest on nutrient-poor sandy soil in the Nature Reserve «De Kalmthoutse Heide (The Kalmthout Heath)» in Kalmthout (4° 25' N, 51° 25' E), situated about 40 km north-west of Antwerp. At the end of February 1995, we erected 21 nestboxes for great tits in a coniferous forest, situated near the border with The Netherlands. The pH-H_2O (1:2) value of the soil in this study site was measured and varied between 3 - 4. The study site in the Kalmthoutse Heide can be considered as a remote area, since nearly all nestboxes were situated more than 500 m from human settlements in the neighbourhood (mainly farms) and it has been shown that great tits do not travel more than 500 m away from their nest site in search of anthropogenic calcium sources (2). In 1995, we also studied the occurrence of egg-shell defects in a great tit population breeding in a nest-box colony (23 nestboxes) on nutrient-rich soil on the University Campus of the University of Antwerp in Wilrijk. The latter control study site, with a pH-H_20 (1:2) value of 6, is situated very close to human settlement. In both study sites nestboxes were checked every 4 to 5 days from the moment nesting material appeared. When eggs were present in the nestbox, the quality of the shell of each individual egg was evaluated. We distinguished between eggs with a normal shell and eggs with a deviant shell. Eggs with deviant shells can be recognized by their rough, dull shell surface and aberrant pigmentation (Fig. 1; Table 1). All eggs in a nest were individually numbered to determine the hatching success of each egg.

TABLE 1

Criteria used to evaluate the shell quality of eggs of great tits (after Graveland, 1993).

normal shell	deviant shell
– Clearly distinguishable spots. Eggs are very rarely entirely white.	– Smeared spots. There is a pink bloom on (part of) the egg. Sometimes eggs are entirely white.
– Shell surface is smooth and glossy.	– Shell surface is rough and dull.
– Pigmentation is usually evenly distributed on shell surface. Sometimes there is a wreath of pigmentation around the blunt top of the egg, but the spots are always clearly and separately recognizable.	– Pigmentation is almost always concentrated around the blunt top of the egg.
– There are never holes in the egg.	– Sometimes holes in the egg are visible with the naked eye.
– Egg is never desiccated.	– Egg is usually in the process of desiccation. Often, the desiccation can already be observed during the laying period because the air chamber enlarges quickly.

Eggs with normal shells never become desiccated. Eggs with deviant shells often become desiccated. As a result of desiccation the content of the egg drops to one side. In extreme cases, the content will become separated from the shell.

Fig. 1. – Deserted clutch of Great Tit containing one egg with a defective shell (top right) and two eggs with normal shells. Photograph: with permission from Jaap Graveland.

Data were analysed using the statistical packages SPSS/PC and StatXact-Turbo with standard techniques (14). Data expressed as percentages were arcsine square-root transformed to normalize.

In total 6 out of 16 (38%) females breeding on nutrient-poor soil in the Kalmthoutse Heide produced eggs with defective shells, compared to none of the 21 females breeding on the University Campus on nutrient-rich soil ($X^2=9.40$, $P<0.01$). When comparing the proportion of aberrant eggs between the two study sites, 19 (14%) out of 138 eggs in the Kalmthoutse Heide had a deviant shell compared to none out of 207 eggs on the University Campus in Wilrijk ($X^2= 30.16$, $P<0.0001$). In the Kalmthoutse Heide the proportion of eggs with defective shells in the six clutches containing deviant eggs was on average 42%, and varied from 9% to 67%. Only 14% (3 out of 19) of the eggs with deviant shells hatched, compared with 98% (115 out of 119) of normal eggs. This difference in hatching success between the two egg categories is highly significant ($X^2=86.42$, $P< 0.0001$). Twelve of the 16 eggs with defective shells that did not hatch, became desiccated during the laying or the early incubation period, two eggs did not hatch due to shell breakage, one egg disappeared from the nest and one egg did not hatch as a result of the clutch being deserted. Within the Kalmthoutse Heide, the hatching success of clutches containing one or more eggs with deviant shells (0.48 ± 0.34 SD, $N=6$) was significantly

lower than that of clutches with only normal eggs (0.99 ± 0.04, N=10; t-test, t=-5.46, df= 14, P<0.0001). The overall hatching success of clutches on the Kalmthoutse Heide was 0.80 ± 0.32 (N=16), compared to 0.97 ± 0.06 (N=21) for clutches on the University Campus in Wilrijk. This difference in hatching success between both study sites is significant (t-test, t=-2.22, df=35, P=0.033).

Although our results are based upon a one-year study, they strongly suggest that soil acidification and calcium deficiency also limit breeding success of great tits breeding in relatively remote areas on nutrient-poor soils in Flanders. We found that 38% of the studied great tit females in acidified forests in the «Kalmthoutse Heide» produced eggs with defective shells, resulting in a significantly reduced hatching success. These results are comparable to those reported by Graveland for the Netherlands (1,15).

In the sixties, reductions in egg-shell thickness were always attributed to DDT and other organochlorines (16,17). However, GRAVELAND & DRENT (2) concluded that it is very unlikely that the increase in eggshell defects in the Netherlands during the past two decades was due to poisoning by organochlorine compounds such as DDT, since raptors are more vulnerable to poisoning than passerines (18) and raptors have fully recovered since the banning of DDT and related compounds in the Netherlands (19).

From Graveland's study, it follows that great tits may be good candidates for use as indicators of the effects of (progressive) soil acidification on birds, and for measuring the effectiveness of measures to be taken to improve the situation (7). It is obvious, however, that more research is needed on a great variety of species. For instance, the black tern, *Chlidonias niger,* has completely disappeared as a breeding species from the 'Kalmthoutse Heide' since the sixties (20, 21), although there have been little or no apparent changes in habitat. It has recently been shown, however, that this species suffers severely from calcium deficiency in certain habitats in the Netherlands (22). Chicks grew well during the first week but then started to develop deformed legs and wings. Postmortem analysis revealed severe rachitis. Additional evidence of calcium deficiency came from higher incidences of incomplete clutches, eggs failing to hatch and the occurrence of eggs with aberrant colouration patterns, as in calcium-deficient great tits (22).

REFERENCES

1. GRAVELAND , J., R. VAN DER WAL, J.H. VAN BALEN & A.J. VAN NOORDWIJK (1994) – Poor reproduction in forest passerines from decline of snail abundance on acidified soils. *Nature*, **368**: 446-448.
2. GRAVELAND, J. & R.H. DRENT (1997) – Calcium availability limits breeding success of passerines on poor soils. *J. Anim. Ecol.*, **66**: 279-288.
3. SMITH, J. (1990) – Acid rain and defective eggshells. *Anser,* **29**: 158.
4. WINKEL, W. & H. HUDDE (1990) – Zum vermehrten Auftreten von «Bruten in leerem Nest»: Befunde am Meisen (*Parus*) und andere Höhlenbrütern aus verschiedenen Untersuchungsräumen Norddeutschlands. *Vogelwarte,* **35**: 341-350.
5. GLOOSCHENKO, V., P. BLANCHER, J. HERSKOWITZ, R. FULTHORPE & S. RANG (1986) – Association of wetland acidity with reproductive parameters and insect prey of the eastern kingbird (*Tyrannus tyrannus*) near Sudbury, Ontario. *Water Air Soil Pollution,* **30**: 553-567.

6. ORMEROD, S.J., K.R. BULL, C.P. CUMMINS, S.J. TYLER & J.A. VICKERY (1988) – Egg mass and shell thickness in Dippers (*Cinclus cinclus*) in relation to stream acidity in Wales and Scotland. *Environ. Pollution*, **55**: 107-121.
7. GRAVELAND, J. (1990) – Effects of acid precipitation on reproduction in birds. *Experientia*, **46**: 962-970.
8. SCHMIDT, K.-H. (1990) – Sprunghafter Anstieg von Brutstörungen bei Höhlenbrütern. *J. Orn.*, **131**: 172-174.
9. CARLSSON, H., L. CARLSSON, C. WALLIN & N.-E. WALLIN (1991) – Great tits incubating empty nest cups. *Ornis Svecica*, **1**: 51-53.
10. GRAVELAND, J. & T. VAN GIJZEN (1994) – Arthropods and seeds are not sufficient as calcium sources for shell formation and skeletal growth in passerines. *Ardea*, **82**: 299-314.
11. GRAVELAND, J. (1996) – Eggshell defects in forest passerines caused by decline of snail abundance on acidified soils. *Vogelwelt*, **117**: 67-73.
12. JONES, P.J. (1976) – The utilization of calcareous grit by laying *Quelea quelea*. *Ibis*, **118**: 575-576.
13. TURNER, A.K. (1982) – Timing of laying by swallows (*Hirundo rustica*) and sand martins (*Riparia riparia*). *J. Anim. Ecol.*, **51**: 29-46.
14. SOKAL, R.R. & F.J. ROHLF (1981) – *Biometry*, 2nd edn. New York: W.H. Freeman.
15. DRENT, P.J. & J.W. WOLDENDORP (1989) – Acid rain and eggshellls. *Nature*, **339**: 431
16. BITMAN, J., H.C. HARRIIS & G.F. FRIES (1969) – DDT induces a decrease in eggshell calcium. *Nature*, **224**: 44-46.
17. RATCLIFFE, D.A. (1970) – Changes attributable to pesticides in egg breakage frequency and egg shell thickness in some British birds. *J. Appl. Ecol.*, **7**: 67-115.
18. NEWTON, I. (1979) – *Population Ecology of Raptors*. Collins, London.
19. OPDAM, P., J. BURGERS & G. MUSKENS (1987) – Population trend, reproduction and pesticides in Dutch sparrowhawks following the ban on DDT. *Ardea*, **75**: 205-212.
20. VANDEGEHUCHTE, M. (1989) – Zwarte stern, *Chlidonias niger. In : Vogels in Vlaanderen: voorkomen en verspreiding.* Vlaamse Avifauna Commissie. Bornem.
21. MAES ,P. & H. VOET (1988) – Zwarte stern, *Chlidonias niger. In:* Devillers, P., Roggeman, W., Tricot, J., del Marmol, C., Kerwijn, C., Jacob, J.-P. & Anselin, A. (eds.). *Atlas van de Belgische broedvogels. Brussel, Koninklijk Belgisch Instituut voor Natuurwetenschappen.*
22. BEINTEMA, A.J., T. BAARSPUL & J.P. DE KRIJGER (1997) – Calcium deficiency in Black Terns *Chlidonias niger* nesting on acid bogs. *Ibis*, **139**: 396-397.

Received : 3 June 1997

GUIDE FOR AUTHORS

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

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

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

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

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

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

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

General information on the society can be found on http://www.uia.ua.ac.be/u/morfo/kbvd.htlm

CONTENTS

Short note

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

ISSN: 0777-6276