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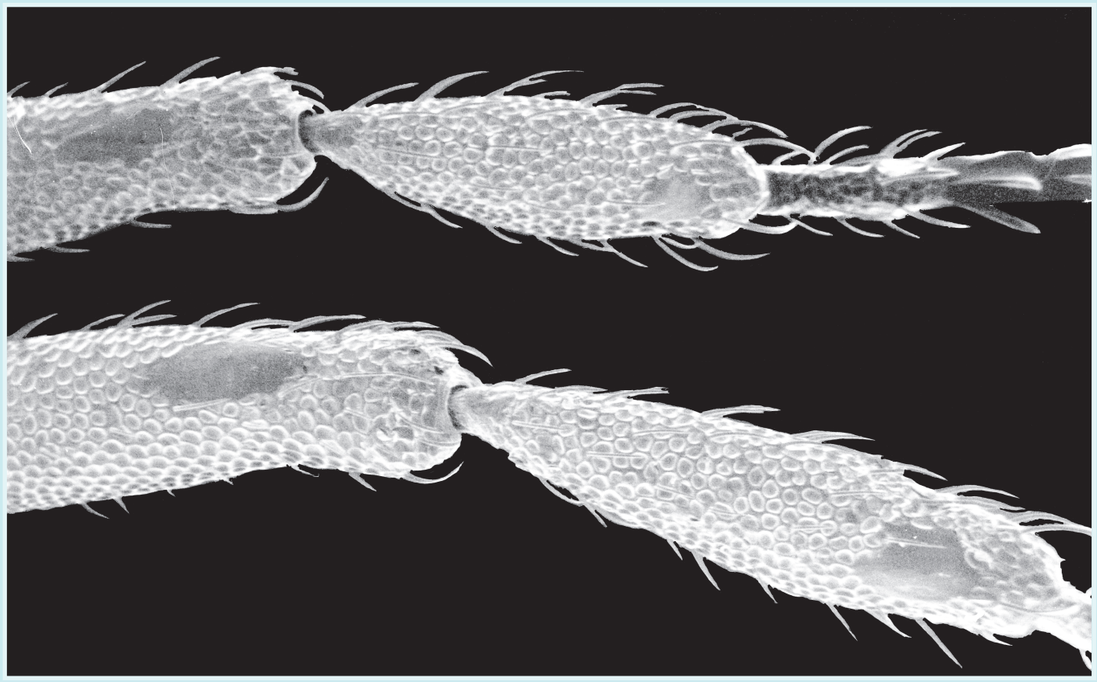

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BELGIAN JOURNAL OF ZOOLOGY

Belgian Journal of Zoology

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Rice plant damage distribution and home range distribution of the ricefield rat *Rattus argentiventer* (*Rodentia: Muridae*)

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ABSTRACT. The patterns of rice plant damage distribution and the home distribution of the ricefield rat *Rattus argentiventer* were studied in a two hectare experimental field and in a two hectare enclosure. Damaged rice plants were randomly (Poisson) distributed during the vegetative stage of rice plant growth but tended to be aggregated or contiguous during the generative stage. Analysis of the spatial distribution of the rats' home ranges, using MORISITA's index of dispersion and nearest neighbor distances, indicated that (i) males showed a slightly stronger tendency to congregate than females, and (ii) females showed a uniform distribution pattern and had mutually exclusive home ranges from the birth season through the lactation period. Before and after the birth season, female rats had a random distribution pattern. Furthermore, an analysis of the degree of home-range overlap between the sexes found that male and female home ranges were distributed almost independently during the non-breeding and early mating seasons. However, early in the breeding season and at the end of the mating season, male and female home ranges overlapped completely. Male and female home ranges were completely exclusive from pregnancy until the end of lactation.

KEY WORDS: Ricefield rat *Rattus argentiventer*, home range, distribution pattern, growth stage of rice plant, rat damage.

INTRODUCTION

Rats can cause substantial economic loss in rice and most other crops, including palm oil, sugar cane, maize, cassava, soybean, groundnut, coconut, mung bean, and sweet potato. The current conservative estimate is that typically between 5 and 15% of the rice crop in rice-growing regions is lost because of rodents (Geddes, 1992).

In Southeast Asia, the reported impact of rodents is highest in Indonesia, where approximately 17% of rice production is lost to the ricefield rat (GEDDES, 1992).

In West Java, especially at the Jatisari field study site, the dominant species of small mammal in rice fields is *Rattus argentiventer* (ROBINSON & KLOSS, 1916). In their five-year study at various locations throughout Indonesia, MURAKAMI et al. (1990) reported that *R. argentiventer* dominated samples of small mammals in rice fields. Less than one percent of the small mammals found were *Bandicota indica* (BECHSTEIN, 1800) or *Rattus rattus diardii* (JENTINK, 1879).

Several authors have discussed the general biology of the ricefield rat (LAM, 1983), and its breeding and control (HARRISON, 1951; BUCKLE et al., 1985; LAM, 1983; SINGLETON, 1997; SOEKARNO et al., 1978). TRISTIANI (1999) examined ricefield rat population parameters, especially birth and immigration, as well as the relationship between rat reproduction and the stages of growth of the rice plant (TRISTIANI et al., 1998). However, little is known of the behavior of this species in relation to population demography. Specifically, there has been little research to clearly reveal the relationship between spatial patterns of damage distribution, the rats' home range distribution, and the stages of growth of the rice plant.

Damage to rice plants by rats is by far the greatest agricultural problem in Indonesia (GEDDES, 1992; SINGLETON, 1997). An understanding of the rats' population dynamics,

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habitat use and distribution pattern, and of the factors that influence their breeding, survival, and movement, is essential for developing an effective, economic, and sustainable management program (FIEDLER & FALL, 1996; SINGLETON & PETCH, 1994; SINGLETON, 1997).

An interesting aspect of the spatial pattern of damage distribution and the home range distribution is the effect on rat sampling and control strategies. A quantitative description of spatial pattern is essential, not only to understand the spatial or dispersion dynamics of populations, but also to develop appropriate sampling strategies for population surveys (IWAO, 1977). We examined the behavior of ricefield rat populations, focusing on rat damage from feeding and on individuals' home ranges, and on the relationship between the rats' reproduction and the growth stages of the rice plant.

The study had two objectives: 1) To determine the pattern of distribution of rat damage. 2) To examine changes in the home-range pattern with the stage of growth of the rice plant, in both a two-hectare experimental field and in a two-hectare enclosure. This information is essential in deciding how far apart poisoned bait should be placed, how to develop better farm management practices, and how to assess the likely transmission rates of potential biological control agents.

MATERIAL AND METHODS

This study was carried out in a 2 ha experimental field, and in an enclosed 2 ha ricefield. The two sites were used to enable us to study the effect of migration on the rats' distribution pattern. In the experimental field, immigration and birth were the major factors in the growth of the rat population (TRISTIANI, 1999), whereas migration could not affect the rat population in the enclosed field. The field work was conducted from 1988 to 1990 at the Jatisari Forecasting Center in West Java, about 120 km east of Jakarta. The Center is an agricultural research station, and rice is the main crop grown.

Surrounding the enclosed field is a permanent concrete wall embedded into the ground to a depth of 0.6 m. On this is a wire-mesh fence 0.6 m high, topped with a second 0.4 m zinc fence. In addition, a 0.2 m zinc barrier was placed along both sides of the fence, at a vertical angle of 45º to effectively prevent any possibility of emigration or immigration. The effectiveness of this barrier was tested with an intensive capture-recapture survey, conducted both inside and outside the enclosure. The results showed that there was no rat migration into or out of the enclosed area.

The enclosure and the experimental field were cultivated during the December to May rainy season, and again during the June to October dry season. The field was left fallow in November. The rats in the enclosure were exterminated by trapping intensively for about one month until there was no evidence of bait being consumed by rats. The enclosure was then deemed rat-free. Two weeks after transplanting the rice in the1989 rainy season, which started in December 1988, thirty adult male and thirty adult female rats were released in the enclosure. All the rats used in the experiment were individually marked with a combination of toe and ear clips. Once the maximum population was reached at the end of each season, the non-tagged rats were removed from the enclosure to prevent severe rat damage to the ricefield.

Single capture live-traps made of wire mesh (200 x 110 x 110 mm: 100 mm^2 mesh) were used to conduct a census of the rat population. The traps were placed in the 10 x 10 m sections of a large grid (110 x 180 m). Each trap was baited with whole grain, which was wrapped with cotton mesh and suspended from the center of the trap. This is a proven method for live-trapping this species (MURAKAMI et al., 1990). For five days, the traps were set at about 5:30 p.m. and then checked the next morning. All of the traps were then removed for a ten-day period. This cycle was maintained throughout the study, meaning that trapping was conducted twice monthly.

Each morning, all the captured rats were transported to a processing station. Rats caught for the first time were marked with a combination of toe and ear clips. The rats were counted, weighed, sexed, and examined externally to determine their reproductive condition. They were then released before sunset, at about 5:30 p.m., at their respective capture sites.

Rats were classified as adult males (≥110 g), adult females (≥60 g), sub-adult males (40-109 g), sub-adult females (40-59 g) or juveniles (≤40 g).

Monitoring rat damage

The study area was a ricefield that was already divided into experimental and enclosed fields. There were 10800 rice plant hills in the enclosure and 7200 hills in the experimental field, at regular intervals of 0.25 m, each with three rice seedlings, transplanted together.

Rice plants were sampled randomly on different days and from different hills. Damaged tillers were recorded and counted. The location of each damaged plant was marked with a bamboo cane and color-coded tape that indicated when it was damaged. The spatial pattern of damage was analyzed statistically by examining the frequency distribution of damaged tillers per sampling unit.

Six plots (10 by 10 m) were sampled in the experimental field, and nine plots (10 by 10 m) were sampled in the enclosure. Each plot contained 1200 hills. Observations were conducted three weeks after the ricefield was transplanted, in the maximum tillering stage, during panicle primordia initiation, and in the booting, flowering, milky, and ripening stages. The phenology of the rice plant is briefly reviewed in Appendix 1.

Spatial distribution pattern of rat damage

To interpret the general pattern of rat damage at each stage of rice plant growth, the overall distribution of the damage was analyzed. Earlier studies of spatial point patterns were primarily concerned with comparing area (or quadrate) counts to a Poisson distribution; departures indicate that the pattern is not completely spatially random (CRESSIE, 1993). Furthermore, CRESSIE (1993) stated that the degree of departure is usually measured by an index based on the quadrate count (e.g., FISHER et al., 1922; DAVID & MOORE, 1954; MORISITA, 1959; LLOYD, 1967).

We tested the goodness of fit of the spatial distribution of rat damage to (1) the Poisson distribution, (2) the negative binomial distribution, and (3) IWAO's patchiness regression (IWAO, 1968).

Goodness of fit of the Poisson distribution

The test for the goodness of fit of the Poisson distribution used the test statistic $x^2 = ss/\bar{x}$ with n-1 degree of freedom or $x^2 = ss/\mu$ with $\mu = n$, if μ is known (ZAR, 1999). To determine whether the distribution of each developmental stage departed from random, the number of damaged tillers per sampling unit was examined in terms of the variance-mean ratio. The null hypothesis in Poisson goodness of fit testing is one of random distribution of entities in space or time (ZAR, 1999). Furthermore ZAR (1999) stated that rejection of the hypothesis of randomness might result from one of two situations. First, the population distribution may be uniform; that is, each unit of space (or time) has the same number of entities. Second, the population may be arranged in what is referred to as a clustered, aggregated, or contiguous, distribution. If a population has a random distribution, $\sigma^2 = \mu$ and $\sigma^2/\mu = 1.0$. If the population distribution is more uniform than random (sometimes called "underdispersed"), $\sigma^2 < \mu$ and $\sigma^2/\mu < 1.0$. If a population is distributed contiguously (sometimes termed "overdispersed"), $\sigma^2 > \mu$ and $\sigma^2/\mu > 1.0$. The departure of the rat damage distribution pattern from randomness (Poisson distribution) was tested by calculating the index of dispersal, *I*, for every sampling as

$$I = \frac{s^2}{\bar{x}}$$

where s^2 represents variance and represents mean of damaged tillers.

Fit to the negative binomial distribution

The mathematical distribution that is sometimes used to describe contiguous distributions of biological data is the negative binomial distribution (ZAR, 1999). The negative binomial distribution has two parameters: (1) $\bar{x}$, the mean number of individuals per sampling unit or the mean number of damaged tillers per sampling unit and (2) *k*, a parameter related to the degree of clumping (LUDWING & REYNOLDS, 1988). The goodness of fit of the rat damage distribution for each stage of rice development was examined using ANSCOMBE's statistic T (ANSCOMBE, 1950):

$$T = \frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})^3 - s^2\left(\frac{2s^2}{\bar{x}} - 1\right)$$

where *n* is the number of sampling unit, x_i is the number of damaged tillers in the *i*th sample unit, and $\bar{x}$ represents the mean number of damaged tillers. The value of *T* was compared with its standard error:

$$S.E.(T) = \sqrt{\frac{2\bar{x}\,(k+1)\,\frac{\bar{x}^2}{k^2}\left(1+\frac{\bar{x}}{k}\right)^2\left\{2\left(3+5\frac{\bar{x}}{k}\right)+3k\left(1-\frac{\bar{x}}{k}\right)\right\}}{n}}$$

where $k = \frac{\bar{x}^2}{s^2 - \bar{x}.}$

T/S.E.(T) should lie between ±1.96 if the negative binomial distribution is a satisfactory model.

IWAO's patchiness regression

In LLOYD's (1967) "mean crowding", $\overset{*}{x}$, indicates the average number of events sharing a quadrate with an arbitrary event, and is calculated as:

$$\overset{*}{x} = \bar{x} + \left(\frac{s^2}{\bar{x}} - 1\right)$$

where $\bar{x}$ represents the mean number of damaged tillers and s^2 represents variance. IWAO (1968) found that LLOYD's (1967) mean crowding is linearly related with the mean density over a range of different densities in a wide variety of situations, including both theoretical and biological distribution. The relationship is shown by

$$\overset{*}{x} = \alpha + \beta\bar{x},$$

where α is the intercept on the $\overset{*}{x}$-axis and β is the regression coefficient. The index of basic contiguity, α, is the number of other damaged tillers in the same sampling unit. The index β (the density-contiguity coefficient) is a measure of the distribution of rat damage, and takes values of <1, =1, and >1 for uniform, random and aggregated distribution, respectively (IWAO & KUNO, 1971). Furthermore, IWAO & KUNO (1971) showed that estimation of α and β for a set of distributions can be made by the usual least squares method, and the fitness to the linear regression may conveniently be indicated by the coefficient of determination, r^2 (r: correlation coefficient).

Spatial distribution of home range

The area of each individual's home range was determined by a modification of the minimum-convex-polygon method (MARES & LACHER, 1987). The distribution of the trap stations at which a given number of different individuals (i.e., 0, 1, 2, or 3 separate rats) were recorded during each survey period was examined. It was assumed that a given rat could be caught in all traps within its home range, even though in practice some were not caught in all of the stations within their home range. Juveniles were

excluded from this analysis, because they may continue to remain within their mothers' home ranges (MURAKAMI, unpublished observations). The dispersion pattern was assessed using MORISITA's index of dispersion, I_B, index (MORISITA, 1962), calculated as:

$$I_B = I_\delta \frac{n}{n - 1}$$

$$\text{with } I_\delta = \frac{n\sum_{i=1}^{n} x_i(x_i - 1)}{n\bar{x}(n\bar{x} - 1)}$$

where n is the total number of observations and x_i is the number of individuals in the ith quadrate.

The degree of spatial correlation or the degree of home range overlap relative to an independent distribution was determined using IWAO's ω-index (IWAO, 1977). The value of ω changes from its minimum of -1 for complete exclusion, through 0 for independent occurrence, to the maximum of +1 for complete overlapping.

RESULTS

Distribution pattern of rat damage

During the rice plants' vegetative growth stage, the variance-mean ratio never exceeded the critical value of p=0.05, for a random distribution. During the generative stage, however, the variance-mean ratio always exceeded the critical value of p=0.05. Fig. 1 summarizes the goodness of fit to a random distribution. The relation of $s^2/\bar{x}$ to $\bar{x}$ in Fig. 1 indicates that the variance-mean ratio increased linearly with the mean in every generative stage, and almost always exceeded unity. It is obvious from Fig. 1 that the variance-mean ratio during the generative stage departs from randomness.

If spatial randomness (sr) during the generative stage is rejected, the next step in a spatial analysis is to measure the departure from sr. Table 1 summarizes the goodness of fit to the negative binomial model for all the observed data during the generative stage. The results show that the value of *T/S.E.(T)* for each stage during the generative stage of rice plants lies between ±1.96 or the value of *T* is always smaller than the standard error. The results show that during the rice plants' generative growth stage, the distribution of rat damage followed the negative binomial distribution.

Analysis of the goodness of fit of IWAO's patchiness regression showed that the values of α (index of basic contiguity) and β (the density-contiguity coefficient) are $\alpha \cong 0$ and $\beta \cong 1$ during the rice plants' vegetative growth stage (Table 2). This indicates that the distribution of rat damage was approximately random during the vegetative stage. However, during the generative stage (from panicle initiation to ripening) the patterns changed to $\alpha > 1$ and $\alpha > 1$, indicating aggregated or contiguous distribution.

TABLE 1

Summary of the goodness of fit of the rat damage distribution to the negative binomial distribution for all rice plant stages, calculated by ANSCOMBE's T-statistic. S.E. is standard error (S.E.) of T.

Stage of **rice plant**	**1989 dry season T ± S.E**	**1989 rainy season T ± S.E**	**1990 dry season T ± S.E**	**1990 rainy season T ± S.E**
Enclosure				
panicle initation	0.15 ± 19.05	3.84 ± 17.96	3.76 ± 12.79	7.47 ± 17.20
booting	-0.63 ± 8.70	0.74 ± 6.98	-1.43 ± 7.08	0.58 ± 13.26
flowering	-0.99 ± 7.12	0.27 ± 7.17	7.11 ± 13.45	7.47 ± 17.20
milky	6.31 ± 7.10	5.34 ± 13.31	0.29 ± 0.32	0.09 ± 0.26
ripening	-0.03 ± 7.28	12.25 ± 13.31	0.98 ± 1.67	-0.47 ± 0.50
Experimental field				
panicle initation	-0.27 ± 2.82	2.91 ± 14.98	0.44 ± 1.08	1.77 ± 6.27
booting	5.95 ± 11.51	3.74 ± 16.08	-1.71 ± 2.85	0.98 ± 9.26
flowering	-0.56 ± 2.78	0.31 ± 12.01	4.68 ± 21.91	9.89 ± 16.78
milky	5.52 ± 8.10	3.54 ± 16.13	1.44 ± 2.08	0.77 ± 5.23
ripening	-0.74 ± 11.52	8.24 ± 10.31	0.57 ± 1.69	-1.16 ± 6.30

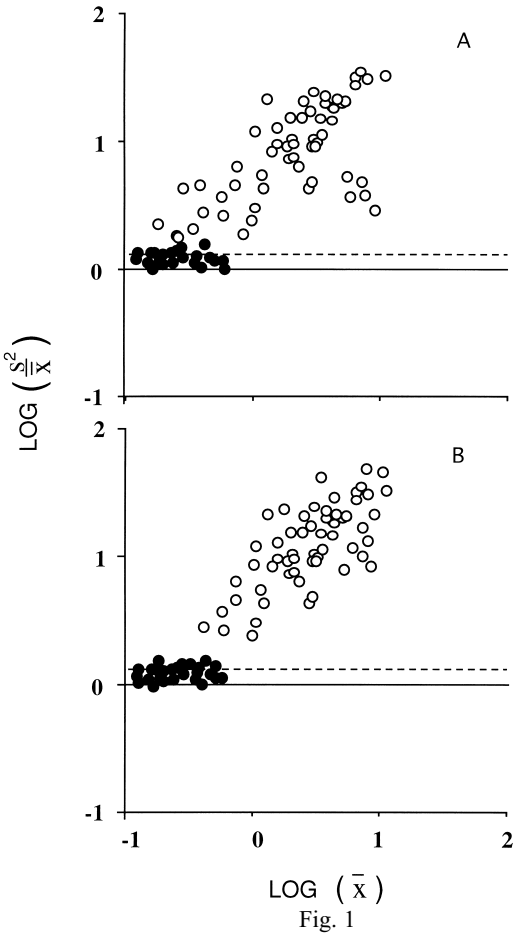

Fig. 1. – Relation of the variance-mean ratio to the mean in enclosure (A) and experimental field (B). The broken line is the critical value at p=0.05 for random distribution. Black circles indicate vegetative stage rice plants, white circles indicate generative stage. s^2 represents variance and $\bar{x}$ represents mean number of damaged tillers.

Fig. 1

TABLE 2

Statistics of IWAO's patchiness regression (mean crowding vs. mean) for the different growth stages of rice plants in both the enclosed and experimental fields. The value of α represents the index of basic contiguity and β is the density-contiguity coefficient. r^2 represents the coefficient of determination (r: correlation coefficient).

Stage of Rice plant		Enclosure α	Enclosure β	Enclosure r^2	Experimental field α	Experimental field β	Experimental field r^2
			1989-dry season				
Tillering	$\alpha \cong 0$	0.028	1.021	0.882	0.015	0.823	0.862
Max.tillering	$\beta \cong 1$	-0.096	1.085	0.948	-0.086	1.045	0.914
Panicle initation		2.078	7.576	0.879	7.018	1.536	0.909
Booting	$\alpha > 1$	1.385	8.941	0.965	7.988	2.042	0.912
Flowering	$\beta > 1$	1.439	7.831	0.951	7.193	1.431	0.941
Milky		1.614	6.607	0.819	1.114	6.206	0.839
Ripening		1.578	6.897	0.828	2.272	3.417	0.923
			1989-rainy season				
Tillering	$\alpha \cong 0$	-0.058	0.777	0.828	-0.047	0.731	0.891
Max.tillering	$\beta \cong 1$	-0.046	0.972	0.816	-0.041	0.911	0.848
Panicle initation		6.696	2.424	0.998	7.162	1.624	0.944
Booting	$\alpha > 1$	8.581	1.874	0.912	8.089	1.984	0.931
Flowering	$\beta > 1$	6.947	1.523	0.806	7.218	1.622	0.902
Milky		7.487	6.777	0.968	7.061	6.422	0.898
Ripening		7.107	6.972	0.957	7.114	6.611	0.899
			1990-dry season				
Tillering	$\alpha \cong 0$	0.016	0.832	0.874	0.019	0.933	0.901
Max.tillering	$\beta \cong 1$	-0.077	1.032	0.974	-0.069	1.011	0.899
Panicle initation		7.249	1.623	0.951	7.157	1.824	0.934
Booting		8.106	2.398	0.941	7.989	2.197	0.954
Flowering	$\alpha > 1$	7.547	1.524	0.993	7.441	1.403	0.925
Milky	$\beta > 1$	1.182	6.467	0.841	1.197	6.263	0.867
Ripening		2.359	3.731	0.969	1.999	3.086	0.918
			1990-rainy season				
Tillering	$\alpha \cong 0$	0.014	0.955	0.931	0.016	0.842	0.827
Max.tillering	$\beta \cong 1$	0.025	0.773	0.888	0.021	0.699	0.899
Panicle initation		7.545	1.985	0.952	7.023	1.976	0.945
Booting		7.219	1.656	0.914	7.112	1.656	0.914
Flowering	$\alpha > 1$	6.545	1.985	0.952	6.545	1.875	0.915
Milky	$\beta > 1$	7.219	2.656	0.814	7.107	2.214	0.902
Ripening		0.258	6.648	0.984	0.301	3.041	0.912

Home range distribution pattern

The patterns of dispersion for male and female rats were analyzed separately for the two fields (Table 3). In terms of the I_B-index, males had a slightly stronger tendency to congregate than females. For most females, the I_B-index was approximately zero from early-March to early-May and from early-August to early-October. From the birth season through the lactation period, females showed a uniform pattern of distribution and their home ranges tended to be mutually exclusive. They had a random distribution pattern before and after the birth season.

The ω-index was used to examine the degree that the home ranges of the sexes overlapped in each survey period. The results show that at the peak of the birth seasons, from March to mid April and from August to mid September, the lack of home-range overlap between males and females amounted to almost complete exclusion (most of the ω-indices approximately equal to -1, Table 4). However, during the early mating seasons (early-January and early-June) and non-breeding season (November and December) most of the ω-indices were less than $0.5 \cong 0$, indicating that the degree of home-range overlap between males and females was an independent occurrence. From the end of the mating season until the pregnancy (February and July) and lactating periods (May and October), most of the ω-indices were approximately equal to +1, indicating that male and female home ranges completely overlapped.

TABLE 3

Summary of home range distributions in the enclosure and in the experimental field calculated by the value of the I_B-index.
I and II refer to the first and second survey periods for each month, respectively. M, male; F, female.

		January		February		March		April		May		June		July		August		September		October		November		December	
		I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
Enclosure																									
1989	M	1.71	2.02	2.31	2.22	4.21	4.14	5.48	2.12	2.33	1.59	2.29	2.52	2.71	7.38	5.10	4.16	6.28	5.95	4.15	2.41	1.58	1.38	1.84	1.85
I_B-index	F	0.61	0.99	0.53	0.71	0.08	0.07	0.09	0.04	0.06	0.72	0.70	0.64	0.50	0.54	0.07	0.05	0.08	0.03	0.09	0.82	0.59	0.81	0.50	0.90
1990	M	1.51	2.41	1.72	2.51	2.30	4.12	3.22	1.22	1.52	1.53	2.51	3.23	2.82	6.03	4.98	4.26	3.96	4.14	5.51	2.14	1.76	2.57	1.46	1.94
I_B-index	F	0.76	0.85	0.58	0.73	0.06	0.09	0.04	0.02	0.09	0.81	0.74	0.81	0.61	0.52	0.06	0.08	0.05	0.02	0.06	0.57	0.54	0.63	0.58	0.86
Experimental field						*birth season*										*birth season*									
1989	M	2.53	2.51	3.89	2.99	3.72	3.21	5.83	6.70	2.60	3.57	3.08	4.07	3.08	6.40	4.10	4.49	2.51	5.18	3.88	4.94	3.80	1.50	2.86	1.65
I_B-index	F	1.31	1.20	1.36	0.62	0.06	0.03	0.04	0.02	0.08	0.23	2.90	2.80	0.14	0.61	0.04	0.09	0.03	0.18	0.08	0.80	0.70	0.50	0.53	1.01
1990	M	2.70	1.98	1.80	1.91	3.20	3.13	3.47	4.11	4.32	5.38	6.28	6.51	2.70	4.37	5.09	4.15.	4.27	5.94	5.14	6.40	4.37	4.37	2.83	2.83
I_B-index	F	1.30	1.38	1.10	0.89	0.08	0.07	0.09	0.08	0.09	0.42	1.40	1.50	1.46	0.59	0.07	0.10	0.09	0.07	0.05	0.82	1.76	1.70	0.59	0.96

TABLE 4

Summary of the degree of home-range overlap between the sexes in the enclosure and in the experimental field, calculated by the value of the ω -index.
I and II refer to the first and second survey periods for each month, respectively. M, male; F, female.

	January		February		March		April		May		June		July		August		September		October		November		December	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
Enclosure																								
(M and F)					*birth season*										*birth season*									
ω																								
1989	0.58	0.59	0.84	0.97	-0.87	-0.79	-0.60	0.81	0.83	0.49	0.41	0.78	0.96	0.91	-0.82	-0.90	-0.99	0.97	0.73	0.89	0.28	0.21	0.11	0.14
1990	0.44	0.61	0.71	0.97	-0.76	-0.82	0.87	0.96	0.87	0.72	0.43	0.60	0.81	0.92	-0.89	-1.04	-0.96	0.79	0.74	0.71	0.26	0.25	0.08	0.12
Experimental field																								
1989	0.39	0.68	0.57	1.21	-1.22	-0.89	-1.03	0.99	0.89	1.02	0.44	0.96	1.09	1.07	-0.93	-1.05	-1.31	0.98	1.05	0.96	0.15	0.34	0.42	0.35
1990	0.48	0.69	0.84	1.01	-1.04	-0.99	-1.02	-1.06	0.99	0.86	0.39	1.01	0.99	1.02	-0.97	-1.00	-0.98	0.42	0.89	0.82	0.47	0.35	0.01	0.09

DISCUSSION

During the vegetative stage (from the tillering stage to the maximum tillering stage), rat damage was randomly distributed. However, during the generative stage it generally tended to be aggregated or contiguous. As pointed out by many authors, both plants and animals are seldom distributed at random (IWAO & KUNO, 1971). Among the species of animals and plants showing aggregated distribution patterns (LUDWING & REYNOLDS, 1988, IWAO & KUNO, 1971), three major types can be distinguished by the combination of α and β: (1) $\alpha>0$ and $\beta\cong1$; (2) $\alpha\cong0$ and $\beta>1$; and (3) $\alpha>0$ and $\beta>1$ (IWAO & KUNO, 1971). The distribution of ricefield rat damage belongs to type (3). IWAO & KUNO (1971) stated that type (3) seems to be the most common among animals and plants, as many organisms tend to be distributed in colonies or clumps. This is both because of their characteristic modes of reproduction and dispersal, and because they respond to the heterogeneous conditions of their habitat. The aggregation that occurs during the generative stage of rice plant growth might be due to heterogeneous conditions in the rats' habitat and/or rat behavioral factors.

There were no significant differences in the distribution patterns of the ricefield rat for the rainy and dry seasons. It is difficult to draw any definite conclusions from this result, since data are only available for four consecutive seasons. Long-term studies are needed to provide more detailed information on the influence of season on the distribution of rat damage.

The present study revealed that males have a stronger tendency to congregate (i.e., a larger I_B value) than females, which seems to result from the male tendency to gather around females. Thus, some males were successful in approaching females, while others apparently were not. Interestingly, females showed a tendency toward underdispersion or uniform distribution, suggesting that females achieve higher reproductive success by not congregating. The I_B value of females tended to be zero during the birth season, indicating that the home ranges of females tended to be mutually exclusive. During this period, females may become more aggressive to establish territories and compete for resources. Among small mammals, reproductively active females generally maintain individual territories during the breeding season, which they actively defend from other females (BATZLI, 1985; REICH & TAMARIN, 1980). ANDERSON (1989) stated that females are unlikely to have to compete for copulation. Instead, the fitness of resident females may be limited by competition for territories that will provide the nutritional resources required for gestation and lactation, along with secure nest sites. Females' nutritional requirements are greatest during gestation and lactation (SADLEIR et al., 1973).

The degree of home-range overlap between the sexes was similar in both years. Our results indicate that during the non-breeding and early mating seasons, male and female home ranges were distributed almost independently. However, at the end of the mating season and early in the breeding season, male and female home ranges overlapped completely. From pregnancy until the end of lactation, male and female home ranges were completely exclusive. ANDERSON (1989) reported that resident males compete for females, while females compete for nutritional resources, nest sites, or for whatever parental investment is permitted in the mating system. This study also found that the home range of reproductively active adult females tended to be mutually exclusive during the breeding season. Female home ranges are more commonly exclusive of same-sex breeding residents than are those of males (JANNETT, 1980; MADISON, 1980a, 1980b; REICH & TAMARIN, 1980, 1984). During this period, the home ranges of adult males were almost completely independent. OSTFELDS (1985) argued that male territoriality is dependent on female strategy, and that male behavior should be territorial when female ranges are mutually overlapping, and non-territorial when female ranges are mutually exclusive. Adult males have been found to occupy home ranges that overlap those of females, but not those of other adult males, in marmots (ARMITAGE, 1974), house mice (FITZGERALD et al., 1981), woodrats (MACMILLEN, 1964), and voles (JANNETT, 1980, 1981; WOLFF, 1980). Our study shows that there was extensive home-range overlap between males and females during the breeding season, as well as considerable intrasexual home-range overlap between neighboring males. In summary, our study demonstrates the importance of considering temporal habitat use when interpreting spatial patterns of rat damage distribution and the home-range distribution of the ricefield rat.

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APPENDIX 1

Phenology of the rice plant (TRISTIANI et al. 1998).

The IR64 variety of rice takes about 113 days from germination to harvest and there are no significant differences in its developmental periods during the dry or wet seasons. The development is usually divided into two main categories: the growing or vegetative stage and the generative stage. The first stage (vegetative stage) lasts approximately one and half months and is characterized by: 1) germination period (±21-25 days), from when the seeds are sowed until plants are seedlings in the nursery; 2) tillering stage (±30-35 days), from when the seedlings are transplanted into the field; the number of seedlings planted per hill varies depending on the size and soil quantity of the field ; 3) maximum tillering stage (±5-7 days), when the tillers per hill have developed. The generative stage lasts approximately two months and is characterized by five developmental events: 1) initiation of the panicle primordia (±7-10 days), when the bulb of the rice plant initially develops; 2) the booting stage (±10-14 days), when maximum bulb and stalk growth take place ; 3) the flowering stage (±7-12 days), when flowers appear; 4) the milky stage (±5-8 days) , when seeds form in a milky liquid ; and, finally, 5) the ripening stage (±7-10 days), when the seeds ripen for harvest

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Rare, or simply overlooked? Practical notes for survey and monitoring of the small glow-worm *Phosphaenus hemipterus* (Coleoptera: Lampyridae)

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ABSTRACT. *Phosphaenus hemipterus* (Fourcroy, 1785) is considered a very rare glow-worm and has consequently been studied very little. This paper unites the scattered data on the known distribution of *P. hemipterus* and gives descriptions of habitat use, phenology and activity patterns at recently discovered sites in Belgium. Adult males were found from mid-June to mid-July and were most abundant on warm days, with a clear diurnal activity pattern. Only a few adult females were found, mainly around dusk and in or near crevices. Larvae are mainly nocturnal and glow spontaneously as do most lampyrid larvae, but many were also found during the day. The larvae appear to feed only on earthworms. Typical features of the habitat of *P. hemipterus* are loamy soils and abrupt transitions from dense vegetation into bare patches. Apparently many of these features are present in areas with severe human disturbance such as in gardens, parks, car parks and at field edges. However, most survey studies on glow-worms are carried out in nature reserves, which may explain why *P. hemipterus* is mostly missed. The species may actually be not as rare as presumed, and, moreover, it occurs in areas that are not considered important for conservation managment.

KEY WORDS: distribution, phenology, habitat use, diurnality, behaviour, survey studies, conservation, Lampyridae

INTRODUCTION

For over thirty years, many warnings have been given about the decline of glow-worm populations (WOOTTON, 1971; TYLER, 1982-84, 1994). Recently, survey projects for the common glow-worm *Lampyris noctiluca* L. have been started in Great Britain (TYLER, 1994) and in the Benelux (DE COCK, unpubl.) to enable assessments of the species' distribution and state of decline. In these countries another species occurs, the small glow-worm *Phosphaenus hemipterus* (Fourcroy, 1785), which has been little studied. In Great Britain *P. hemipterus* is listed as a Red Data Book species (SHIRT, 1987). Records are confined to a few localities in Sussex and Hampshire (WOOTTON, 1971). The suggestion in TYLER (1994) that *P. hemipterus* might be extinct proved to be wrong (DENTON, 1995(1996)). Although the species no longer seems to occur on the site described by DENTON (1995(1996)), it is quite likely to survive on the site from which rubble was taken to DENTON's site (TYLER, pers. com.). In Belgium the known distribution of *P. hemipterus* is extremely scattered and limited when compared to other lampyrid species (MAGIS, 1977). However, the species might be more common but simply overlooked (TYLER, 1994). One reason may be its assumed diurnal behaviour (JENNER, 1883; WEBER, 1909; AIRY-SHAW, 1961; MAGIS, 1977; TYLER 1994), with the consequence that it cannot be located by its glowing behaviour, the usual detection method for nocturnal glow-worm surveys. Secondly, females are extremely rarely found since they appear to hide in the soil or under stones (WEBER, 1909) whereas males can easily be confused with staphilinid beetles as they bear shortened wing cases. Finally, the species may just be overlooked because of its small size (< 10 mm). Furthermore, as a unique case among Lampyridae, both sexes are flightless, which restricts their dispersal and in turn may result in a more localised distribution.

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Most of the descriptions of habitat of *P. hemipterus* are obsolete and rather superficial, such as: the sunny face of a loamy potato-field (MÜLLER, 1805), a wall in a town garden (JENNER, 1883; BUTLER, 1880), pavement in a garden (MORRIS, 1893), on stones in a rock garden (AIRY-SHAW, 1961), the border of a lettuce field and gardens (WEBER, 1909), an earthen path in a park, on bare plots, a town centre, in an orchard (MAGIS, 1977), in detritus around tombstones in a churchyard (CRIBB, 1991). Information on the geographic distribution of *P. hemipterus* is also fragmented and vague. A literature survey shows that *P. hemipterus* is roughly distributed from the Iberian peninsula in the southwest to the western part of Russia in the east, and from Romania and North Italy in the south, to southern England in the northwest and Sweden, Finland and Karelia in the north (Table 1). This suggests that among glow-worm species, *P. hemipterus* has the second largest distribution in Europe after *Lampyris noctiluca* (TYLER, 1982-1984).

In order to gain a more profound insight into the opportunities for conservation of *P. hemipterus*, one should first know where and when to search for it. In this paper, I present data on the phenology, habitat use and behavioural patterns on several sites in Belgium, which arose from searches for populations of *P. hemipterus* since 1995. These data are an example of what can be collected and may be of practical use for planning survey studies, e.g. when to start studies, habitats of interest and behavioural patterns to consider.

TABLE 1

References to the occurrence of *P. hemipterus* in different countries and regions

Country, region	Authors
Belgium	JACOBSON (1911), MAGIS (1977)
Denmark	JACOBSON (1911), LUNDBERG (1995)
England	JACOBSON (1911), WOOTTON (1981), TYLER (1994), DENTON (1995(1996))
Estonia	JACOBSON (1911), HABERMAN (1960), REMM (1967), ELBERG (1989), LUNDBERG (1995)
Finland	JACOBSON (1911), LUNDBERG (1995)
France	PERRIER (1971)
Karelia (NW Russia)	JACOBSON (1911), LUNDBERG (1995)
Latvia	TELNOV (1997)
Lithuania	LUNDBERG (1995)
The Netherlands	EVERTS (1903), JACOBSON (1911), DE KEER (1930), BRAKMAN (1966)
North Italy	JACOBSON (1911)
Poland	RAZOWSKI (1991), BURAKOWSKI *et al.* (1985)
Quebec & Nova Scotia (introduced)	BOUSQUET (1991), TYLER (1994)
Saint-Petersburg gouv., Russia	JACOBSON (1911)
Spain	MAGIS (1977), JACOBSON (1911)
Sweden	JACOBSON (1911), LUNDBERG (1995), BJÖRCK (1998)
West Russia	MAMAEV *et al.* (1976)

MATERIAL AND METHODS

Following from MAGIS' (pers. comm.) initial work, searches for *P. hemipterus* populations were started in a park south of Brussels called 'Tervurenpark' in 1995 and 1996, and in the adjacent forest 'Zoniënwoud' where the species has been previously recorded (MAGIS, 1954). These areas were carefully scoured for populations of *P. hemipterus*. In 1997, the species was also discovered by chance and studied at our home campus of the University of Antwerp (U.I.A.) in Wilrijk. From experience I learned that males are best detectable along paths and walls, and while searching for new populations special attention was paid to such places. When one male was found, usually many more were detected in its vicinity.

P. hemipterus has accidentally been caught in pitfall traps (LUC CREVECOEUR, BAS DROST, BERND FRANZEN, KONRAD H. MACIEJEWSKI, pers. comm; see further). Pint glasses or plastic beakers (0.5 litre) filled with a 1 to 5% formaldehyde or glycerol solution, sunk to the rim in the soil and placed (in a row) at distances of 5 metres, will do as pitfalls. The use of pitfall traps seems to be a succesful method for a survey, but might be too drastic if the studied population is in danger of extinction as nothing is known about its effect on population dynamics. The data presented here are therefore based on simple visual counts. A promising technique to capture live specimens is to use pitfalls with drain bottoms and covered with a funnel to avoid escape.

The phenology data are based on the numbers of adult males found daily during a systematic search at the UIA campus in June and July 1997. The daily activity pattern of males was studied in detail on two different days (26.VI.1996 and 28 .VI.1996) and at two sites in the Tervuren park, by counting males several times per day. Activity was expressed as the number of individuals found per unit of searching time, in order to compare between search sessions. Air temperature at the surface and moisture were recorded immediately after each sample session with a mercury thermometer and hair hygrometer (Lufft) in 1996, and a digital thermo-hygrometer (TFA) in 1997. If captured, males were marked with a dot of correction fluid or nail varnish to avoid double counts. The behaviour of some males was also observed in captivity. Following WEBER's (1909) suggestion, females were sought under stones, clods of earth and in the soil up to 10 cm depth, under leaf litter (CHINERY, 1988), and in crevices and openings in walls. Special attention was paid to spots with high male density. During the night, larvae could easily be located by their spontaneous glowing behaviour. Since there is some uncertainty on the diurnal activity of females and larvae (WEBER, 1909; CHAPPELL, 1879-80; MORRIS, 1893), environmental light intensity was measured whenever a female or a larva was found during the day.

Since *P. hemipterus* seems to prefer loamy soils, soil samples were taken at sites where the species was found and compared with samples from sites where *L. noctiluca* was found. The proportions of sand and silt/clay fractions were determined by baking the samples for three hours at 300°C to destroy all organic matter, weighing the sample and sifting it over a sieve of 50 µm to wash out silt and clay. The remaining sand fraction was dried and weighed.

RESULTS

Phenology

Fig. 1 shows the daily abundance of adult males. Moderate numbers of males were already found from the start of the census on 16.VI.1997, but they were most abundant towards the end of June. Few males were found by the start of the second week of July. In comparison with MAGIS' (1977) study on material from Belgian collections, the maximum abundance was observed about one week later. This may be explained by the weather, which was unusually wet and cold during this period, as shown by the below-average daily temperatures from 19.VI.1997 till 07.VII.1997 (KMI, 1997). The number of males was positively correlated with temperature within the peak period of abundance, i.e. from 21.VI.1997 to 31.VI.1997 (Spearman rank correlation, $r_S = 0.68$, $t_9 = 2.9$, $P < 0.01$).

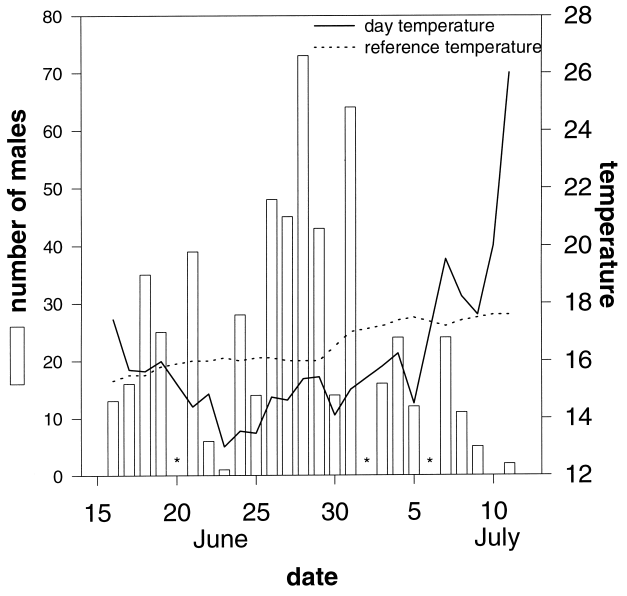

Fig. 1. – Seasonal activity pattern of *P. hemipterus* males at the UIA-campus site in 1997, with daily maximum temperatures and reference mean temperature in June calculated from data between 1921 and 1993 (KMI, 1997). * = no observations done.

Habitat use

P. hemipterus was found on three sites, the habitat of which is described below. Examples of habitats are illustrated in Fig. 2.

Tervurenpark (A): This public park, which houses the Museum of Central Africa, is situated south of Brussels. It contains a mixture of patches of beech forest, mixed forest, ponds and canals, broad strips of lawn and asphalted access roads. Between the Museum buildings and the main park there is a French garden laid out in a stair-like fashion. The soil in these parts of the park is strongly mixed, though the texture would be comparable to the adjacent parts, which have loam soils with a strongly-stained B horizon (DUDAL, 1956). This whole area was searched for *P. hemipterus*, which was found in three separate sites:

French garden (A.1; Fig. 2a, 2b): Males were found in an isolated shrubbery of the French garden. The site is surrounded by lawn and on two sides borders on hardened paths. The spot has a dense vegetation of indigenous shrubs such as hazel (*Corylus avellana*), hawthorn (*Crataegus monogyna*) and elder (*Sambucus nigra*), and exotic shrubs like snowberry (*Symphoricarpos albus*), Oregon grape (*Mahonia neubertii*), *Rhodondendron spp.* and at the borders some herbs, especially ground ivy (*Glechoma hederacea*) and stinging nettle (*Urtica dioica*) and mosses. At the edges and among the vegetation there are bare plots of crumbly loam with many cracks and holes in the soil, which contains no obvious litter layer (Fig. 2b). At less than forty metres from the site there is beech woodland. In 1995, 42 ♂♂ were observed in a four day period, and in 1996, 24 ♂♂ were marked during a similar period. Neither ♀♀ nor larvae were found here.

Car park site (A.2; Fig. 2c, 2d): This site is situated near one of the museum buildings that has a lawn in front, which in turn is surrounded by an access road, pavement and several parking lots. Next to the pavement and roads there are hedgerows (*Symphoricarpos albus*) with a dense thicket behind. Males were mostly found at the transitions of vegetation into more open areas (Fig. 2d). However, most males were found along a low, 60 metre-long wall of concrete blocks, separating a macadam road from shrub and beech woodland on a slope (Fig. 2c). A four-day search period in 1996 resulted in 142 marked ♂♂, three ♀♀ and three day-active larvae. A two-hour search on two days in 1997 resulted in 17 ♂♂ and one day-active larva. A search for larvae by night was impossible because of the confounding light of street lamps spread over the site.

Agroforestry site (A.3; Fig. 2e): This site is along an access road of the park with, on one side, plantations of young trees and on the other side a steep, grassy verge. Next to this verge lay a moist field with overgrown rubbish-heaps, a small afforestation of horse-chestnut (*Aesculus hippocastaneum*) and a somewhat bigger, mixed wood of sweet chestnut (*Castanea sativa*), common and sessile oak (*Quercus robur, Q. petraea*), locust tree (*Robinia psuedo-acacia*), hornbeam (*Carpinus betulus*), and especially maple (*Acer pseudoplatanus*). The horse-chestnut wood has a 5 to 10 centimetre thick leaf-litter layer, whereas in the mixed wood up to 60% of bare patches of loamy soil are visible between areas of the thin

litter layer. On open spots and on an overgrown path between the woodlots there are dense patches of nettles (*Urtica dioica*) and brambles (*Rubus fruticosus*). Behind these afforestations there is a beech forest. In 1995, 6 ♂♂ were found on the road. In 1996, none was seen, though in both years over 20 larvae were found in the horse-chestnut and mixed wood.

Fig. 2. – Examples of habitats for *P. hemipterus*. (a) French garden. Overview of the shrubbery. (b) French garden. Microhabitat with crumbly loam soil. (c) Car park site. Low wall of concrete blocks. (d) Car park site. Transitions of vegetation into more open areas. (e) Agroforestry site. (f) adult male of *P. hemipterus*. (photograph taken by Dr. F. Adriaensen, UIA)

The common glow-worm *L. noctiluca* also occurs in this park, the highest densities being found along verges and in vegetation on a pond shore. They were also found on clear places in the beech forests and even in or very close to *P. hemipterus* sites, but always in grassy or leaf litter habitats.

Zoniënwoud (B): This beech forest is situated south of Brussels on typical löss soils with moist loamy depressions surrounded by alfisol on the interfluvia (DUDAL & BAEYENS, 1959). Undergrowth consists of ferns in the wood and sparse vegetation along paths and forest roads. Depending on the slope the soil is completely bare or covered by a thick litter and humus layer (up to 10 cm). Some wood clearings with pasture occur throughout the forest. No males or females were discovered during a day of searching in 1995. In October 1996, 11 larvae were found glowing by night on and next to a sunken path at the forest edge near Watermael-Bosvoorde. Another two were found on the same spot in July 1997, during field work on another glow-worm *Lamprohiza splendidula*, which also occurs in these forests.

Antwerp University, U.I.A.-campus site (C): *P. hemipterus* was found by chance on the U.I.A.-campus site near Antwerp. It has never been reported before in this region. The site is situated in a closed park landscape with garden-like features. The buildings, parking lots, roads and paths are for the greater part surrounded by dense shrub woods, hedges and lawns. The terrain has been severely dug up, but formerly it had moderate to strongly gleyey sandy loam soils with a strongly stained texture B horizon (BAEYENS, 1971). Sites where the species was recorded are spread over the whole campus, but are separated by roads and paths, so that one might envisage at least five (sub)populations. These populations mostly occur at the edges of small low woods. The leaf litter layer is very thin here and more than 50% of the soil is uncovered. In June 1997, two ♀♀ were found and 159 ♂♂ were marked on 23 days. During the same period 20 day-active larvae were found. Over 20 larvae were seen glowing in wooded parts on a damp night in September. *L. noctiluca* occurs in a less managed, adjacent part of the campus where *P. hemipterus* has not been recorded yet.

Microhabitats

In all areas, the males mostly crawled on roads, paths and especially along kerbs and road borders (Fig. 2d) or at the base of walls (Fig. 2c), either in the sun or in shadow. In woodland, along hedgerows, beneath shrubs, etc., they were found on bare spots or on loamy soil with a thin leaf-litter cover (Fig. 2b). Occasionally they crawl a few centimetres high on stems, twigs or other elevations.

Only three ♀♀ were found in 1996 and another two in 1997. Three were found in front of very thin fissures (<2 mm wide) at the base of a wall, one in an excavation of a wooden log and another one on top of a fallen branch. No ♀♀ were found in the soil or under leaf litter, wood, stones or lumps.

Larvae were mostly found on leaf litter in the more wooded or densely vegetated parts of sites, but also crawling over bare moist surfaces, for example along kerb stones, patches of bare earth or at the base of walls.

Table 3 shows data on soil samples taken from sites where glow-worms have been found. *P. hemipterus* was never found on pure sand soils, whereas *L. noctiluca* was found on sandy as well as more colloidal soils. A Mann-Whitney test shows that in general soil samples from *P. hemipterus* sites contain more silt and clay than those of exclusive *L. noctiluca* sites ($P < 0.01$).

Other recent records in the Benelux

There is a recent record on 09.VI.1990 from Ekeren, north of Antwerp (K. JANSSENS, pers. comm.) where a male was found in a bathroom. In Genk (Limburg, Belgium), *P. hemipterus* has been trapped in pitfalls since 1995, on a southeast facing, steep slope between fields and the border of a canal. The stony, chalk-loamy soil shows many bare patches. At about 10 metres from the pitfall traps there is a woodlot and on top of the slope there is a path (L. CREVECOEUR, pers. comm.). Over three years more than 100 ♂♂ have been trapped at Lingedijk (area between the rivers Maas and Rijn in Gelderland, the Netherlands) in a frequently-pruned afforestation of ash (*Fraxinus excelsior*) with intermediate rows of poplar and willow, and dense hawthorn bushes at the edges. The rich clay soil grows nettles, but among the vegetation there are bare spots, which contain many crevices in summer (B. DROST, pers. comm.).

Activity pattern and behaviour

Fig. 3 shows the diurnal activity pattern of males on two days and in two parts of the car park site. On 26.VI.1996, males were most abundant in the early afternoon shortly before temperatures reached a maximum. However, the observations -particularly on the street side- suggest that male activity declined when the air became too dry. This site received more direct sunlight for a longer period, which explains the lower moisture level. On 28.VI.1996, when both temperature and moisture fluctuated less than on 26.VI.1996, male activity also fluctuated less markedly, except for a notable increase after an evening shower, which may be explained by the sudden rise in moisture at a fairly high temperature. After this peak male activity declined rapidly as dusk set in (Fig. 3).

When crawling, the males sometimes stop abruptly or climb on stems or twigs and start scanning the air by turning their heads and waving antennae, and continue in the same or a new direction. Males mostly aggregated on places where females were present or were observed shortly after. In captivity, males became inactive just after sunset and tended to hide under lumps of loam, leaf litter or in cracks in the soil until the next morning.

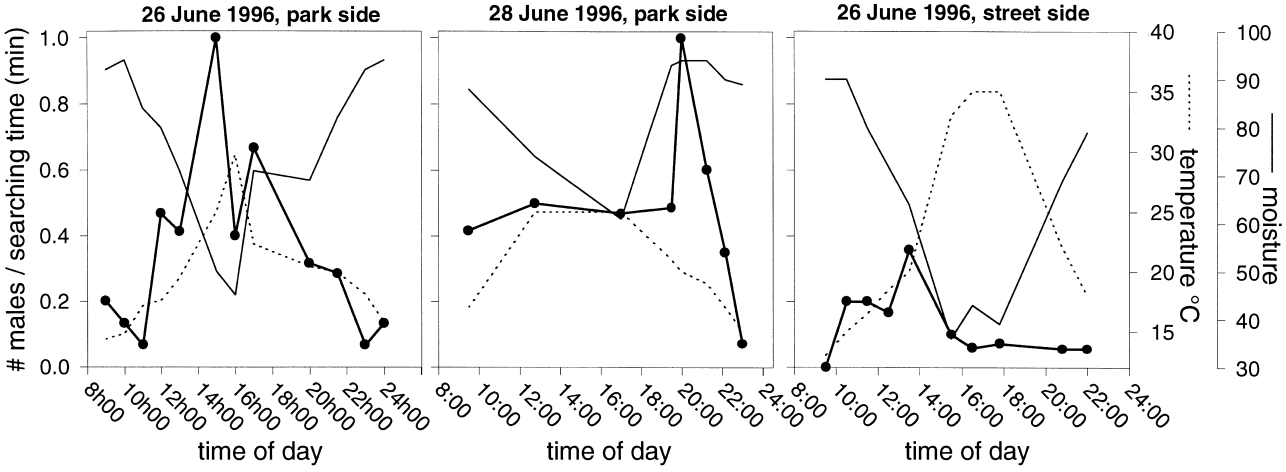

Fig. 3. – Diurnal activity patterns of male *P. hemipterus* and environmental measures at the car park site on two different days.

Two ♀♀ were found crawling at night in copula in front of very thin fissures at the basis of a wall, at respectively 23:11 p.m. (26.VI.1996; 0.5 lux) and 21:28 p.m. (27.VI.1996; 170 lux). When disturbed, they fled into these fissures. Two others were found at noon on drizzly days, in front of a wallcrack (27.VI.1996; 1000-2000 lux), and in a niche of a wooden log (25.VI.1997; 1480 lux). A fifth one was found sitting on top of a fallen branch surrounded by searching males in a dense thicket at 18:30 p.m. (28.VI.1997). Neither the females nor the males were seen glowing spontaneously. They only glowed for a few seconds when disturbed.

In captivity larvae would readily attack earthworms, but always ignored the offered snails and slugs (*Oxychilus spp., Helix rotundatus, Succinea* sp.*, Cepaea nemoralis, Deroceras* sp. and *Arion* sp.), which are however the favourite prey of other European species (Schwalb, 1961; Tyler, 1994). Also in natural conditions larvae were seen feeding on earthworms, which indicates that *P. hemipterus* specialises on this type of prey. As in other European glow-worm species, the adults do not seem to feed (Mĭ˘ski´c, 1981).

TABLE 2

The number of observations of diurnal larvae of *P. hemipterus* in relation to light intensity in lux

lux	# observations
500-1000	3
1000-2000	8
2000-3000	1
3000-4000	2
4000-5000	1
5000-6000	0
>6000	2

A final observation is that in total more than 20 larvae were found in broad daylight, up to light intensities of 10774 lux. However, Table 2 shows that most of 17 diurnal larvae appeared at environmental light intensities between 1000-2000 lux, thus in shadowy places or on cloudy days. These larvae mostly crawled very fast along borders. Most larvae have been found during the night when they glowed spontaneously.

DISCUSSION

Surveys for *P. hemipterus* should best be planned from June till the beginning of July when the adults are most abundant. Occassionally a few males can still be found in August and even September (Magis, 1977). Larvae can be found throughout the year, except of course in winter when they hibernate, probably in the soil. However, the chance to come across adult males is likely to be higher, firstly because they are day-active, secondly because they seem to be more mobile and readily move from patches where they developed as larvae, and thirdly they often aggregate when searching for a female. The data suggest that males will be most abundant on afternoons of sultry days or after a thunder-storm when it is hot and damp. If one surveys in these conditions and on places with appropriate habitats, undiscovered populations can easily be detected visually. When one is solely interested in finding this species, it would be more time consuming to put pitfalls than simply searching visually for it. On the other hand, if *P. hemipterus* occurs in an area where a pitfall study is going on, then it is very likely that males will be trapped. For a monitoring study I presume that the use of pitfalls is the most succesful if they are put in rows along walls, road borders or other such edges where males and (diurnal) larvae were frequently found. However, females are very difficult to find since they seem to hide most of the time. Their appearance does not seem to be restricted to certain parts of the day. The easiest way to locate an emerged female is by following a congregation of males. Frequently males crawl on the same spot for several days, which suggests that a female is hidden in their vicinity. However, it appears to be nearly

impossible to find such hidden females. Females of *P. hemipterus* were found at environmental light intensities ranging from 0.5 to 2000 lux, while females of other glow-worm species only start activity when light intensity dips under 1.4 lux after sunset (DREISIG, 1971). A possible non-disturbing way to monitor a known population is by counting the number of glowing larvae at night. However, we do not know yet if this larval glowing is strongly correlated with habitat type, season, activity pattern or other environmental variables that may affect this method.

P. hemipterus is a unique glow-worm species in several respects. Its most striking feature is that both sexes are flightless and that at least the male adults are diurnal. The behaviour and morphology of males, with their long, broad antennae and small eyes, and the fact that they appear to be attracted to and assemble on places where females are hiding, strongly suggest that this species uses pheromones rather than light signals in sexual communication, as in other diurnal lampyrids (LLOYD,1972; MATSUDA & OHBA, 1991). Another unique feature of *P. hemipterus* is that the larvae seem to be specialised in feeding on earthworms. The larvae of some North American *Photinus* and *Photuris* spp. also feed on earth worms, though not exclusively, and also possess more hooked mandibles, which contrast with the curved, scythe-like ones of specialised snail preators (MCDERMOTT 1964; WING, 1989; BUSCHMANN, 1984).

In spite of its remarkable features and large geographic distribution, knowledge about *P. hemipterus* remains restricted because of its assumed rarity. However, this scarceness may be the result of the interest of today's entomologists in habitats not favoured by *P. hemipterus*. Characteristic habitats for *P. hemipterus* are places with a considerable amount of human disturbance like parks, gardens, (forest)roads and paths, which are mostly encountered in (sub)urban habitats. Apparently, such places were surveyed more often in the past. Another common feature is the presence of compact, loamy or clayey soils (see Table 3). Even the Antwerp population occurs on loam, while sand is the prevalent soil type in this region. This preference can be explained by two factors. Firstly, loam is also preferred by earthworms (EDWARDS et al., 1972), which are prey to *P. hemipterus* larvae, and secondly, the colloidal nature of these soil types helps it to keep moist and crumbly with many cracks and openings, which are ideal hiding places. Another recurring feature of *P.* hemipterus sites is an abrupt transition from dense vegetation or humus layer towards open, bare terrain, e.g. pavement, roads, walls, stones, fields. The preference of *P. hemipterus* for such human designed environments suggests that the species might originate from rocky or at least partly bare, but humid habitats with sufficient vegetated patches. Other European glow-worm species usually live between the vegetation or on leaf litter in moist forests, edges of wood, grasslands, along river banks, lake shores and verges (SCHWALB, 1961; TYLER, 1994; WUNSCH, 1995). Most of these habitats are present in nature reserves, which until now have received most attention concerning glow-worm survey studies (e.g. WUNSCH, 1995). This difference in habitat use is noticeable as *P. hemipterus* and other glow-worms occur sympatrically in our study areas. It is possible that *P. hemipterus* occurs in some nature reserves, but as it has such a particular habitat preference, it is more likely to be found in areas with more disturbance. Since such habitats are plentiful, the species might be not as rare as presumed. However, this can only be ascertained with further survey studies.

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TABLE 3

Sand fractions after sifting (50 µm) and fractions of washed out silt and clay, and soil texture for soil samples from 22 sites where glow-worm species occur (LN = *Lampyris noctiluca*, LS = *Lamprohiza splendidula*, PH = *Phosphaenus hemipterus*). A, B, C refer to sites described in the text. Soil classes were determined with Bradshaw & Weaver's (1993) soil texture diagram

site	% sand	% silt/clay	soil texture	species
A.1 FrG	20	80	silt	PH
A.2a ISOa	59	41	sandy loam	PH
A.2b ISOb	54	46	(sandy) loam	LN, PH
A.3 ZW	18	82	silt	LN, PH
A. lake	51	49	sandy/silt loam	LN
A. wood	58	42	sandy loam	LN
B. Zoniënwoud	65	35	sandy loam	LS, PH
C. UIA P3	39	61	silt loam	PH
C. UIA Stele	41	59	silt loam	PH
C. UIA D	56	44	sandy loam	PH
C. UIA Anim.	55	45	sandy loam	PH
C. UIA Fort	47	53	silt loam	LN
C. UIA Home	80	20	loamy sand	LN
Wijnegem kloof	87	13	sand	LN
Wijnegem Np	87	13	sand	LN
Wijnegem oever	86	14	sand	LN
Wijnegem Vp	89	11	sand	LN
Wijnegem KAST	86	14	sand	LN
Wijnegem Bp	88	12	sand	LN
Hoboken bos	71	29	loamy sand	LN
Hoboken pad	88	12	sand	LN
Hoboken oever	96	4	sand	LN

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Rhabdocoela (Platyhelminthes) from the Weddell Sea (Antarctica) with the description of eight new species

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ABSTRACT. In this contribution we present eleven species of Rhabdocoela (Platyhelminthes) collected during the Antarktis VII/4 (Epos leg. 3) expedition. Six of them belong to the family Trigonostomidae (Typhloplanoida). Five of these are new to science: *Trigonostomum messoplanoides* n. sp., *Proxenetes trispinosus* n. sp., *Messoplana globulifera* n. sp., *M. minuta* n. sp. and *M. spiralis* n. sp. All the species can be recognised from congeneric species by the shapes of their stylets. *Ceratopera axi* (Riedl, 1954) Den Hartog, 1964 also was found and is discussed briefly. The other five species belong to the family Polycystididae (Eukalyptorhynchia). Three of them are new to science and belong to the genus *Austrorhynchus* Karling, 1952: *A. magnificoides* n. sp., *A. antarcticus* n. sp. and *A. biserratus* n. sp. They differ from other *Austrorhynchus* species in having a triangular shape of the accessory organ of the male system. Particular differences in shape of the stylet and accessory organ clearly distinguish each of the new species. Unlike the other two species, *A. antarcticus* lacks a hook on the stylet. The genus *Austrorhynchus* is briefly discussed. The collected material of *Gyratrix hermaphroditus* Ehrenberg, 1831 and *Porrocystis assimilis* (Levinsen, 1879) Karling, 1952 is also briefly discussed.

KEY WORDS: Turbellaria, Rhabdocoela, Typhloplanoida, Trigonostomidae, Eukalyptorhynchia, Polycystididae, Weddell Sea, Antarctica.

INTRODUCTION

The first thorough survey of Turbellaria from Antarctic and subantarctic waters can be found in WESTBLAD (1952). Apart from giving a history of research on South Polar Turbellaria, this author discussed and described all the Turbellaria collected in subantarctic waters during the Swedish Antarctic Expedition (1901-1903), except for the Kalyptorhynchia. This latter group was dealt with by KARLING (1952). No more work was done on Antarctic or subantarctic Turbellaria after 1952, except for the splitting of *Austrorhynchus pectatus* Karling, 1952 into different species by BRUNET (1965) and KARLING (1977). This raised the number of species in the (sub) Antarctic region from 60 to 64.

This contribution presents the rhabdocoelan Turbellaria from the southern part of the Weddell Sea collected during leg 3 of "European Polarstern Study" (12 January 1988 - 10 March 1989). Sample localities include transects off Halley Bay and Kapp Norvegia, some localities off Vestkapp and around Mount Spiess. As such it represents the first findings of Typhloplanoida and Kalyptorhynchia in real Antarctic waters. Apart from the species described, a number of other species were found of which the material is so bad that an accurate description is impossible. Some of these were species of Eukalyptorhynchia from about 2000 m deep, the greatest depth at which Kalyptorhynchia have ever been found. These specimens were collected in Halley Bay, between 74°09.5'S 029°41.4'W and 74°08.0'S 030°03.3'W with an Agassiz trawl. Preliminary results can be found in DAHMS et al. (1990).

MATERIAL AND METHODS

The samples were taken with different gear, but Turbellaria were almost exclusively found in samples

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taken with the Agassiz trawl or a multicorer. A large amount of sediment brought in with the trawls was filtered over a net of 80 µm mesh width and the animals were picked out from the remaining filtrate, still containing sediment and kept on ice.

Collected animals were first studied alive and whole mounted. The movements of the ship did not allow accurate observations at magnifications higher than 25x and made photographing impossible. Moreover, the animals are very sensitive to temperatures above 0°C. Therefore, study of the living animals had to be done very quickly. No specimens could be gathered for sectioning, except for some specimens of *Porrocystis assimilis* (Levinsen, 1879) Karling, 1952.

All hard structures (stylet, bursal appendage) are measured along their central axis.

Type material will be deposited in the collection of the Research Group Zoology of the Limburgs Universitair Centrum (LUC), Diepenbeek, Belgium.

SAMPLING LOCALITIES

The following list enumerates all the localities where the species mentioned in this paper were collected. A list of all the sampling localities of the expedition can be found in Dahms et al. (1990).

Loc.1: Kapp Norvegia. Station 223; between 71°14.2'S 012°35.9'W and 71°14.9'S 012°40.8'W; 380-384 m deep (25/01/1989).

Loc.2: Halley Bay. Station 229; between 75°14.9'S 026°12.5'W and 75°15.5'S 026°16.5'W; 500-509 m deep (29/01/1989). Very thin layer of flocullent sand with stones.

Loc.3: Halley Bay. Station 230; samples from different localities around 75°14.0'S 26°70.0'W; around 265 m deep, (30/01/1989). Very hard sediment.

Loc.4: Halley Bay. Station 234; 75°52.5'S 027°45.6'W, 416m deep, (30/01/1989).

Loc.5: Halley Bay. Station 235; 75°10.6'S 027°35.4'W; 399 m deep (31/01/1989). Very thin flocullent layer of fine sand with stones.

Loc.6: Halley Bay. Station 241; between 75°07.1'S 027°59.5'W and 75°04.7'S 028°00.4'W; 457-462 m deep (01/02/1989). Upper 2 cm very fine sand, between 2 and 7 cm sand and deeper coarse sand with pebbles.

Loc.7: Halley Bay. Station 245; between 74°39.7'S 029°41.6'W and 75°40.4'S 029°37.2'W; 483m deep, (02/02/1989). Upper 2 cm with Bryozoa, deeper medium sand with gravel.

Loc.8: Halley Bay. Station 248; between 74°39.9'S 029°31.3'W and 74°39.3'S 029°34.4'W; 599-600 m deep (03/02/1989). Less Bryozoa than in Loc. 7 with small stones, between 5 and 12 cm coarse sand. At 12 cm medium sand. Deeper stiff clay.

Loc.9: Halley Bay. Station 258; between 74°40.2'S 029°36.6'W and 74°38.9'S 029°42.6'W; 484-509 m deep (09/02/1989).

Loc.10: Vestkapp. Station 271; between 73°17.0'S 020°59.4'W and 73°16.4'S 020°54.6'W; 352-399 m deep (12/02/1989).

Loc.11: Kapp Norvegia. Station 277; between 71°40.0'S 012°35.9'W and 71°39.8'S 012°34.9'W; 405-407 m deep (16/02/1989). Small stones.

Loc.12: Kapp Norvegia. Station 284; between 71°12.0'S 013°14.0'W and 71°12.2'S 013°16.8'W; 402-412 m deep (18/02/1989).

Loc.13: Kapp Norvegia. Station 291; between 71°06.1'S 012°33.5'W and 71°05.9'S 012°34.8'W; 499-515 deep (19/02/1989).

Loc.14: Mt. Spiess. Station 312; between 54°43.9'S 000°06.3'E and 54°47.7'S 000°05.3'E; 320-471 m deep (03/03/1989).

DESCRIPTIONS

***Trigonostomum messoplanoides* n. sp.**
(Fig. 1)

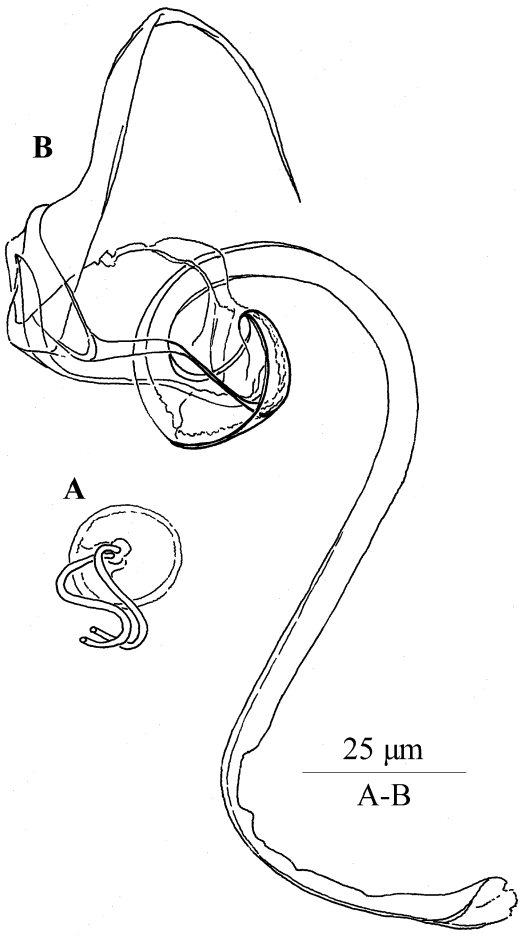

Fig. 1. – *Trigonostomum messoplanoides* n. sp. – A. Bursal appendage. – B. Copulatory organ

Type locality. Loc.13.

Material. One specimen studied alive and whole mounted (in bad condition) (holotype LUC nr. 207).

Etymology. The stylet bears a strong resemblance to the stylets found in species of the genus *Messoplana* Den Hartog, 1966.

Description

The specimen measures 0.4 mm in whole mount. The pharynx is situated in the first half of the body. The copulatory organ is characterised by a very large prostate vesicle. The stylet is about 225 µm long, tubiform, and proximally curved. A 75 µm-long flagelliform spine is attached to the proximal end of the stylet. As such, the whole stylet resembles the stylets of some *Messoplana* species more than it does that of the other *Trigonostomum* species.

The bursal appendage consists of two straight tubes that are fused at the base. It is only 22 µm long.

Discussion

Although the shape of the stylet suggests a *Messoplana* species, this animal can easily be identified as a *Trigonostomum* species by the position of the pharynx and the clearly visible triangular ventral invagination ("proboscis") in front of the brain. The latter was obvious in the living animal as well as in the whole mount.

***Proxenetes trispinosus* n. sp.**
(Fig. 2)

Type locality. Loc. 8

Material. Two specimens studied alive and whole mounted, one of them designated holotype (LUC nr. 201), the other paratype (LUC nr. 202).

Etymology. The species name refers to the three distal spines of the stylet.

Description

Pharynx approximately in the middle of the body. One pair of eyes and well-developed rhabdite tracts present.

The sclerotized parts of the copulatory organ consist of (1) the curved stylet, (2) the thin-walled mantle surrounding the proximal semicircular part of the stylet (less obvious in the paratype), and (3) three slim spines that are attached to the mantle near the proximal base of the stylet. The stylet is 20-22 µm wide proximally and 80-83 µm long, ending distally in a sharp point. The three spines measure 56, 53 and 42.5 µm long in the holotype and 52.5, 46 and 52 µm long in the paratype. At their bases, the spines are approximately 3.5 µm wide.

The bursal appendage has a thick wall showing faint striae. It is 35 µm long and consists of a basal single tube without a ring. Distally it splits into two tubes of about 20 µm. In the paratype the base measures 29 µm and the distal tubes 11.5 µm.

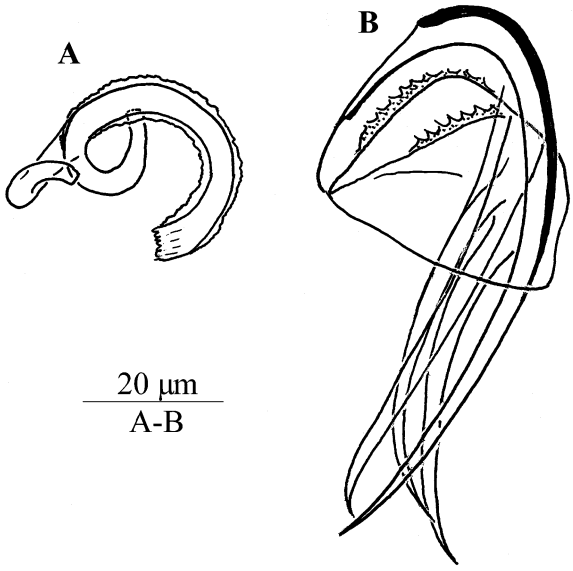

Fig. 2. – *Proxenetes trispinosus* n. sp. – A. Bursal appendage. – B. Copulatory organ

Discussion

The stylet shows some peculiarities that suggest a relationship with the species of the subgenus *Paraxenetes* Ax, 1971. Shared features are the semicircular proximal part of the stylet surrounded by the mantle, and the mantle showing a number of distal spines that reach the distal tip of the stylet (see Ax, 1971).

This new species differs from the other two species of the subgenus in the relatively short and thick distal tubes of the bursal appendage. In the other two species they are more slender and much longer than the single basal tube. In *P.* (*Paraxenetes*) *quadrispinosus* Den Hartog, 1966 the mantle around the stylet bears four short spines proximally as well as distally. The proximal spines are lacking in *P.* (*Paraxenetes*) *ampullatus* Ax, 1971 and *P.* (*Paraxenetes*) *trispinosus*. In *P. ampullatus* there is only one short distal spine, while there are three spines in *P. trispinosus*. These three spines resemble this of *P. ampullatus*, but are inserted more at the proximal end of the stylet. Moreover, the stylet is much smaller in *P. ampullatus*, being only ± 45 µm long.

***Messoplana globulifera* n. sp.**
(Fig. 3)

Type locality. Loc.2

Other localities. Loc.4, Loc.6.

Material. Three specimens were studied alive and whole mounted, one of them designated holotype (LUC nr. 203).

Etymology. The species name refers to the globular part of the bursal appendage.

Description

Animals approximately 0.6 mm in length (whole mounts) without eyes. The living specimens are opaque

grey. The adenal rhabdites are organised in two long tracts in the frontal part of the body. The pharynx is situated in the second half of the body.

The hard parts of the copulatory organ are very hard to study in the thick whole mounts. The stylet proper is 55 - 72 µm long (m = 60; n = 3), proximally bent, with a thicker convex side. The accessory spine is straight or curved, 41 – 50 µm long (m = 45; n = 3). A common basal piece connects stylet and accessory stylet. In living animals and less squeezed animals, the convex sides of the accessory and prostate stylets lie closer to each other than observed in the whole mounts.

The large seminal receptacle is connected to the common genital atrium by a muscular bursal canal. The bursal appendage is a single tube, 94 – 98 µm (m = 96; n = 2) long and about 4 µm broad. In its middle it has a ± 17 µm long swollen part, which is twice as broad as the rest of the tube. There is no ring-like structure at the base of the bursal appendage where it leaves the seminal receptacle.

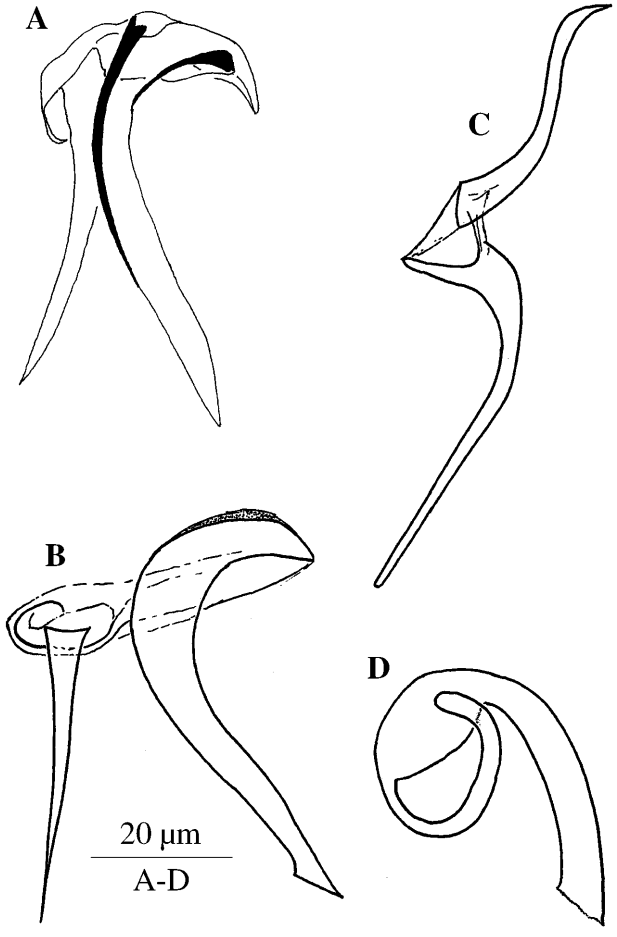

Fig. 3. – *Messoplana globulifera* n. sp. – A. Copulatory organ of a slightly squeezed living animal. - B, C. Copulatory organs from whole mounts (C. from the holotype). - D. Bursal appendage.

***Messoplana minuta* n. sp.**
(Figs 4A, 4B)

Type locality. Loc.10.

Material. Two specimens studied alive and whole mounted. One whole mount designated holotype (LUC nr. 204), the other paratype (LUC nr. 205).

Etymology. The species name refers to the very small stylet.

Description

Animals 0.3-0.4 mm long (whole mount) with two eyes that are widely separated from each other. The pharynx is situated in the middle of the animal.

The prostate vesicle is much smaller than the seminal vesicles. The stylet is rather thin-walled, slightly bent in the proximal half. The accessory spine is a thick-walled hook attached to the proximal base of the prostate stylet. The stylet is 32 µm long in both specimens; the hook is about 20 µm long.

The seminal receptacle is about the same size as the seminal vesicles. In the paratype it shows a weak constriction (not found in the holotype). The spiralled, tubiform bursal appendage, measuring 53 µm in the holotype and 58 µm in the paratype, gradually tapers towards the fecundatorium. Distally it splits into two short tubes that are 1/5 as long as the total length of the bursal appendage. The base of the bursal appendage at the seminal receptacle lacks a ring.

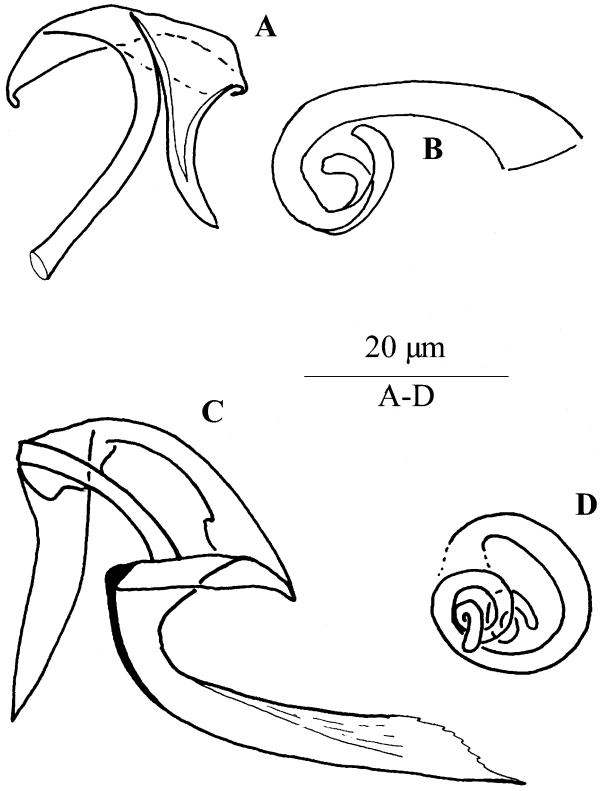

Fig. 4. – *Messoplana minuta* n. sp. – A. Copulatory organ. – B. Bursal appendage. – *Messoplana spiralis* n. sp.: C. Copulatory organ. – D. Bursal appendage.

Messoplana spiralis n. sp.
(Figs 4C, 4D)

Type locality. Loc.9.

Material. One whole mounted individual (holotype LUC nr. 206).

Etymology. The species name refers to the characteristic spiral bursal appendage.

Description

There are no observations on the living animal available because the animal was too damaged after extraction. There are no eyes.

The thin-walled stylet is 52 µm long. Proximally it bends 90°. The accessory stylet is a 27 µm long massive spine. Stylet and spine are connected by a ± 27 µm long basal part. As in *M. globulifera*, this basal part probably is very flexible, giving the stylet different appearances depending on how hard the specimen is squeezed.

The tubiform bursal appendage is strongly spiralled. It is 46 µm long. At its proximal end it is very thin-walled. Distally it splits into two tubes, which are ± 1/4 of the total length of the appendage long. There is no basal ring where the bursal appendage leaves the seminal receptacle.

Discussion

The three new species here described differ from the other *Messoplana* species by the absence of a ring at the base of the bursal appendage where it leaves the seminal receptacle. This is unique within the genus *Messoplana* and even within the Trigonostominae. This feature could be put forward as a likely synapomorphy for the three species described above.

The stylets of the three new species are all of the same basic construction: a relatively short and curved stylet with a curved hook attached to its base. The convex sides of hook and stylet face each other. This stylet construction is shared with other representatives of the genus e.g. *M. canariensis* Ehlers & Ehlers, 1980 and *M. floralis* Ehlers, 1974.

M. globulifera is unique within the genus as it has a single tubiform bursal appendage. Split bursal appendages are found in all of the other *Messoplana* species, in species of the genus *Proxenetes* and in some species of the genus *Trigonostomum*.

Ceratopera axi (Riedl, 1954) Den Hartog, 1964

Proxenetes axi Riedl, 1954

Ceratopera bifida Ehlers & Ax, 1974

New localities. Weddell Sea: Loc.9. La Réunion: Cap la Houssay, on short algae in the surf zone, 30/10/1992.

Distribution. Gulf of Naples and Sicily (Italy) (Riedl, 1954); Galapagos (Ehlers & Ax, 1974); Falkland Islands and California (Karling, 1986).

Material. Observations on living specimens and two whole mounts (one from each new locality).

Discussion

The stylet of the specimen from the Weddell Sea is 124 µm (86 µm if measured as in Ehlers & Ax, 1974). The stylet of the Réunion specimen is 105 µm (74 µm) long. The bursal appendage is not visible in the whole mounted specimen from the Weddell Sea. In the specimen of La Réunion it is 77 µm long and (apparently) distally not split. One of the differences between *C. axi* and the "*C. bifida*" mentioned by Ehlers & Ax (1974), apart from the split bursal appendage, is the length of the stylet. In *C. axi* it measured 90 µm, in *C. bifida* 70 µm. The measurements on our material lie in between. The length of the stylet in the La Réunion specimen is close to that of *C. bifida*, but the bursal appendage is not split. Karling (1986) considered *C. bifida* a junior synonym of *C. axi*. He based this conclusion on the variability in length and shape of the stylet between different populations and on the fact that the distal split in the stylet is mostly very difficult to observe. Our observations support Karling's (1986) point of view.

Austrorhynchus magnificoides n. sp.
(Figs 5A, 5B)

Type locality. Loc.12.

Other localities. Loc.14.

Material. Observations on four living animals that were all whole mounted. One of the whole mounted specimens designated holotype (LUC nr. 208), two others designated paratypes (LUC nr. 209 & 210).

Etymology. The name refers to the overall resemblance with *A. magnificus* Karling, 1952.

Description

Animals of ± 0.7 mm long, without eyes. The internal organisation as seen on living animals is identical to that of other *Austrorhynchus* species.

The sclerotized parts of the male organ consist of a prostate stylet and an accessory stylet (further called A-organ as in Karling, 1977). The prostate stylet is double-walled, with the inner stylet restricted to the tubiform part of the outer stylet. The tube is straight, almost double the length of the basal funnel. The stylet is 45-50 µm long (m = 47, n = 4). A large hook is present at the transition of funnel to tube, and is obviously a protrusion of the outer stylet. The hook is 15 – 20 µm long (m = 19, n = 4). The A-organ is a triangular plate. It is 88 -101 µm long (m = 92, n = 4) and 45 - 52 µm broad (m = 49, n = 4) at its broadest, proximal end. In this end there is a deep slit, resulting in a division of the proximal end into a pronounced style and foot (terminology of Karling, 1977, see our figure 5B). The style is connected to the foot by a rather thin bridge, leaving a "window" in the A-organ. One side of the A-organ is

combed and tapers into the flagellum, which itself is combed up to its most distal, swollen end.

***Austrorhynchus biserratus* n. sp.**
(Figs 5C, 5D)

Type locality: Loc.9.

Material: Observations on one living animal that was whole mounted (holotype LUC nr. 212).

Etymology: the species name refers to the two combed parts of the A-organ: bi (Lat.): double, serratus (Lat.): serrated.

Description

Animals of 0.6 mm long, with two eyes. The internal organisation as seen on living animals is identical to that of other *Austrorhynchus* species.

The double-walled stylet is 31 µm long. The tubiform part of the stylet is straight, a little longer than the basal

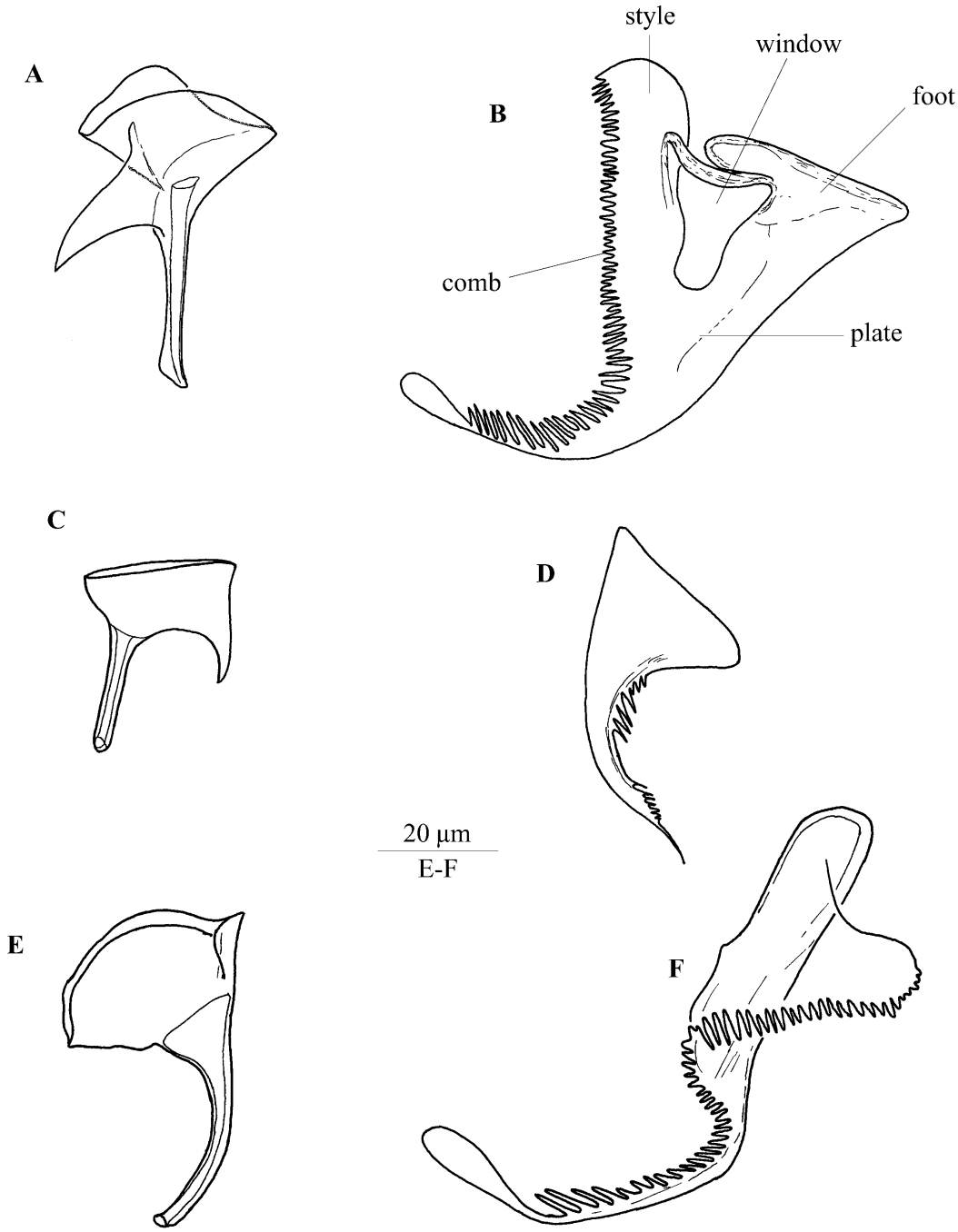

Fig. 5. – Stylets and A- organs of the *Austrorhynchus* species – A, B. *A. magnificoides* n. sp. – C, D. *A. biserratus* n. sp. – E, F. *A. antarcticus* n. sp.

funnel. Distally, the basal funnel is exceptionally broad (27 µm) bearing a 13 µm long hook. Tube and hook are widely separated from each other. The A-organ is rather small; being only 60 µm long and 32 µm broad at its broadest (proximal) end. It is a simple triangular plate, with a long curved, pointed distal end. One side has two separate combed parts, one almost in the middle, the other at the distal tip. Proximally there is no division into a style and a foot.

Austrorhynchus antarcticus n. sp.
(Figs 5E, 5F)

Type locality. Loc.1.

Material. Observations on one living animal that was whole mounted (holotype LUC nr. 211).

Etymology. The name refers to Antarctica.

Description

Animals of 0.8 mm long, without eyes. The internal organisation as seen on the living animal is identical to that of other *Austrorhynchus* species.

The double-walled stylet is 53 µm long. The slightly curved tubiform part is twice as long as the basal funnel. The internal stylet is restricted to the tubiform part. There is no hook. The shape of the A-organ is obscured by the fact that it is folded in its proximal part. It is 121 µm long. Apparently it is basically triangular in shape. The combed side tapers into the flagellum, which itself bears a comb to its most distal swollen end.

Discussion

The three species described above are the first to be found in real Antarctic waters, the ones mentioned by KARLING (1952) being from the Falklands and South Georgia, and apart from *A. magnificus* they are the first from deep (› 100m) sublittoral habitats. They differ from all of the other *Austrorhynchus* species by having a triangular A-organ with a combed side that tapers into the flagellum. As a result, a free flagellum is not seen (uncertain for *A. antarcticus*: see above). Of the three species described above, the most complicated A-organ is found in *A. magnificoides*. It is most comparable with that of *A. magnificus* Karling, 1952, but is substantially smaller (92 µm long in *A. magnificoides*, 190-220 µm in *A. magnificus* see KARLING, 1977). Moreover, in *A. magnificus* the combed side of the plate stops abruptly. This gives the A-organ a more rectangular shape and leaves the short uncombed flagellum free. Although similar in shape, the prostate stylets of *A. magnificus* and *A. magnificoides* differ greatly in dimensions. The 47 µm of *A. magnificoides* is comparable with lengths of the stylets of congeneric species, whereas the stylet of *A. magnificus* is exceptionally long as noticed by KARLING (1952) (up to 170 µm). In both species, the stylet bears a hook. This hook is relatively much longer and more heavily built in *A. magnificoides* than in *A. magnificus* (40 % of the stylet in the former species, ± 10 % in the latter).

The A-organ in *A. biserratus* is one of the most simple found in the genus, being a simple triangular plate. It is unique in having two separate combed parts on one of its sides. Furthermore, the wide separation between the tubiform part of the stylet and the hook has not been observed in any of the other *Austrorhynchus* species, where the hook is found attached to the base of the tube.

We cannot say anything definitive about the A-organ of *A. antarcticus*, but it is reminiscent of that of *A. magnificoides*. *A antarcticus* differs clearly, however, from that species in lacking a hook on the stylet. It shares this feature with three other species of the genus: *A. pectatus* Karling, 1952, *A. pacificus* Karling, 1977 and *A. galapagoensis* Artois & Schockaert, 1999. Although the A-organ of the only specimen of *A. antarcticus* is not very clear, it surely differs from that of these three species. Therefore we conclude that *A. antarcticus* is a separate taxon.

Gyratrix hermaphroditus Ehrenberg, 1831
(Fig. 6)

Locality. Loc.11.

Distribution. Cosmopolitan and euryhaline species.

Material. Observations on one living animal that was whole mounted.

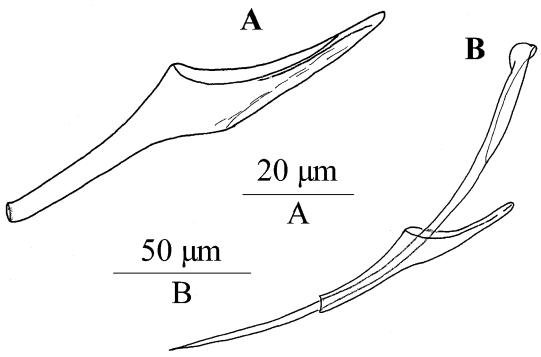

Fig. 6. – *Gyratrix hermaphroditus* Ehrenberg, 1831. – A. Sheath. – B. Stylet with sheath.

Discussion

For some time, detailed research has shown this species to be a complex of sibling species of very complicated constitution (CURINI-GALLETTI & PUCCINELLI, 1989, 1990, 1994, 1998). The stylet of the only individual found is 157 µm long. The sheath is 38 µm long with a rather short stalk of 36 µm. The stalk tapers towards its proximal tip. These dimensions lie within the range as given for the species by KARLING & SCHOCKAERT (1977). Only the stalk appears to be exceptionally short, even shorter than the sheath. Most probably, the Antarctic population represents yet another species within the complex. It is eyeless. Lack of eyes has previously been observed in only one other

marine population (Galapagos) (see ARTOIS & SCHOCKAERT, 2000).

Porrocystis assimilis (Levinsen, 1879) Karling, 1952

Localities. Loc.3. Loc.4, Loc.7, Loc.9, Loc.12, Loc.13.

Distribution. Found in extreme southern and northern waters. Gauss-station (south-east from Kerguelen), 350 m deep (REISINGER, 1926). South Georgia and the Falklands 12-30m (different localities) (KARLING, 1952). Chile, two littoral localities at the north coast of Chiloé, (MARCUS, 1954). Arctic localities: West-Greenland (Egedesminde and Jacobshaven) (LEVINSEN, 1879); Greenland: Godthaab and Godhavn (STEINBÖCK, 1932).

Material. Observations on living animals. Several whole mounts from the different Weddell Sea localities. Nine specimens serially sectioned; in very bad condition.

Discussion

The only differences from the descriptions of KARLING (1952) and MARCUS (1954) that we observed in our material are the dimensions of the hard parts in the male atrial system. The stylets of our animals measured 86-113 µm (m = 104, n = 8). This is a little larger than the measurements found in literature: 60-80 µm (KARLING 1952) and 48 µm (MARCUS 1954). The hooks in our material are 26-40 µm long (m = 30 ; n = 8) with a basal plate of 25-31 µm long (m= 27, n = 9). This is more or less the same as mentioned by KARLING (1952) (21-27 µm long basal plate with a slightly shorter hook), but longer than measured by MARCUS (1954) on the material from Chile (hook 20 µm, basal plate 11 µm).

ACKNOWLEDGEMENTS

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Femoral and tibial glands in the ant genus *Strumigenys* (Hymenoptera, Formicidae)

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ABSTRACT. The distal part of the femur and tibia of the six legs of workers, queens and males of five of the six *Strumigenys* species examined is characterized by a dorsally occurring epithelial gland. In *S. rogeri*, we could find this gland only on the midleg femur and on the hindleg femur and tibia of queens, whereas it appears absent in workers. The glandular area is externally visible as an apparently smooth oval patch with numerous minute pores with a diameter around 100 nm. Both the femoral and tibial glands are formed by a considerable thickening of the tegumental epidermis in a region where the overlaying cuticle is reduced to approximately half the thickness it has elsewhere. The glandular epithelium is formed by secretory cells with basally located rounded nuclei and with an irregular microvillar differentiation of the apical cell membrane. Their cytoplasm contains numerous secretory vesicles of variable size and electron density, and scattered free ribosomes. The function of these hitherto unknown leg glands so far remains unknown.

KEY WORDS: morphology, ultrastructure, exocrine glands, femoral glands, tibial glands, *Strumigenys*, Formicidae.

INTRODUCTION

Ants are known for the overwhelming variety of glands that make up their exocrine system (HÖLLDOBLER & WILSON, 1990; BILLEN & MORGAN, 1998). Although most attention has been given to the glands in the head, thorax and abdomen, also the legs were recently found as a possible location for exocrine glands. Among these leg glands, the pretarsal glands represent a common exocrine structure for the six legs in the various castes of all ant species (BILLEN, 1993), while other glands are only found in ants of a particular phyletic group, caste and/or leg pair, like the metatibial gland, that is characteristic for the hindlegs of workers in the doryline section (BOLTON, 1990; HÖLLDOBLER et al., 1996). The glands that may occur in a particular leg segment moreover do not need to be homologous. The tibial glands in *Crematogaster* ants, for instance, are formed by an internalized epithelium surrounding a central reservoir (LEUTHOLD, 1968; BILLEN, 1984), while a tibial gland formed by bicellular secretory units has been reported in the army ant *Dorylus molestus* Gerstaecker, 1858 (BILLEN, 1997). Still another tibial gland is the metatibial gland in ants of the doryline section, that represents a glandular modification of the ventral tegumental epidermis (HÖLLDOBLER et al., 1996). In this paper, reference is made to a personal communication by B. Bolton that also the myrmicine *Strumigenys* displays a tibial gland, although its dorsal position makes it not homologous with the ventrally occurring metatibial gland in the doryline section. The present contribution deals with the morphology and ultrastructure of this tibial gland and of a structurally similar but hitherto unknown femoral gland in *Strumigenys*.

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MATERIAL AND METHODS

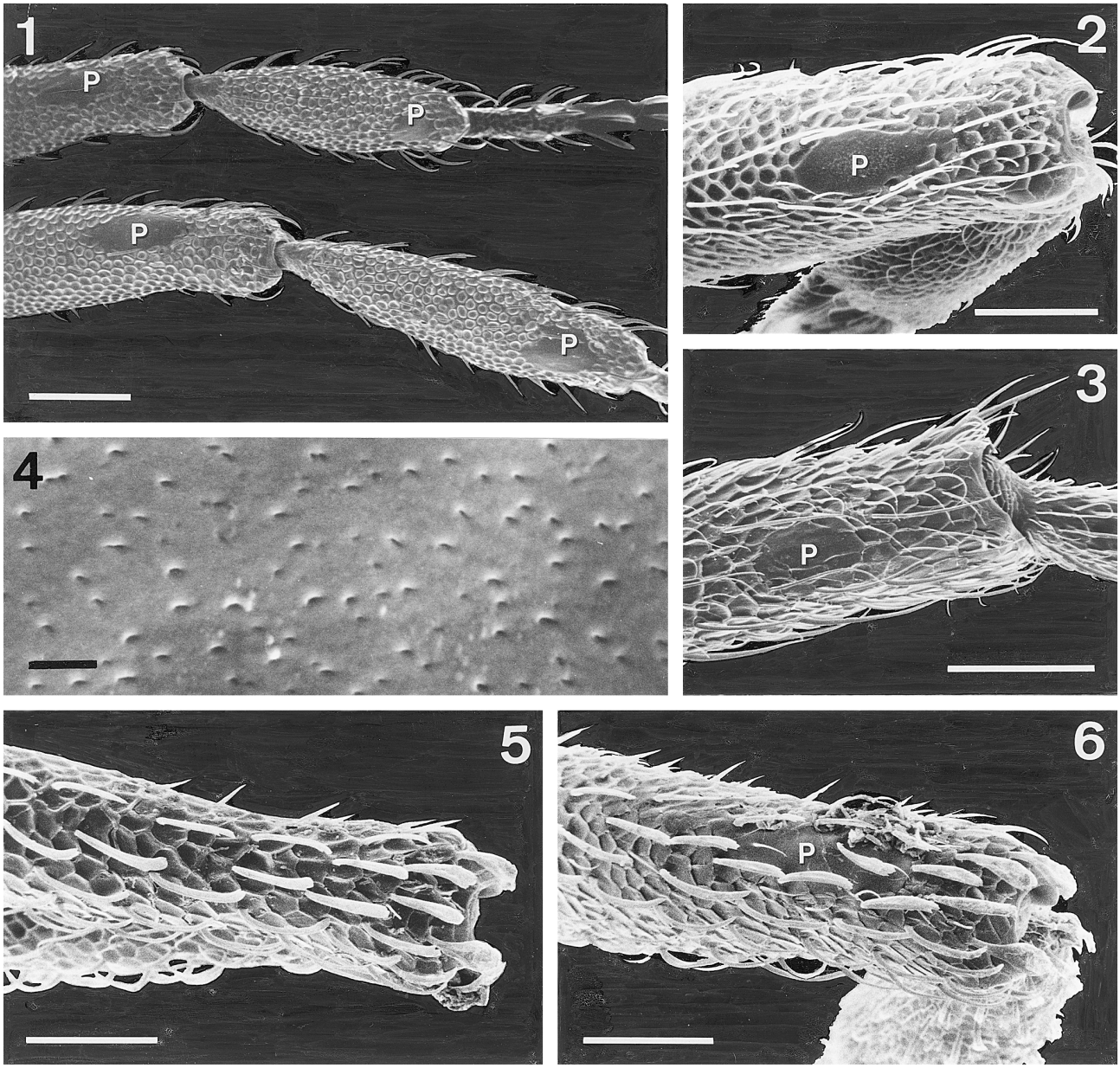
Strumigenys lewisi Cameron, 1886 was collected in Takamatsu, Japan. Colonies of the other species for this study were collected in Indonesia and brought to Belgium for further morphological work. Colonies of *S. koningsbergeri* Forel, 1905 were collected at Mt. Sarak, Bogor, *S. nanzanensis* Lin & Wu, 1996 in Ujung Kulon, and *S. rogeri* Emery, 1890, *S. signeae* Forel, 1905 and *Strumigenys* sp. (colony code FI97-586, belonging to the *S. lyroessa* group) in Kebun Raya. Voucher specimens of

the Indonesian species have been deposited in the Bogor Zoological Museum and in the London Natural History Museum.

Leg parts were fixed in 2% cold glutaraldehyde buffered with 50 mM Na-cacodylate and 150 mM saccharose and postfixed in 2% osmium tetroxide. After dehydration in a graded acetone series and embedding in Araldite, they were sectioned with a Reichert Ultracut E microtome. Semi-thin sections for light microscopy were stained with methylene blue and thionin, double stained thin sections were viewed in a Zeiss EM900 electron microscope. Air-dried specimens for scanning microscopy were coated with gold and observed with a Philips SEM 515 microscope.

RESULTS

Near the distal part of both the femur and tibia of the six legs of the workers, queens and males of the *Strumigenys* species studied, we found an apparently smooth dorsal patch amidst the hairy leg sculpturation (Figs 1-3). Only in *S. rogeri*, the presence of this structure is considerably



Figs 1-6. – Scanning electron micrographs illustrating the position of the dorsally occurring smooth cuticular patches (P) overlying the epithelial glands in femur and tibia. – 1. Mid- and hindleg femora and tibiae of a queen of *Strumigenys* sp. FI97-586, scale bar 100 µm. – 2. Foreleg femur of *S. lewisi* queen, scale bar 50 µm. – 3. Hindleg tibia of *S. lewisi* male, scale bar 50 µm. – 4. Detail of cuticular pores in smooth patch of midleg femur of *S.* sp. FI97-586 worker, scale bar 1 µm. – 5. Distal part of hindleg tibia in worker of *S. rogeri* showing absence of smooth patch, scale bar 50 µm. – 6. Same region of hindleg tibia in queen of *S. rogeri* showing presence of smooth patch, scale bar 50 µm.

more restricted as we only found it on the midleg femur and on the hindleg femur and tibia of the queen. It could not be found on the legs of *S. rogeri* workers (Figs. 5,6 - males of this species were not available for examination).

On the femur, the patch occurs at approx. 50 µm from the articulation with the tibia, and measures approx. 25 x 15 µm in the worker and 50 x 25 µm in the queen. The hindleg femoral patch is larger, measuring 35 x 17 µm in the worker and 80 x 25 µm in the queen. On the tibia, it occurs approx. 20 to 30 µm from the articulation with the basitarsus, and for the three leg pairs measures approx. 25 x 15 µm in the worker and 30 x 15 µm in the queen. At high magnification, these patches show very numerous pores with a diameter of approx. 100 nm and a pore density between 1.5 and 2.5 pores/μm^2 for both the femoral and tibial patches (Fig. 4).

The glandular epithelium in both the femur and tibia reaches a thickness of 7 to 10 µm, whereas the non-glandular epidermal lining of the leg tegument has a thickness of less than 1 µm. In the region of the glandular epithelium, the overlaying cuticle is reduced to a thickness of 3 to 5 µm, which is approximately half the thickness it has elsewhere in the leg (Figs 7-10). In this part overlaying the gland, numerous irregular channels traverse the cuticle and open at the exterior surface through minute pores (Figs 4, 9, 11). Due to the small size and high number of these pores, the cuticular surface that corresponds with each secretory cell shows approx. 100 such pores. The secretory cells have basally located rounded nuclei with a diameter of 1.5 to 3 µm, and display an irregular microvillar differentiation of their apical cell membrane (Figs 9, 11). Their cytoplasm is characterized by numerous rounded secretory vesicles with a diameter ranging from 0.1 to 2 µm. Vesicles of variable electron density occur, and become confluent with the intermicrovillar spaces apically (Fig. 9). Numerous scattered free ribosomes and few mitochondria occur (Fig. 12), while endoplasmic reticulum and other cytoplasmic organelles are scarce.

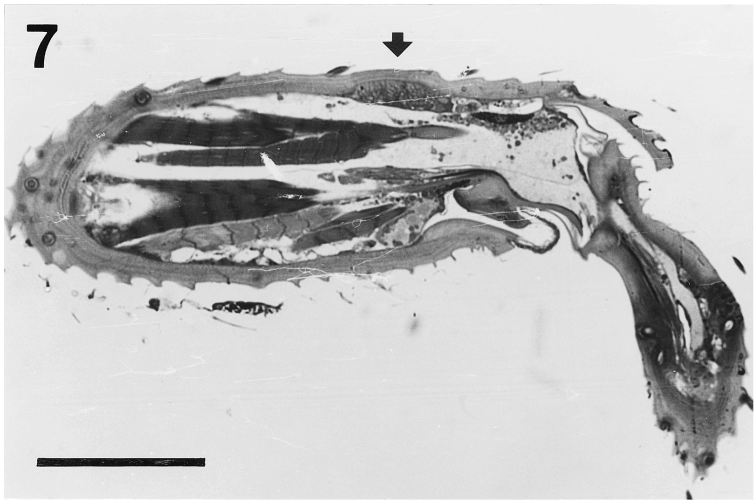
DISCUSSION

The presence of exocrine glands in the tibia is known for a number of ants, and even comprises various glands with different structural organization and/or location within the tibia. The epithelial gland that is here described on the distal part of the tibia in several *Strumigenys* species, however, is not homologous to any of the other known tibial glands in ants. It is structurally most similar to the metatibial gland that is characteristic for the hindlegs ants of the doryline section (HÖLLDOBLER et al., 1996), although the gland in the myrmicine *Strumigenys* species occurs in a dorsal instead of a ventral position. It moreover occurs in the three leg pairs and can be found in workers, queens and males. Tibial glands with externally visible pore plates have also been reported for the six legs of some rhinotermitid termites (BACCHUS, 1979), but these larger and less numerous pores each represent the opening of an individual duct cell and associated secretory cell. The much smaller pores of *Strumigenys*, however, represent cuticular channels, and therefore do not have any relationship with the number of secretory cells.

The femoral glands of *Strumigenys* appear as the structural homologues of the tibial glands with a similar occurrence in the femur of all legs and in all castes. They also represent the first example of an exocrine gland in the femur of ants. The occurrence of glands in the femur was recently reported for some meliponine bees, although these are some scattered unicellular glands occurring in the three castes as well as an internal sac-like gland that is only found in queens (CRUZ LANDIM et al., 1998).

The presence of these femoral and tibial glands is a phylogenetically useful feature, as it represents a synapomorphy of all the genera in the *Strumigenys*-group (subtribe Strumigenyiti), while the glands are absent from all other groups of Dacetonini. In workers of some *rogeri*-group species the gland is obvious but in others, including *S. rogeri* itself and some other African members of the group, it is vestigial or even absent. Despite this, the presence of the glands is an apomorphy of the strumigenyiform clade of genera (BOLTON, 1999). Subsequent reduction or loss of the gland has occurred in a number of individual species and a few entire species-groups, both in *Strumigenys* and its sister genus, *Pyramica*.

The structural organization of both the tibial and femoral glands is a clear example of the epithelial type (class 1 secretory cells according to the classification of NOIROT & QUENNEDEY, 1974). Compared to other epithelial glands, these leg glands in *Strumigenys* are fairly small, both in terms of external surface and gland volume. Cell height hardly reaches 10 µm, whereas the metatibial gland epithelium e.g. may have a thickness of up to 80 µm (HÖLLDOBLER et al., 1996). The apparently poor occurrence of endoplasmic reticulum and other cytoplasmic organelles make it difficult to judge about the metabolic potential of these leg glands, although the obvious presence of vesicles and their structural association with the apical microvilli should be indicative for the secretory activity. Also the presence of hundreds of transcuticular channels that open as minute pores at the surface is in agreement with an effective mechanism for release of the secretory products to the outside. The chemical composition of the secretion and its function so far remain highly speculative. The general presence of the gland on the six legs in the three castes may make a pheromonal function less probable. Also a function as lubricant, that is often postulated for glands with unknown function, seems not very likely for these tibial and femoral glands considering their position that is not directly associated with any articulation where such smearing function may be more meaningful. The high number of ribosomes scattered in the cytoplasm may indicate the secretion has a protein fraction, although further research will be needed to find out about the function of these peculiar glands in *Strumigenys* ants.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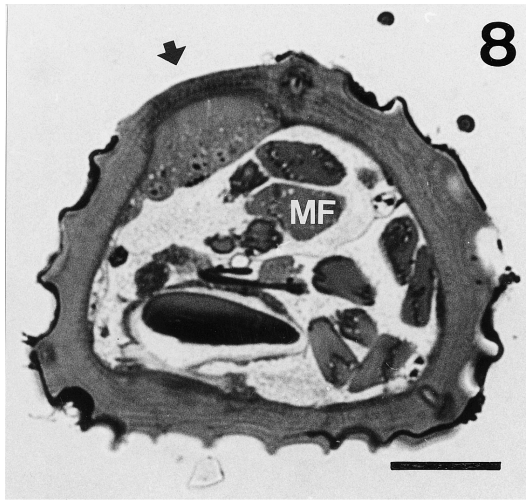
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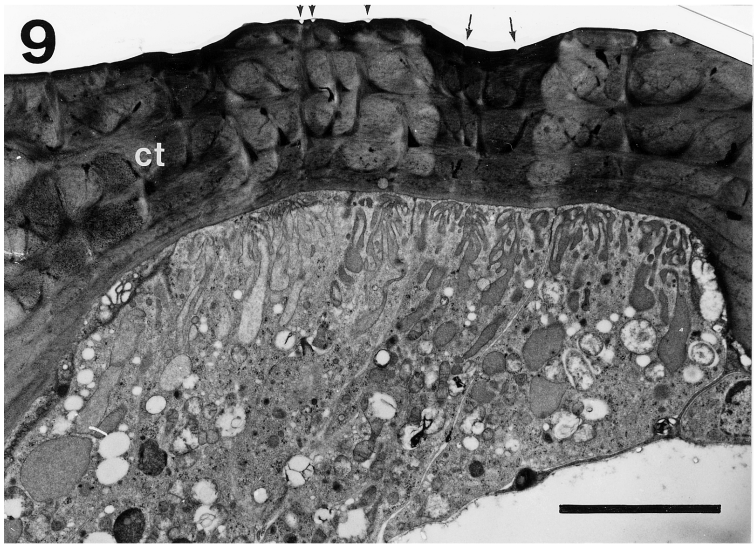
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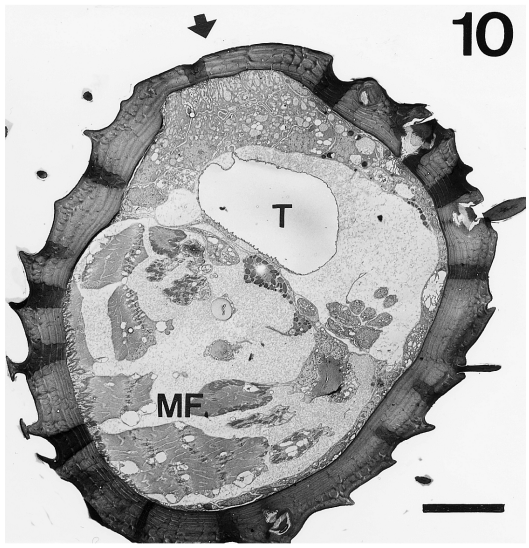
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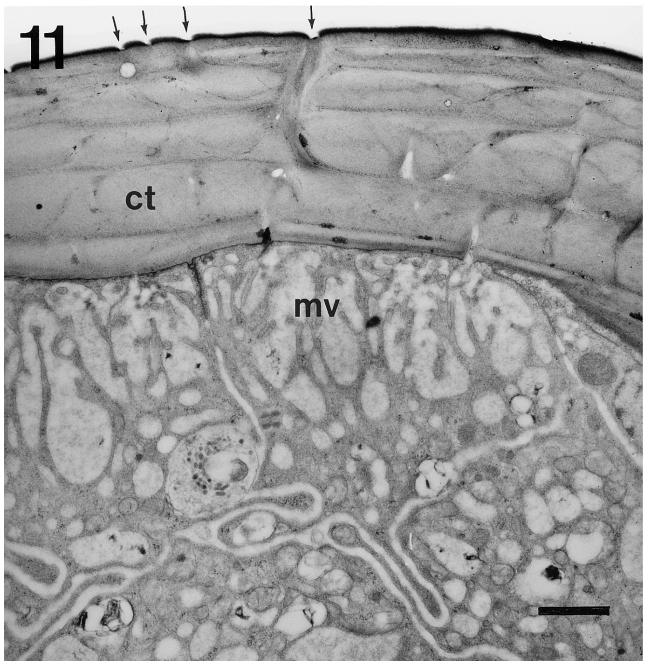
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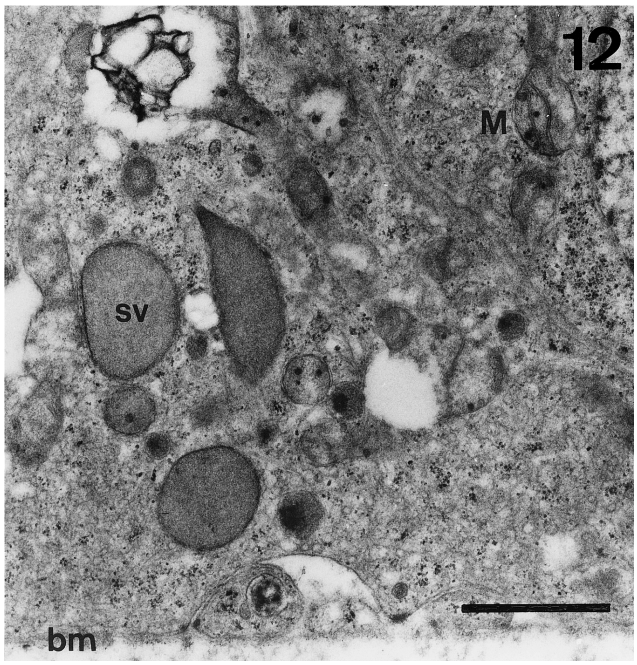
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Legends to the figures (see opposite page)

Fig. 7. – Longitudinal semithin section through midleg tibia and basitarsus of queen of *Strumigenys nanzanensis*, showing dorsal tibial gland epithelium (arrow). Scale bar 50 µm.

Fig. 8. – Transverse semithin section through distal part of hindleg femur of *S. koningsbergeri* worker with dorsal femoral gland (arrow). MF = muscle fibres, scale bar 10 µm.

Fig. 9. – Electron micrograph of epithelial gland in hindleg femur of *S.* sp. FI97-586 worker. Note irregular vertically orientated transcuticular channels and their opening at surface via small pores (arrows). ct = cuticle, scale bar 5 µm.

Fig. 10. – Electron micrograph showing transverse section through distal part of hindleg tibia of *S. nanzanensis* queen with occurrence of epithelial gland (arrow). MF = muscle fibres, T = trachea, scale bar 10 µm.

Fig. 11. – Detail of cuticle (ct) and apical cytoplasm with irregular microvilli (mv) in epithelial gland of hindleg tibia of *S. nanzanensis* queen. Note pores opening on cuticular exterior side (arrows), scale bar 1 µm.

Fig. 12. – Detail of cytoplasm in basal region of glandular epithelium in midleg femur of *S.* sp. FI97-586 worker. bm = basement membrane, M = mitochondria, sv = secretory vesicle, scale bar 1 µm.

Belg. J. Zool., 130 (1): 117-130 January 2000

On the anatomy and function of the cephalic structures in *Phractura* (Siluriformes: Amphiliidae), with comments on some striking homoplasies occurring between the Doumeinae and some loricaroid catfishes

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ABSTRACT. The morphology and function of the cephalic structures related to the feeding mechanism – movements of the mouth, suspensorium, opercular series, hyoid arch, maxillary barbels and mandibular barbels – was studied in two catfish species of the genus *Phractura* (Amphiliidae: Doumeinae), *P. intermedia* and *P. brevicauda*. For comparison, other species of the family Amphiliidae, as well as a large number of other catfishes, were also studied. The morpho-functional analysis pointed out that *Phractura*, as well as the other doumeins, present several unusual morphological modifications, which are very likely related to two main functional specialisations: the ability to attach the body to the substrate with an oral sucker, and the ability to scrape this substrate. The comparison with other siluriforms revealed an impressive number of morpho-functional homoplasies occurring between the African doumeins and the South-American callichthyids, scoloplacids, astroblepids and loricariids.

KEY WORDS: Amphiliidae, catfish, Doumeinae, ecomorphology, evolution, feeding mechanisms, functional morphology, homoplasies, *Phractura*, Siluriformes.

INTRODUCTION

The Siluriformes, with their already described 2584 species, represent about 32% of all freshwater fishes (TEUGELS, 1996). They are "one of the economically important groups of fresh and brackish water fishes in the world: in many countries, they form a significant part of inland fisheries; several species have been introduced in fish culture; numerous species are of interest to the aquarium industry where they represent a substantial portion of the world trade" (TEUGELS, 1996: 10).

Among the 34 siluriform families, the African family Amphiliidae – which is subdivided in two subfamilies, Amphiliinae (*Amphilius* and *Paramphilius*) and Doumeinae (*Andersonia*, *Belonoglanis*, *Doumea*, *Phractura* and *Trachyglanis*) –, is surely one of the less studied (HE, 1997). Despite the large number of papers concerning catfish anatomy (REGAN, 1911; KINDRED, 1919; ALEXANDER, 1965; GAUBA, 1966; 1970; JAYARAM, 1966; 1970; CHARDON, 1968; GOSLINE, 1975; HOWES, 1983ab; 1985; JAYARAM & SINGH, 1984; LUNDBERG & MCDADE, 1986; FAGADE, 1980; ARRATIA, 1987; 1990; 1992; BORNBUSCH, 1991a,b; MO, 1991; DE VOS, 1995; ADRIAENS & VERRAES, 1994; 1997a,b,c; DIOGO et al., 1999b; etc.), the only ones in which the morphology of amphiliids is described with some detail are those of REGAN (1911), HARRY (1953), CHARDON (1968), SKELTON (1986), SKELTON et al. (1984), HE (1997), DIOGO & CHARDON (1998; 1999; in press-a,b) and DIOGO et al. (1999a). Moreover, as these descriptions are restricted to the osteology and external aspect of these fishes, their myology, arthrology and functional morphology are unknown. This complicates any attempt to study the phylogenetic relationships between the amphiliid genera and also between the different catfish families.

The aim of this study is to describe the anatomy (osteology, myology and arthrology) and function of the cephalic structures, particularly those related to the feeding mechanism (movements of the mouth, suspensorium, opercular series, hyoid arch, maxillary barbels and mandibular barbels), in the amphiliid *Phractura*, in order

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to pave the way for further anatomical, functional, ecological, ethological and phylogenetical studies on amphiliids, as well as to improve our global knowledge on catfish biology.

MATERIAL AND METHODS

The specimens studied (Table I) are from the private collection of the Laboratory of Functional and Evolutionary Morphology of Liège (LFEM), from the "Musée Royal de l'Afrique Centrale" of Tervuren (MRAC), from the "Université Nationale du Bénin" (UNB), from the "Muséum National D'Histoire Naturelle" of Paris (MNHN) and from the National Museum of Natural History of Washington (USNM). Anatomical descriptions are made after dissection of alcohol fixed or trypsin-cleared and alizarine-stained (following Taylor & Van Dike's 1985 method) specimens. Dissections and morphological drawings were made using a Wild M5 dissecting microscope equipped with a camera lucida. Functional hypotheses are based on anatomical evidence, biomechanical principles and manipulation of the dissected specimens.

TABLE 1

Specimens studied in the present work, their mode of conservation and their provenience (LFEM: private collection of the Laboratory of Functional and Evolutionary Morphology, Liège; MNHN: "Muséum National D'Histoire Naturelle", Paris; MRAC: "Musée Royal de l'Afrique Centrale", Tervuren; UNB: "Université Nationale du Bénin", Cotonou; USNM: National Museum of Natural History, Washington).

Amphiliidae	*Andersonia leptura* Boulenger 1900	Alcohol	MNHN 1961-0600
	Amphilius brevis Boulenger, 1902	Alcohol	MRAC 89-043-P-403
	Belonoglanis tenuis Boulenger 1902	Alcohol	MRAC P.60494
	Paramphilius trichomycteroides Pellegrin, 1917	Alcohol	LFEM
	Doumea typica Sauvage, 1879	Alcohol	MRAC 93-052-p-152
	Phractura brevicauda Boulenger, 1911	Alcohol	MRAC 90-057-P-5145
	Phractura brevicauda Boulenger, 1911	Alizarine	MRAC 92-125-P-386
	Phractura intermedia Boulenger, 1911	Alcohol	MRAC 73-016-P-5888
	Trachyglanis minutus Boulenger 1902	Alcohol	LFE
Ariidae	*Arius gigas* Boulenger, 1891	Alcohol	LFEM
Austroglanidinae	*Austroglanis sclateri* Boulenger, 1901	Alcohol	LFEM
Bagridae	*Bagrus docmak* Forsskall, 1775	Alcohol	MRAC 86-07-P-512
	Hemibagrus wickii Bleeker, 1858	Alcohol	LFEM
	Pseudomystus bicolor Fowler, 1934	Alcohol	LFEM
Callichthyidae	*Callichthys callichthys* Linnaeus, 1758	Alcohol	LFEM
Clariidae	*Clarias gariepius* Burchell, 1822	Alcohol	MRAC 93-152-P-1356
	Heterobranchus longifilis Valenciennes, 1840	Alcohol	LFEM
Claroteidae	*Auchenoglanis occidentalis* Cuvier & Valenciennes, 1840	Alcohol	MRAC 73-010-P-3536
	Chrysichthys auratus Geoffroy Saint-Hilaire, 1809	Alcohol	UNB
	Clarotes laticeps Rüppell, 1829	Alcohol	MRAC 73-13-P-980
	Liauchenoglanis thomasi Boulenger, 1916	Alcohol	MRAC 73-010-P-3540
	Parauchenoglanis ansorgii Boulenger, 1911	Alcohol	LFEM
Diplomystidae	*Dyplomystes chilensis* Molina, 1782	Alcohol	LFEM
Loricariidae	*Lithoxus fimbriatus*, Steidachner 1917	Alcohol	LFEM
Plotosidae	*Plotosus lineatus* Thunberg, 1787	Alizarine	USNM 200226
Pimelodidae	*Pimelodella serrata* Eigenmann, 1917	Alcohol	LFEM
Shilbeidae	*Shilbe intermedius* Rüppell, 1832	Alcohol	MRAC 90-30-P-24
Trichomycteridae	*Trichomycterus guianensis* Eigenman, 1909	Alcohol	LFEM

LIST OF ABBREVIATIONS

af-	articulatory facet …
af-I	a.f. neurocranium-autopalatinum
af-II	a.f. neurocranium-hyomandibulare
af-III	a.f. anguloarticulare-quadratum
af-IV	a.f. operculare-hyomandibulare
af-V	a.f. interoperculare-ceratohyale
afo	anterior fontanel
c-	cartilago …
c-apal-a	c. autopalatinus anterior
c-Meck-ho	c. Meckeli: horizontal portion
c-ex-mnd-b-mp	c. externus mandibularis tentaculi: mobile part
c-ex-mnd-b-sp	c. externus mandibularis tentaculi: supporting part
fr-V-VII	trigemino-facialis foramen
l-	ligamentum …
l-an-ch	l. angulo-ceratohyale
l-an-iop	l. angulo-interoperculare
l-ent-vm	l. entopterygoideo-vomerale
l-meth-apal	l. mesethmoideo-autopalatinum

l-meth-prmx	l. mesethmoideo-praemaxillare
l-prmx-apal	l. praemaxillo-autopalatinum
l-prmx-mx	l. praemaxillo-maxillare
l-puh-hh	l. parurohyalo-hypohyale
l-q-prmx	l. quadrato-praeomaxillare
m-	musculus …
m-A1-OST-1	m. adductor mandibulae A1 (Ostariophysi): section 1
m-A1-OST-2	m. adductor mandibulae A1 (Ostariophysi): section 2
m-A2	m. adductor mandibulae A2
m-A3	m. adductor mandibulae A3
m-ad-mnd	m. adductor mandibulae
m-ad-ap	m. adductor arcus palatini
m-ad-op	m. adductor operculi
m-dil-op	m. dilatator operculi
m-dp-in-mnd-b	m. depressor interni mandibularis tentaculi
m-ex-t	m. extensor tentaculi
m-ex-t-1	m. extensor tentaculi: section 1
m-ex-t-2	m. extensor tentaculi: section 2
m-ex-t-3	m. extensor tentaculi: section 3
m-hh-inf	m. hyohyoideus inferior
m-intt	m. intertentacularis
m-l-ap	m. levator arcus palatini
m-l-op	m. levator operculi
m-obl-inf	m. obliquus inferioris
m-pr-h	m. protractor hyoidei
m-pr-h-l	m. protractor hyoidei pars lateralis
m-pr-h-v	m. protractor hyoidei pars ventralis
m-pr-ex-mnd-b	m. protractor externi mandibularis tentaculi
m-re-ex-mnd-b	m. retractor externi mandibularis tentaculi
m-re-in-mnd-b	m. retractor interni mandibularis tentaculi
m-re-t	m. retractor tentaculi
m-sh	m. sternohyoideus
mnd	mandible
mnd-b-ex	external mandibular barbel
o-	os …
o-ang-art	o. angulo-articulare
o-apal	o. autopalatinum
o-boc	o. basioccipitale
o-ch-a	o. ceratohyale anterior
o-ch-p	o. ceratohyale posterior
o-cl	o. cleithrum
o-com	o. coronomeckelium
o-den	o. dentale
o-ent	o. entopterygoideum
o-eoc	o. exoccipitale
o-epot	o. epioticum
o-fr	o. frontale
o-hh	o hypohyale
o-hm	o. hyomandibulare
o-iop	o. interoperculare
o-leth	o. latero-ethmoideum
o-meth	o. mesethmoideum
o-mp	o. metapterygoideum
o-mx	o. maxillare
o-op	o. operculare
o-osph	o. orbitosphenoideum
o-para	o. parasphenoideum
o-pop	o. praeoperculare
o-post-scl	o. posttemporo-supracleithrum
o-prmx	o. praemaxillare
o-prot	o. prooticum
o-psph	o. pterosphenoideum
o-pt	o. pteroticum
o-puh	o. parurohyale
o-q	o. quadratum
o-soc	o. supraoccipitale
o-soc-pp	o. supraoccipitale: posterior process
o-sph	o. sphenoticum
o-vm	o. vomerale
pap	papillae
pfo	posterior fontanel
r-br-VI	branchiostegal ray VI

RESULTS

With the exception of the external aspect, the differences between the two species of *Phractura* (Boulenger, 1900) studied, *P. brevicauda* (Boulenger, 1911) and *P. intermedia* (Boulenger, 1911), are not significant. Therefore, our description will be based on *P. brevicauda*. The visual information given in the figures have the preponderance over the textual one, and, thus, this latter will be brief.

Osteology

The nomenclature for the osteological structures basically follows that used by DAGET (1964).

Os mesethmoideum. Unpaired (Figs 1, 2, 3). It is connected to the palatine and to the premaxillary by two small ligaments (Fig. 4).

Os lateroethmoideum. Paired. With a lateral articulatory facet for the palatine (Figs 1, 3).

Os vomerale. Unpaired. In the shape of T, but with very small antero-lateral processes (Fig. 3).

Os orbitosphenoideum. Paired. Posterior to the lateral ethmoid (Figs 1, 3, 5).

Os parasphenoideum. Unpaired. The longest bone of the cranium (Fig. 3).

Os pterosphenoideum. Paired, posterior to the orbitosphenoid (Figs 1, 3).

Os sphenoticum. Paired. It presents, together with the pterotic, an articulatory facet for the hyomandibula (Figs 1, 3).

Os pteroticum. Paired. Well-developed bone associated posteriorly to the posttemporo-supracleithrum (Figs 1, 2, 3).

Os prooticum. Paired. The foramen of the trigemino-facial nerve complex is situated between this bone, the pterosphenoid and the parasphenoid (Fig. 3).

Os epioticum. Paired. Well-developed, situated on the posterior surface of the neurocranium (Figs 1, 3).

Os exoccipitale. Paired. Situated laterally to the basioccipital (Fig. 3).

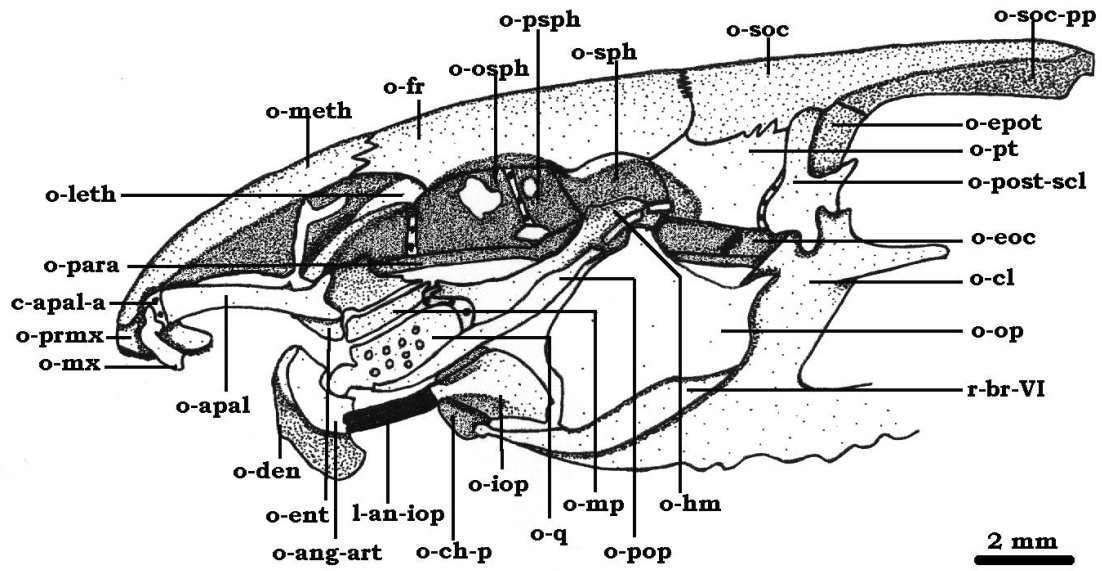

Fig. 1. – Lateral view of the skull of *Phractura brevicauda*. Infraorbital series removed.

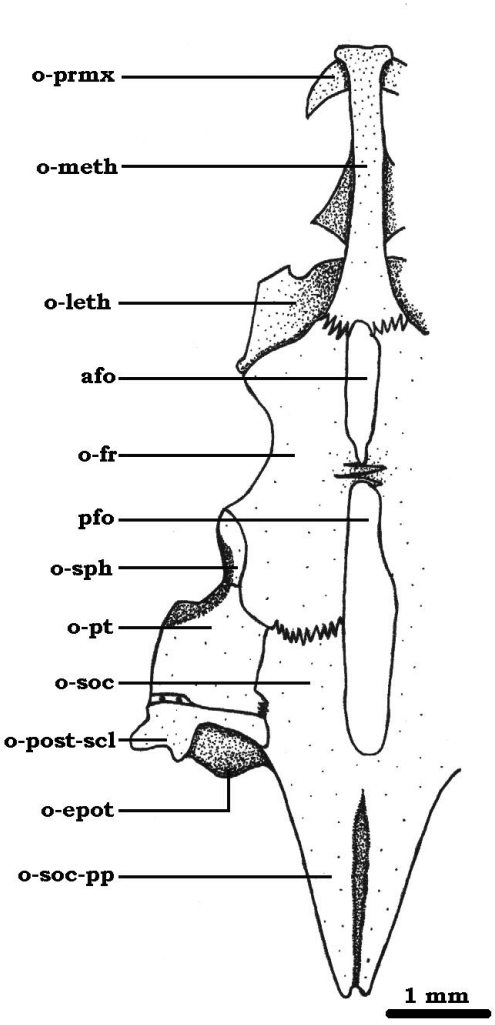

Fig. 2. – Dorsal view of the neurocranium of *Phractura brevicauda*.

Os basioccipitale. Unpaired. It presents two well-developed ventro-lateral processes associated by means of a thick ligamentous tissue with the ventro-medial limbs of the posttemporo-supracleithrum (Fig. 3). It also presents two postero-ventral processes, which join to similar processes of the first vertebra (Fig. 3).

Os frontale. Paired. The two frontals are largely separated by the well-developed anterior and posterior fontanels, and suture with each other medially only halfway along their length *via* a bony bridge, which separates the fontanels (Fig. 2).

Os supraoccipitale. Unpaired. Large bone with a long posterior process (Figs 1, 2). The posterior fontanel extends on its anterior surface (Fig. 2).

Os posttemporo-supracleithrum. Paired. Its dorso-mesial limb is firmly connected with the pterotic, epiotic and supraoccipital. The extrascapular, normally associated with this limb, is missing (Figs 1, 3). Its thin ventro-mesial limb is firmly attached to the basiocccipital by a strong and short ligament (Fig. 3). Its ventro-lateral limb is forked, forming an articulating groove for the upper edge of the cleithrum (Fig. 1). A prominent posterior process (Figs 1, 2) is present on the postero-dorsal surface of the posttemporo-supracleithrum.

Os operculare. Paired. Broad bone (Figs 1, 6 A) antero-dorsally articulated with the hyomandibular by means of two articulatory surfaces (Fig. 6 A). Its ventral edge is quite far from the suspensorial bones (Fig. 1).

Os interoperculare. Paired. It presents a well-developed mesial articulatory surface for the posterior ceratohyal (Figs 6 C, 7). Its fore end is connected to the back of the mandible by a long, strong ligament (Figs 1, 6 C, 7).

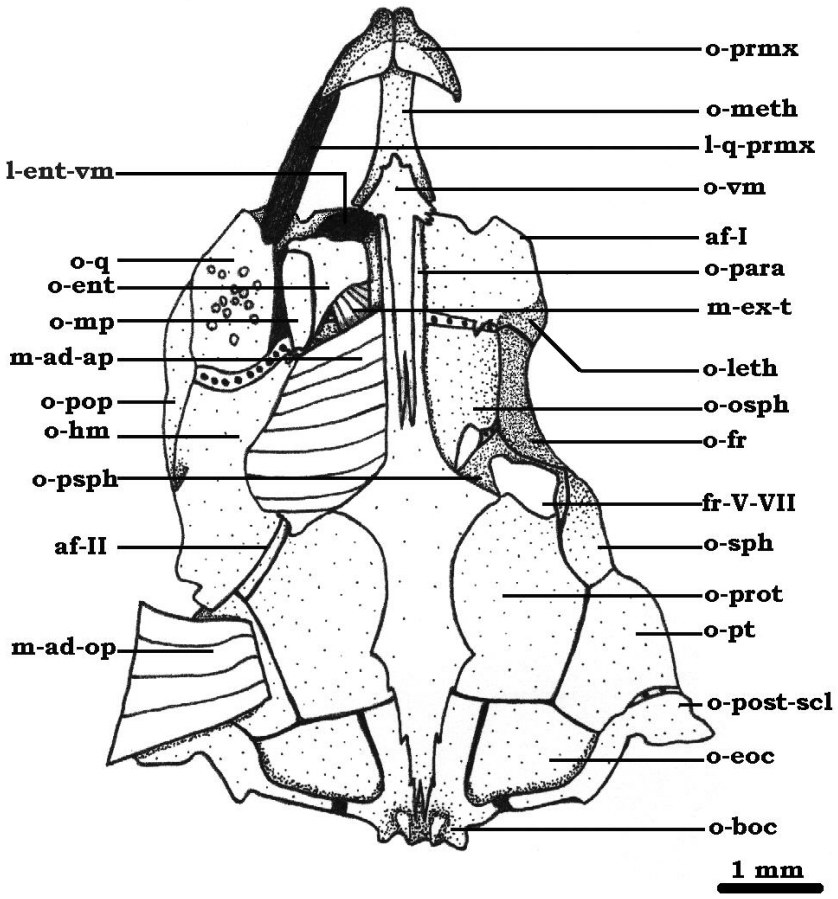

Fig. 3. – Ventral view of the neurocranium of *Phractura brevicauda*. On the left side the suspensorium, the adductor arcus palatini, the adductor operculi and the extensor tentaculi are also illustrated.

Os praeoperculare. Paired. Long and thin bone firmly attached to the hyomandibula and to the quadrate (Figs 1, 3).

Os hyomandibulare. The homology, and, thus, the correct denomination, of this bone, as well as of the other suspensorium components of catfish, has been the subject of endless controversies (McMurrich, 1884; De Beer, 1937; Hoedeman, 1960ab; Gosline, 1975; Arratia et al., 1978; Arratia & Menumarque, 1981; 1984; Howes, 1983ab; 1985; Arratia, 1987; 1990; 1992; Howes & Teugels, 1989; etc.). We hope to bring the solution in an extensive and detailed article actually proposed for publication. However, for the time being, we will describe the suspensorium bones by their most accepted names (see Arratia, 1992). The paired hyomandibulas articulate dorsally with the paired pterotics and sphenotics (Fig. 3).

Os quadratum. Paired. Its fore end presents a well-developed anterior articulatory facet for the angulo-articular (Fig. 1) and is connected, by means of a long, strong ligament to the premaxillary (Fig. 3). Its posterior surface is separated from the hyomandibular by means of a large cartilage (Figs 1, 3). Mesially it is firmly attached, by means of a very short ligament, to the metapterygoid and entopterygoid bones (Fig. 3). It presents a large number of pores in all its surface (Figs 1, 3).

Os metapterygoideum. Paired. Small, rectangular bone firmly attached to the entopterygoid by means of a very short ligament (Fig. 3).

Os entopterygoideum. Paired. The paired entopterygoids are broader than the metapterygoids (Figs 1, 3). The fore end of each entopterygoid presents a short, strong ligament that attaches to each antero-lateral process of the vomer (Fig. 3). The ectopterygoids are absent.

Os autopalatinum. Paired. It articulates with the maxillary and the lateral ethmoid, respectively, by its anterior cartilage and by its postero-medial surface (Fig. 1). Its fore end is associated, by means of short ligaments, to the antero-ventro-lateral surface of the mesethmoid and to the postero-dorsal edge of the premaxillary (Fig. 4). Posteriorly, the palatine is bifurcated (Figs 1, 5).

Os maxillare. Paired. They support the paired maxillary barbels.

Os praemaxillare. Paired. Both premaxillaries present a well-developed postero-lateral process (Figs 1, 2, 3, 8),

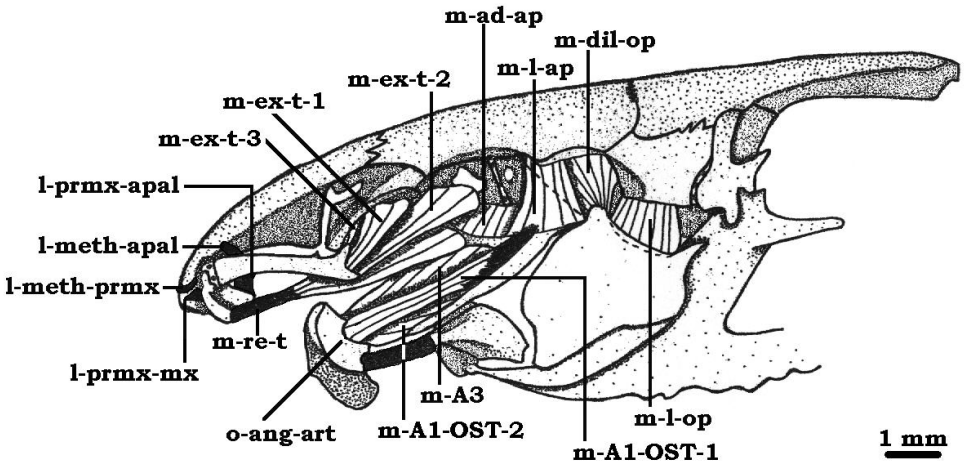

Fig. 4. – Lateral view of the cephalic musculature of *Phractura brevicauda*.

forming an inverted U-shaped complex (Figs 2, 3, 8). Each of them is dorsally linked by a strong ligament to the proximal extremity of the paired maxillaries (Fig. 4) and bears ventrally a large tooth-plate (Fig. 1).

Os angulo-articulare. Paired. This bone, together with the dentary, coronomeckelian and Meckel cartilage, constitute the mandible (Fig. 9B). The rear end of the angulo-articular presents an articulatory surface for the quadrate (Fig. 9B) and is linked to the interopercular and to the posterior ceratohyal by two long, strong ligaments (Fig. 7).

Os dentale. The paired dentaries are firmly connected, by means of a large number of short and thin fibres, to the supporting parts of the cartilages associated with the mandibular barbels (Fig. 8). The fore end of each dentary is deeply incurvated ventrally and presents only two small teeth, which confers to the mandible a very peculiar shape (Fig. 9B, C, D).

Os coronomeckelium. Paired. Small bone lodged in the medial surface of the mandible. Posteriorly it bears a crest for attachment of the jaw muscle (Fig. 9A).

Os ceratohyale posterior. Paired. The ceratohyale posterior, ceratohyal anterior and hypohyal bones have a very peculiar shape. The ceratohyale posterior is a quadrangular bone linked by ligaments to the fore end of the mandible (Fig. 7) and to the mesial surface of the suspensorium. Postero-laterally it articulates with the interopercular (Fig. 7).

Os ceratohyale anterior. Paired. Together with the posterior ceratohyal it supports the branchiostegal rays (Fig. 7).

Os hypohyale. Paired. Well-developed bone (Fig. 7).

Os parurohyale. The parurohyal (see ARRATIA & SCHULTZE, 1990) is a single shuttle-like bone lying medially behind the symphysis of the two hypohyals and connected to these bones by means of two short and thick ligaments (Fig. 7).

Myology

The myological nomenclature is mainly based on WINTERBOTTOM (1974). However, for the different adductor mandibulae subdivisions, we follow DIOGO & CHARDON (2000b), since recent works have pointed out that, with respect to these subdivisions, WINTERBOTTOM's (1974) nomenclature presents serious limitations (see

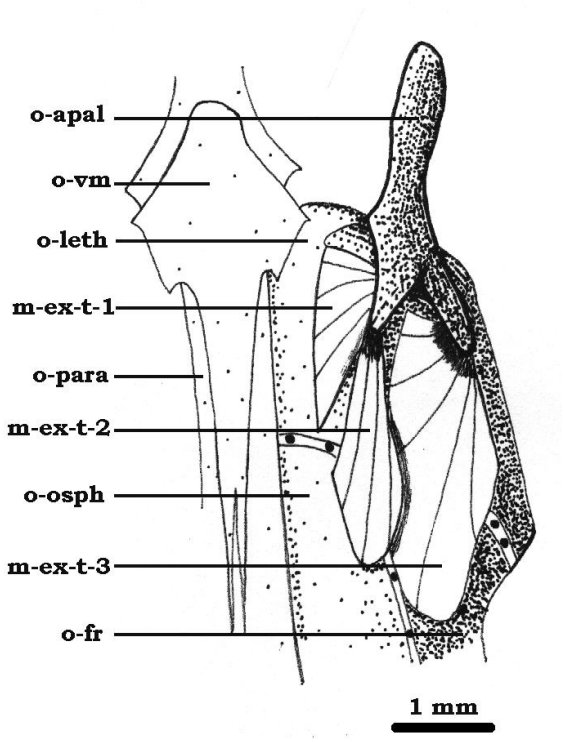

Fig. 5. – Palatine and extensor tentaculi of *Phractura brevicauda*, ventral view.

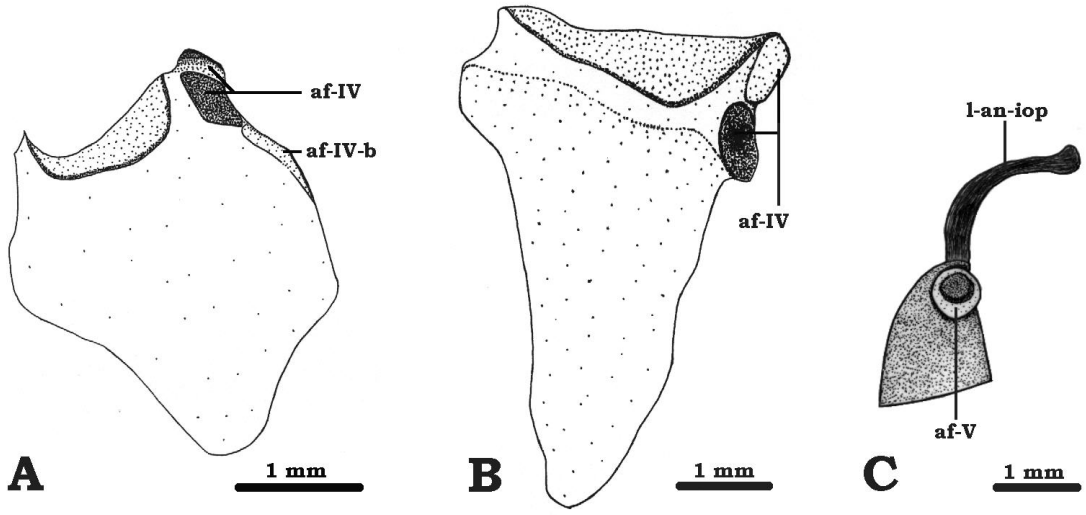

Fig. 6. – (A) Opercular of *Phractura brevicauda*, medial view. (B) Opercular of *Diplomystes chilensis*, medial view. (C) Interopercular of *Phractura brevicauda*., medial view.

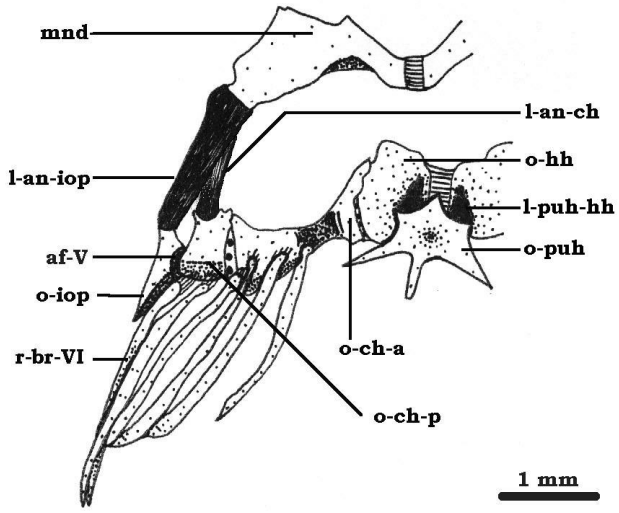

Fig. 7. – Ventral view of the splanchnocranium of *Phractura brevicauda*.

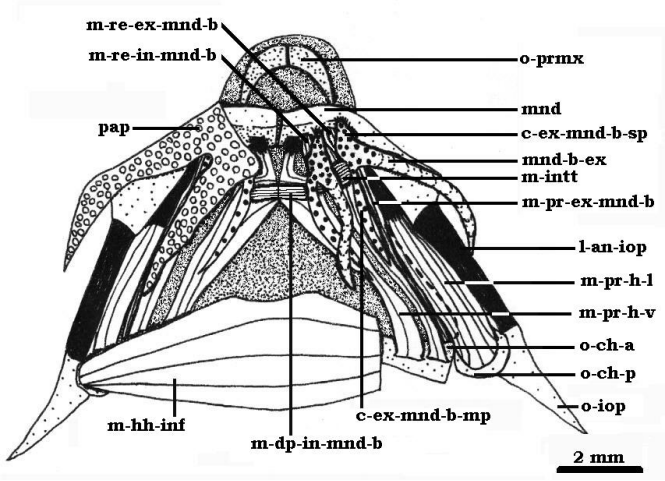

Fig. 8. – Ventral view of the cephalic musculature of *Phractura brevicauda*. On the right side, the skin enveloping the mandibular barbels was removed and the hyohyoideus inferior was cut.

Gosline, 1989; Diogo & Chardon, 2000b). In relation to the muscles associated with the mandibular barbels, which were not studied by Winterbottom (1974), we follow Diogo & Chardon (in press-b).

Musculus adductor mandibulae. Paired. The configuration of the adductor mandibulae is rather simple. The A1-OST is sub-divided into a lateral A1-OST-1 and a medial A1-OST-2 part (Figs. 4, 9A). The A1-OST-1 originates on the preopercular and the A1-OST-2 on the preopercular and quadrate (Fig. 4). They insert on the dorso-lateral surface of the angulo-articular (Figs 4, 9A). The A2, which lies medially to the A1-OST, attaches caudally on the preopercular and rostrally on the medial surface of the mandible (Fig. 9A). The deeper bundle of the adductor mandibulae, A3, runs from the preopercular, hyomandibular and quadrate (Fig. 4) to the coronomeckelian bone (Fig. 9A). The Aω is lacking.

Musculus levator arcus palatini. Paired. It originates on the ventro-lateral surfaces of the frontal and sphenotic and inserts on the dorso-lateral face of the hyomandibula (Fig. 4).

Musculus dilatator operculi. Paired. Thick muscle medial to the levator arcus palatini (Fig. 4). It originates on the ventral surfaces of the frontal, sphenotic and pterotic and on the lateral surface of the pterosphenoid and inserts on the antero-dorsal face of the opercular (medial to the preopercular but lateral to the articulatory facet of the opercular for the hyomandibula) (Fig. 4).

Musculus levator operculi. Paired. Very thin muscle dorsally linked to the ventro-lateral surface of the pterotic

and ventrally associated to the dorsal face of the opercular (Fig. 4).

Musculus adductor operculi. Paired. Situated medially to the levator operculi, it originates on the ventro-medial surface of the pterotic (Fig. 3) and inserts on the dorso-medial surface of the opercular.

Musculus adductor arcus palatini. The paired adductores arcus palatini extend from the lateral sides of the orbitosphenoid, pterosphenoid and parasphenoid to the medial sides of the hyomandibula (Figs 3, 4).

Musculus extensor tentaculi. Paired. This muscle is differentiated in four sections. The extensor tentaculi 1 originates on the lateral ethmoid and inserts, by means of a thin tendon, on the postero-medial face of the palatine (Figs 4, 5). The extensor tentaculi 2 originates on the orbitosphenoid and inserts tendinously on the postero-ventro-mesial process of the palatine (Figs 4, 5). The extensor tentaculi 3 extends from the lateral ethmoid and from the ventral surface of the frontal to the postero-dorso-lateral process of the palatine, to which it is linked by means of a thin tendon (Figs 4, 5).

Musculus retractor tentaculi. Paired. Well-developed muscle running from the external surface of the hyomandibular to the maxillary (Fig. 4).

Musculus protractor hyoidei. Paired. This muscle presents three parts. The pars ventralis, originating on the ceratohyal anterior and inserting on the dentary, is differentiated into two bundles associated, respectively, with the cartilages of the external and internal mandibular barbels (Fig. 8). The pars dorsalis originates on the ventral side of the ceratohyals and inserts tendinously on the dentary. The pars lateralis originates on the antero-ventral surface of the posterior ceratohyal and inserts, by means of a strong tendon, on the ventro-medial face of the dentary (Fig. 8).

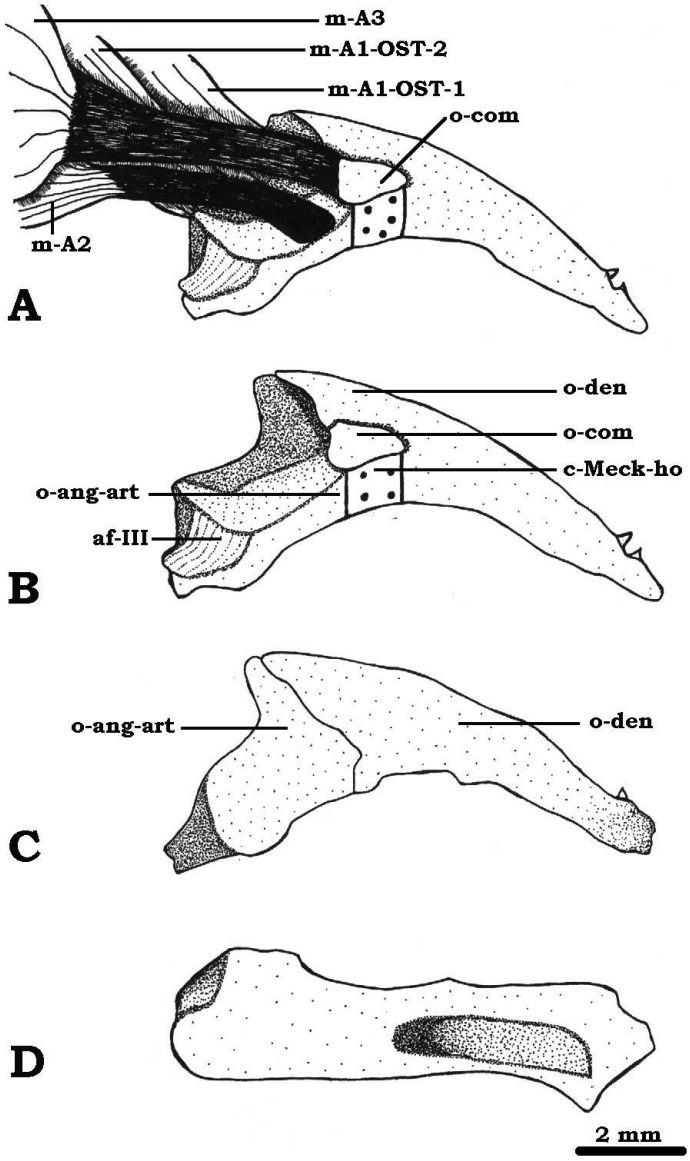

Fig. 9. – *Phractura brevicauda*. (A) Mandible and adductor mandibulae, medial view. (B) Mandible, medial view. (C) Mandible, lateral view. (D) Mandible, ventral view.

Musculus hyohyoideus inferior. Paired. Thick muscle attached laterally on the ventral surface of the ceratohyals and medially on a medial aponeurosis (Fig. 8).

Musculus sternohyoideus. Unpaired. It originates on the anterior region of the cleithrum and inserts on the posterior region of the parurohyal.

Musculus retractor externi mandibularis tentaculi. Paired. Small muscle running from the moving part of the cartilage associated with the outer mandibular barbel to the dentary (Fig. 8).

Musculus retractor interni mandibularis tentaculi. Paired. Small muscle that originates on the moving part of the cartilage associated with the internal mandibular barbel and inserts on the dentary (Fig. 8).

Musculus protractor externi mandibularis tentaculi. Paired. Long muscle extending from the anterior and posterior ceratohyals to the moving part of the cartilage associated with the external mandibular barbel (Fig. 8).

Musculus intertentacularis. Paired. Very small muscle running from the mesial face of the cartilage associated with the external mandibular barbel to the lateral face of that associated with the internal one (Fig. 8).

Musculus depressor interni mandibularis tentaculi. Paired. Small muscle extending from a medial aponeurosis to the mesial surface of the cartilage associated with the internal mandibular barbel (Fig. 8).

DISCUSSION

In this chapter we will firstly present a hypothesis on the function of the cephalic structures related to the feed-

ing mechanisms – movements of the mouth, suspensorium, opercular series, hyoid arch, maxillary barbels and mandibular barbels – in the doumein *Phractura*. Afterwards, we will focus on the striking functional and morphological homoplasies occurring between the Doumeinae and the callichthyid, scoloplacid, astroblepidae and loricariid catfishes.

Function of the cephalic structures related to the feeding mechanisms in Phractura

Opening of the mouth. The mechanism of mouth opening is illustrated in Fig. 10. *Phractura* has a ventrally placed mouth (Figs 1, 8) modified into a sucking disk, with a large number of labial papillae (Fig. 8). The opening of the mouth is realized by movements of both the premaxilla and the mandible (Fig. 10 A→B).

The movement of the premaxilla is associated to the palatine-maxillary system. The contraction of the extensor tentaculi muscle pulls the back of the palatine dorsomedially, what provokes – by means of the "rocking" articulation between this bone with the lateral ethmoid (see GOSLINE, 1975; DIOGO & CHARDON, 2000a) – a ventro-lateral displacement of the anterior extremity of the palatine (Fig. 10 A→B). As this extremity is firmly associated by a large, strong ligament to the posterior edge of the premaxilla (Fig. 4), this edge is also displaced ventrally, thus protracting the premaxilla (Fig. 10 A→B). Although a slightly mobile premaxilla is not unusual in catfishes (see ALEXANDER, 1965; GOSLINE, 1975; SCHAEFER & LAUDER, 1986; 1996; DIOGO & CHARDON, 2000a), a highly protractile premaxilla like that of *Phractura* (Fig. 10 A→B) is only found in the Scoloplacidae, Callichthyidae, Astroblepidae and Loricariidae (SCHAEFER & LAUDER, 1986; SCHAEFER, 1990; MO, 1991). Like in *Phractura* (Fig. 10 A→B), in the scoloplacids, callichthyids, astroblepids and loricariids the protraction of the premaxilla is related with the palatine-maxillary system, namely with the ventral displacement of the fore end of the palatine (see ALEXANDER, 1965; GOSLINE, 1975; VANDEWALLE et al., 1986).

The lowering of the mandible is associated to the retraction of the pectoral girdle. When the obliquus inferioris muscles are contracted, the girdle is retracted, which, through the sternohyoideus, provokes the retraction (without ventral displacement, due to absence of the interhyal: see ADRIAENS & VERRAES, 1994) of the mesial part of the hyoid arch (Fig. 10 A→B). This retraction, by means of the protractor hyoidei muscle and the membranous connection between the mandible and the hyoid arch, promotes the lowering of the mandible (Fig. 10 A→B). This mechanism is one of the four typical mechanisms of mandible depression in catfishes (DIOGO & CHARDON, 2000a). However, the other three typical mechanisms (first and third hyoid mechanisms and opercular mechanism) of lowering of the mandible described by DIOGO & CHARDON (2000a) seem to be lost in *Phractura*. In fact, in *Phractura*, the peculiar configuration of the ceratohyal posterior and of the interopercular (Fig. 7), and principally the well-developed articulatory surface between these two bones (Figs 6C, 7), only allow a rocking movement of the interopercular against the ceratohyal posterior (see below). Therefore, the interopercular cannot be retracted nor rotated dorsally, and, thus, the opercular and the third hyoid mechanism described by DIOGO & CHARDON (2000a) cannot be performed. This hypothesis is supported by the fact that in *Phractura* the levator operculi (which promotes the opercular mechanism of mandible lowering in teleosts) is abnormally thin, seeming to be a vestigial, almost useless muscle. Curiously, in the scoloplacids, astroblepids and loricariids, catfishes that, like *Phractura*, also have a highly protractile premaxilla and a ventrally placed mouth modified into a sucking disk, these two mechanisms are also lost, but for a different reason: the mandibulo-interopercular ligament is absent (SCHAEFER & LAUDER, 1986; SCHAEFER, 1990). The peculiar configuration of the interopercular (Figs 6C, 7) and of all the hyoid arch (Fig. 7), the well-developed interoperculo-ceratohyal articulation (Figs 6C, 7) and the fact that, contrarily to most catfish, the angulo-ceratohyal ligament originates on the anterior (and not postero-dorsal) edge of the ceratohyal posterior (Fig. 7), prevent *Phractura* to perform the first hyoid mechanism described by DIOGO & CHARDON (2000a).

When the premaxilla is protracted and the mandible is lowered at the same time, the mouth is completely opened and can thus adhere to the substrate (Fig. 10 A→B). As in the astroblepids and the loricariids (SCHAEFER & LAUDER, 1986; VANDEWALLE et al., 1986) the high mobility of both the upper and lower jaws probably confers to *Phractura* the possibility to attach to many different types of substrates. After the attachment to the substrate (Fig. 10 A→B), slight movements of the upper (retraction/protraction) and lower (raising/lowering) jaws – which are probably independent, since in *Phractura* the upper and lower jaws are morphologically and functionally uncoupled (see DIOGO et al., 1999a) –, will promote the scraping of the substrate (see Fig. 10B). At the moment, no data on the trophic characteristics of *Phractura*, or of any other Doumeinae, are available (the Amphiliinae present many morpho-functional differences in relation to the Doumeinae – see HARRY, 1953; HE, 1997; DIOGO & CHARDON, 1998; 1999; 2000a –, and, thus, it would be incorrect to make any extrapolation between the trophic characteristics of the members of the two subfamilies). So, the question whether *Phractura* presents the same food habits as those of the other scraping catfishes, namely of the loricarids (detritus, algae) or of the astroblepids (generalist omnivores) (SCHAEFER & LAUDER, 1986), or presents any other type of feeding habit, cannot be answered yet.

Closing of the mouth. The closure of the mouth is realized by the retraction of the premaxilla and the raising of the mandible (Fig. 10 B→A). Premaxilla retraction is performed by the contraction of the antagonist of the exten-

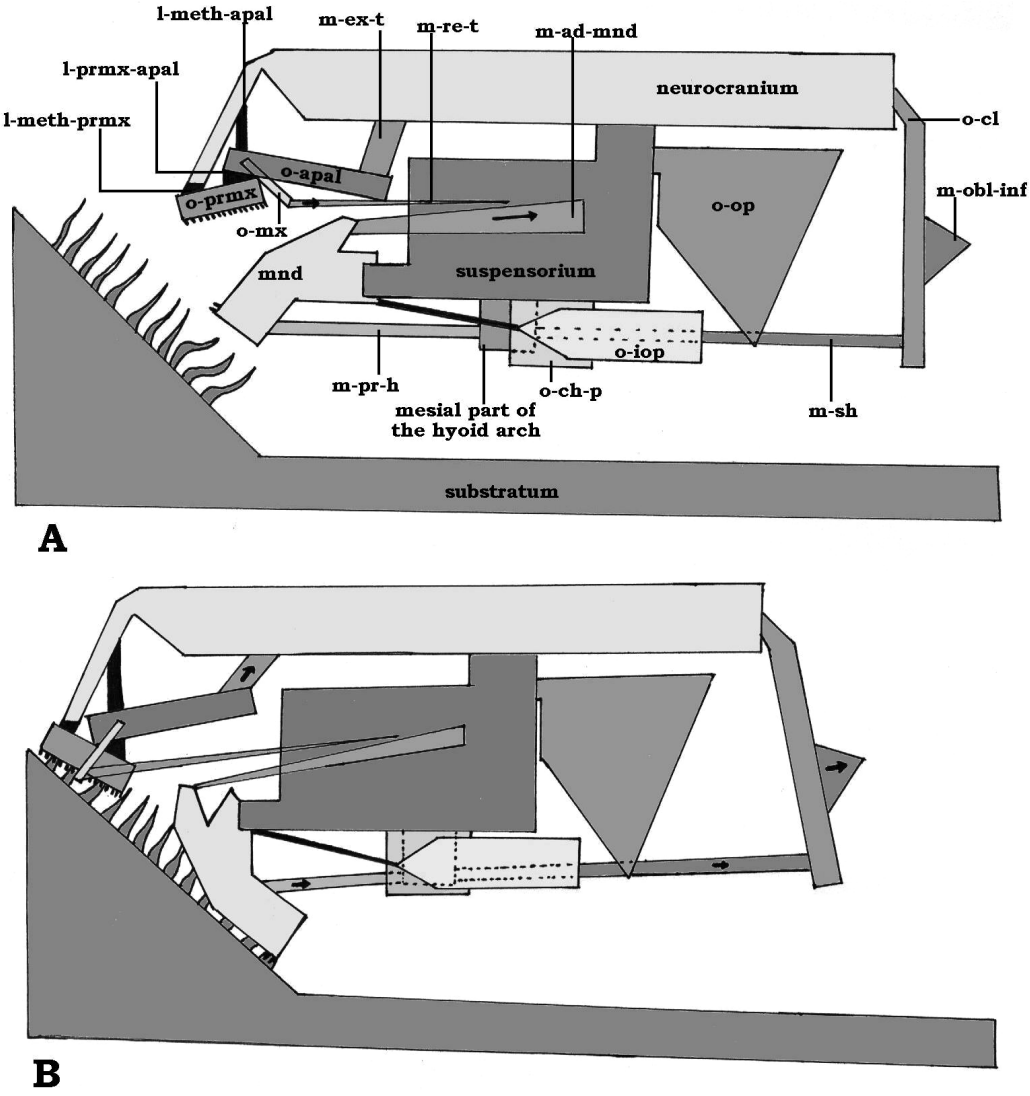

Fig. 10. – Scheme illustrating our hypothesis concerning the mechanisms of mouth closure and mouth opening in *Phractura brevicauda*. (A) The premaxilla is retracted (due to contraction of the retractor tentaculi) and the mandible is raised (due to contraction of the adductor mandibulae). (B) The premaxilla is protracted (due to contraction of the extensor tentaculi) and the mandible is lowered (due to contraction of the obliquus inferioris and/or sternohyoideus and/or protractor hyoidei).

sor tentaculi, the retractor tentaculi, which retracts the maxillary, provoking a dorsal displacement of the fore end of the palatine and, consequently, of the back of the premaxillary (Fig. 10 B→A). This seems also to be the case in the callichthyids, but not in the scoloplacids, astroblepids and loricariids, in which the retraction of the high-mobile premaxillary could be provoked directly by the contraction of specialised muscles directly inserted onto this bone (see HOWES, 1983b; SCHAEFER & LAUDER, 1986; VANDEWALLE et al., 1986; SCHAEFER, 1990). The raising of the mandible is realized by the contraction of the adductor mandibulae (Fig. 10 B→A). The two other typical mechanisms of mandible lifting described by DIOGO & CHARDON (2000a) are lost in *Phractura*.

Abduction of the suspensorium. The mechanism of suspensorium abduction is similar to that of most other catfishes (see DIOGO & CHARDON, 2000a). However, the fact that, of all the catfishes studied by us, *Phractura* is that with a thinner levator arcus palatini, associated with the fact that it is also the only one that does not present an antero-dorsal hyomandibular articulatory spine for the neurocranium (Fig. 1), seems to indicate that in this genus the abduction of the suspensorium is not as important as in most other catfishes. This hypothesis is in agreement with HUGHES (1970). According to this author, in bottom-living fishes (like *Phractura*) the suction pump mechanism is far more important in aquatic respiration than the pressure pump system. For this suction pump mechanism, no extensive abduction of the suspensorium, no elevation of the neurocranium and no depression of the of the hyoid bars are needed. Instead, a negative pressure is generated in the opercular cavity mainly through the dilatation of the opercular cavity (which is particularly important in *Phractura*: see below), thus provoking the sucking of the water from the orobranchial cavity, through the gills, into the opercular cavity.

Adduction of the suspensorium. The mechanism of suspensorium adduction is similar to that of most other catfishes, with the exception that in *Phractura* the contraction of the adductor operculi and the adduction of the suspensorium are not functionally coupled (see DIOGO & CHARDON, 2000a), since the adductor operculi does not insert on the hyomandibular (Fig. 3).

Dilatation of the opercular cavity. As in the other catfishes (see DIOGO & CHARDON, 2000a), the dilatation of the opercular cavity is performed by the contraction of the dilatator operculi. However, a detailed analysis of some peculiar features of the opercular series of *Phractura* seems to indicate that the dilatation of the opercular cavity is a particularly important cephalic mechanism in this genus. This hypothesis is supported by HUGHES' (1970) assumption that in bottom living fishes the suction mechanism is far more important that the pressure pump system (see above). The peculiar features of the opercular series are: 1) compared to other catfishes, in *Phractura* (Fig. 1) the back of the opercular bone lies relatively far from the articulatory surface between this bone and the hyomandibular, not only due to the abnormal width of the opercular bone, which is much larger than that of most other catfishes, but also to the fact that the ventral part of this bone lies abnormally far from the hyomandibular; 2) in *Phractura* there are two (Fig. 6A, B), and not one, articulatory surfaces of the opercular for the hyomandibular; 3) the interopercular presents a peculiar articulatory surface for the ceratohyal posterior (Figs 6C, 7), which allows a large lateral displacement of the back of the former: it is thus likely that both the opercular and the interopercular, and not only the former, would contribute to the dilatation of the opercular cavity when the dilatator operculi muscle contracts; 4) in *Phractura* the adductor operculi only inserts on the opercular (Fig. 3), and not on the opercular and the hyomandibular, as is the case in most other catfish studied by us (see DIOGO & CHARDON, 2000a), what increases the mobility between these two bones; 5) the dilatator operculi and the adductor operculi of *Phractura* are very thick, well-developed muscles.

Adduction of the operculum. As in the other catfishes (see DIOGO & CHARDON, 2000a), the adduction of the opercular bone is performed by the contraction of the adductor operculi.

Abduction of the maxillary barbel. The mechanism of maxillary barbel abduction is quite similar to that of *Chrysichtys* (see DIOGO & CHARDON, 2000a: Fig. 5C→D). So, when the extensor tentaculi muscle (Figs 4, 5) is contracted, the back of the palatine is pulled dorsomesially, what, by means of the "rocking" articulation (see GOSLINE, 1975) between the lateral-ethmoid and the palatine, provokes a ventro-lateral displacement of the fore end of the palatine and, thus, the abduction of the maxillary and its associated barbel (see DIOGO & CHARDON, 2000a: Fig. 5C→D). As in many other specialised catfishes, besides maxillary barbel abduction, the extensor tentaculi could also promote the depression (by means of the contraction of the extensor tentaculi 2) and the elevation (by means of the contraction of the extensor tentaculi 3) of the maxillary barbel (see DIOGO & CHARDON, 1999; in press-a; DIOGO et al., 1999a).

Adduction of the maxillary barbel. As in many other specialised catfishes, the adduction of the maxillary barbel is promoted by the contraction of the retractor tentaculi (see ALEXANDER, 1965; GOSLINE, 1975; HOWES, 1983ab; 1985; DIOGO & CHARDON, 1999; in press-a; etc.), being functionally uncoupled from the movements of the lower jaw (see DIOGO & CHARDON, 1999; in press-a; DIOGO et al., 1999a).

Movements of the mandibular barbels. The movements of the mandibular barbels of Phractura are similar to those of *Chrysichthys*, which are described in detail by DIOGO & CHARDON (2000a).

Homoplasies occurring between the Doumeinae and some loricarioid catfishes

Our morpho-functional analysis pointed out that *Phractura* is a peculiar catfish presenting several unusual morphological modifications, which are probably related to two main functional specilizations: the ability to attach the body to the substrate with an oral sucker, and the ability to scrape this substrate. In order to appraise the phylogenetic and systematic significance of these morphological modifications and functional specialisations, it was decided to study the other genera of Amphiliidae, as well as many other catfish genera, and to compare our results with those available in the literature. Our observations and comparisons with other amphiliid genera indicate that the main morpho-functional specialisations present in *Phractura* are not confined to members of this genus, but are, in reality, also present in the other doumeins (but not in the Amphiliinae, which, together with the Doumeinae, compose the family Amphiliidae: DIOGO & CHARDON, in preparation). In addition, our observations and comparisons with non-amphiliid catfishes (either studied by us or described in the literature) revealed an impressive number of morpho-functional homoplasies occurring between the African doumeins and the South-American callichthyid, scoloplacid, astroblepid and loricariid catfishes (given the scarce data available on higher-level phylogeny of the siluriformes, it is impossible to discriminate whether these homoplasies are the result of a convergence or of an evolutionary parallelism). These homoplasies could be divided in five types: 1) the homoplasies shared by the doumeins, the callichthyids, scoloplacids, astroblepids and loricariids and other specialised catfishes; 2) the homoplasies shared by the doumeins and the callichthyids, scoloplacids, astroblepids and loricariids; 3) the homoplasies shared by the doumeins and the callichthyids and scoloplacids; 4) the homoplasies shared by the doumeins and the scoloplacids, astroblepids and loricariids; 5) the homoplasies shared by the doumeins and the astroblepids and loricariids.

The main homoplasies shared by the doumeins, the callichthyids, scoloplacids, astroblepids and loricariids and other specialised catfishes are: 1) *Presence of a retractor tentaculi muscle.* The functional consequence of this character is that the maxilla, and, thus, the maxillary barbel, could be retracted directly by the contraction of the retractor tentaculi, rather than indirectly by the closure of the mouth and/or the elasticity of the tissues involving the palatine-maxillary system (see ALEXANDER, 1965; GOSLINE, 1975; HOWES, 1983a; DIOGO & CHARDON, in press-a). The differentiation of a retractor tentaculi muscle, which is present in most specialised catfishes, from the adductor mandibulae occurred independently in different catfish lineages (see HOWES, 1983a; DIOGO & CHARDON, 2000b; in press-a); 2) *Absence of the extrascapular bone*. The disappearance of this bone has occurred independently in several catfish lineages (CHARDON, 1968; ARRATIA, 1987). The functional consequence of this character, which is present in most specialised catfishes, seems to be the ankilosis between the posterior region of the neurocranium, the pectoral girdle and the anterior vertebra (CHARDON, 1968); 3) *Mesethmoid without anterolateral cornua*. This character, which has very likely evolved independently in different catfish lineages (MO, 1991), is probably related to a larger mobility of the premaxillary; 4) *Articulatory surface of the palatine for the neurocranium situated in the posterior portion of the palatine*. This character has very likely evolved independently in different catfish lineages and is probably related to a larger efficacity of the palatine-maxillary system (DIOGO & CHARDON, 1998; DIOGO et al., 1999); 5) *Depression of the coronoid process and disappearance of the adductor mandibulae Aw and of the vertical portion of Meckel's cartilage*. These characters have very likely evolved independently in different catfish lineages and are probably associated with the dorso-ventral depression of the mandible, which is probably linked to the adaptation to a benthic life style (by means of a flattening of the skull) (DIOGO & CHARDON, 2000b).

The main homoplastic character shared by the doumeins and the callichthyids, scoloplacids, astroblepids and loricariids is the *highly protractile premaxillary*. Although a slightly, or even moderate (in some plotosids, for example) mobile premaxilla is not unusual in catfishes (see ALEXANDER, 1965; GOSLINE, 1975; SCHAEFER & LAUDER, 1986; DIOGO & CHARDON, 2000a), a highly protractile premaxilla like that of the doumeins (see Fig. 10 A→B) is only found in the Scoloplacidae, Callichthyidae, Astroblepidae and Loricariidae (SCHAEFER, 1990; MO, 1991). This character is however homoplastic, not only since the morphological relation (ligaments, connective tissue) between the mesethmoid and the premaxillary in the doumeins is quite different from that of the scoloplacids, callichthyids, astroblepids and loricariids, but also since these South-American catfishes are more closely related with the trichomycterids and the nematogenyids than with any other catfishes (see, for example, SCHAEFER, 1990). The highly protractile premaxillary is probably associated with the capacity to scrape the substrate.

The main homoplastic character shared by the doumeins and the callichtyids and scoloplacids is the *mandible with few or no teeth*. With exception of the specimens of the genera *Trachyglanis* and *Phractura*, which present very few teeth on the lower jaw, the doumeins studied by us possess an edentate mandible. The lower jaw of the Scoloplacidae bears usually only four to six minute teeth (SCHAEFER, 1990), and that of the Callichthyidae bears usually very few teeth, whereas two genera of this family, *Corydoras* and *Brochis*, possess an edentate mandible (REIS, 1998). The functional significance of this homoplastic character is not clear.

The main homoplastic character shared by the doumeins and the scoloplacids, astroblepids and loricariids is the *loss of the opercular mechanism of lower jaw depression*. The morphological features associated to this functional modification in the doumeins (well-developed articulatory surface of the interopercular for the ceratohyal posterior, etc.) are quite different from those related with the same modification in the scoloplacids, astroblepids and loricariids (loss of the mandibulo-interopercular ligament) (see above). This homoplastic character could be associated to a more important dilatation of the opercular cavity, what could be related to a benthic life style (see above).

The main homoplasies shared by the doumeins and the astroblepids and loricariids are: 1) *Ventrally placed mouth modified into a sucking disk*. This homoplastic character (see, for example, SCHAEFER, 1990) is characteristic of the doumeins, astroblepids and loricariids, although it also occurs in some mochokids and in some sisorids (SCHAEFER, 1990). Its functional consequence is to confer to the fish the possibility to attach to the substrate (see Fig. 10A→B). 2) *Ligamentous connection between the palatine and the ethmoidal region*. In the astroblepids (SCHAEFER, 1990), loricariids (VANDEWALLE et al., 1986) and doumeins, the ethmoidal region is connected to the palatine by means of ligaments. However, this connection is realised in a quite different way in each of these groups. In the doumeins, a thick, short ligament (see Fig. 4) links the anterior region of both the palatine and the mesethmoid. In the loricariids these two bones are also ligamentously linked, but by means of a thin, long ligament (see VANDEWALLE et al., 1986). Finally, in the astroblepids the connection between the ethmoidal region and the palatine is realised by a ligament that runs from the palatine to the lateral ethmoid, and not to the mesethmoid (SCHAEFER, 1990). Although it is difficult to decide whether the ligaments present in the Astroblepidae and the Loricariidae are homologous or not, the close phylogenetic relationship between these catfishes and the other loricaroids (CHARDON, 1968; HOWES, 1983b; SCHAEFER & LAUDER, 1986; SCHAEFER, 1990; MO, 1991; etc.) clearly indicates that these ligaments are, in no way, homologous with those of the doumeins. The functional consequence of this character is not clear.

General conclusions

This study reveals that *Phractura*, as well as the other doumeins, present several unusual morphological modifications, which are probably related to two main functional specilizations: the ability to attach the body to the substrate with an oral sucker, and the ability to scrape this substrate. These morpho-functional specialisations are strikingly similar to those of the callichthyid, scoloplacid, astroblepid and loricariid catfishes, and particularly with those of the two latter groups, which also present the ability to attach to the substrate and to scrape it. However, this study reinforces the idea that most morphological homoplasies, even those that are quite similar, could be discriminated if analysed in a careful, detailed way. It is also stressed that several morphological modifications could be related with a sole functional specialisation. It his hoped that our results could be useful in future evolutionary, functional, morphological, ecological, ethological, systematic and phylogenetic studies concerning not only the amphiliids, but also the catfishes in general.

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Earthworm populations of Roosevelt Avenue (Brussels, Belgium): composition, density and biomass

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ABSTRACT. Earthworms were sampled (electric octet method) in the lawn islets of the 2.5 km long Roosevelt avenue (city of Brussels, Belgium). From a preliminary sampling a total of nine species (five to eight per islet) were found of which four (*Aporrectodea caliginosa, A. longa, A. rosea* and *Lumbricus rubellus*) were present in all the islets. No insularity effect could be found.

A full year sampling was then performed in five selected islets, revealing total density between 89 and 253 individuals/m^2 and biomass between 49 and 153 g/m^2 (yearly averages). Biomass (but not density) correlated best with silt and pH.

KEY WORDS: Earthworm populations, density, biomass, urban soils

INTRODUCTION

Practically all field studies on earthworms have been carried out in natural or semi-natural habitats. In Belgium, apart from the earthworm inventory by BOUCHÉ (1978), recent data on the topic are rare (BOULANGÉ, 1968, GASPAR et al., 1981) or unpublished (SYMOENS[1], HENNUY[2], HIDVEGI[3], MUYS, 1993, MASSARD[4]).

There is especially little information on populations of earthworms in urban localities. According to their situation, the soils of cities present various levels of disturbances: most are made of embanked materials (NUYENS, unpublished data[5]), are polluted with heavy metals and other substances (PIZL & JOSENS, 1995a, BONTYA, unpublished data[6]), and are stamped and compacted (FANNING & FANNING, 1989). Another characteristic of urbanised surroundings is the partitioning of biotopes into smaller parcels, which transforms the biotopes for animals as little mobile as earthworms into kinds of archipelagoes (BEGON et al., 1990; PANHUYSEN, unpublished data[7]).

Populations are thereby broken up, faced with unaccustomed competition conditions and sometimes with species introductions.

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In spite of the various disturbances and pollution to which they are subjected, these surroundings can shelter earthworm communities relatively rich in species and individuals (PIZL & JOSENS, 1995a, b). These populations can provide a good model for studies of population dynamics and competition.

MATERIAL AND METHODS

Locality

This study was carried out on F. D. Roosevelt avenue, which is situated in the south-east of the Brussels-capital Region, Belgium (50° 48' N, 4° 23' E). It includes in its middle a long lawn of 2450 meters surrounded by two roads. The streets that cross the avenue divide the lawn into 12 islets, which were numbered from the city towards the periphery. The islets have a width of 8.3 meters and a length varying from 110 meters for islet 5 to 250 meters for islet 12. The total lawn area is 1.75 ha.

The vegetation of the lawn is dominated by Poaceae (*Poa annua, P. trivialis, Agrostis stolonifera, Lolium perenne* and *Holcus lanatus*). Among dicotyledons, *Trifolium repens*, *Bellis perennis*, *Taraxacum officinale* and *Hypochaeris radicata* are the most abundant (TANGHE, personal communication).

The superficial soil is generally sandy to silt-laden and deep; it is made of anthropic embankments 50 to 100 cm thick, resting on native sands. It is enriched in humus produced by the grass that is mown twice a month from April to October and by dead leaves from the limes that line the avenue. Very few people walk on the islets, except the n° 1, and their soils are, therefore, little trampled.

Islets are isolated by intense automobile traffic: Roosevelt avenue is an important penetration axis into the city and suffers daily traffic jams.

Preliminary sampling and choice of the islets

Two samples were taken from each of the 12 islets in November 1995 according to the method described hereafter. Five out of the 12 islets were then selected according to the composition and density of their earthworm communities.

Definitive sampling

Each islet was sampled once a week from February 1, 1996 to February 26, 1997, with an interruption of one week in August 1996 (breakdown of the extractor) and two weeks in January 1997 (permanent frost).

In the middle of each of the five islets, an area 60 m long by 4 m wide was subdivided into 60 elementary parcels of 2 m x 2 m. The order of sampling of the elementary parcels was determined by a protocol of simple random sampling. So the first random number designated the first parcel to be sampled on the first week (for the five islets), the second number the second parcel on the second week and so forth.

Extraction of earthworms

Because of constraints associated with the residential character of the avenue, the extraction of earthworms was achieved by Thielemann's electric "Oktett-Methode" (1986) with the help of a Worm-Ex extractor of the GefaÖ firm (Heidelberg, Germany).

For each sampling, the elementary parcel of the day was first located, then the lawn was mown to ground level over a surface area of 0.5 m^2 with a Gardena hand-mower. The ring (a circle of 0.125 m^2) of the Worm-Ex extractor was laid down in the centre of this surface. The electrodes (65 cm long) were driven into the soil with all our weight on the handles until the resistance of the soil stopped us from driving them further, and the depth reached was measured. The extraction started with the lowest voltage and was increased every five minutes (successively 200, 250, 300, 350, 400 and 500 V). The speed of rotation of the electric field remained constant at 4 rotations per minute.

Identification and weighing of the earthworms

The worms were rinsed in tap water and sorted out under a lamp and a stereomicroscope. Identification was carried out using the keys of JOSENS & HIDVEGI (unpublished) and of SIMS & GERARD (1985), and we adopted the nomenclature of the latter authors.

Each worm was briefly dried on tissue and weighed (Sartorius Basic balance) with a precision of ± 0.1 mg.

Soil parameters

Five samples were taken per islet, and C and N determined for each sample. Samples were then pooled prior to texture analysis. Carbon content (Strölein's oxidation at 1100° C) and nitrogen content (Kjeldahl's method) as well as the texture analysis (clay, silt and sand contents) were determined in the laboratory of pedology (Prof. Herbauts).

The pH (water) and water content (dried at 105° C) were measured on each worm sampling site (63 measurements per islet).

Parameters of populations

The relative frequency (of a species within an islet) is the ratio of the number of samples where the species is present to the total number of samples.

The dominance (of a species within an islet) is the ratio of the number of individuals of a species to the total number of individuals.

Density and biomass within an islet (means ± standard deviations) are expressed per square meter on the basis of a complete year cycle.

RESULTS

Soil characteristics

These are summarised in Table 1.

Clay content varied within a narrow range (6 to 9%), but silt and sand content varied greatly and inversely.

Among the other soil parameters carbon content (2.7 to 4.4%) correlated positively with clay (r=0.94, p=0.02), inversely with sand (r=-0.88, p=0.05) and weakly with silt (r=0.84, p=0.07). Nitrogen (0.25 to 0.37%) followed the same tendency and correlated strongly with carbon (r=0.98, p=0.003).

The pH varied in a narrow range (6.9 to 7.15) and was correlated negatively and strongly with silt (r=-0.96, p=0.008) and positively with sand (r=0.95, p=0.12), while water content followed the inverse tendency and correlated negatively with pH (r=-0.94, p=0.017).

TABLE 1

Soil characteristics (mean ± standard deviation) of Roosevelt avenue (Brussels, Belgium)

	Islet 3	islet 5	islet 8	islet 9	islet 10	p ANOVA
Clay (%) (n = 1*)	6.0	7.0	9.1	7.3	6.6	
Silt (%) (n = 1*)	24.4	55.8	56.2	30.7	23.1	
Sand (%) (n = 1*)	63.7	30.3	26.7	55.2	64.8	
Carbon (%) (n = 5)	2.71 ± 0.40	3.76 ± 0.14	4.41 ± 0.46	3.72 ± 0.78	2.98 ± 0.39	0.0001
Nitrogen (%) (n = 5)	0.25 ± 0.02	0.33 ± 0.02	0.37 ± 0.04	0.32 ± 0.05	0.29 ± 0.040	0.001
C/N (n = 5)	11.5 ± 0.6	11.6 ± 0.82	11.9 ± 1.5	11.6 ± 1.0	10.5 ± 1.0	0.28 (ns)
pH (n = 63)	7.10 ± 0.25	6.91 ± 0.27	6.95 ± 0.30	7.13 ± 0.29	7.15 ± 0.26	< 0.0001
Water (%)(n = 63)	29.9 ± 10.41	36.8 ± 11.56	38.8 ± 13.31	29.5 ± 11.94	24.8 ± 8.56	< 0.0001
remark	carbonates				carbonates	

* 5 samples mixed

Preliminary sampling and choice of islets for definitive sampling.

On the basis of two samples per islet in November 1995, the specific richness varied between four and seven species (Table 2), and the whole lawn of Roosevelt avenue sheltered nine species, of which four (*Aporrectodea caliginosa, A. longa, A. rosea* and *Lumbricus rubellus*) were present in all islets. There was

TABLE 2

Results of preliminary earthworm sampling in the lawns of Roosevelt avenue (Brussels, Belgium, November 1995)

islet	*Allolobophora chlorotica*	*Aporrectodea caliginosa*	*Aporrectodea icterica*	*Aporrectodea longa*	*Aporrectodea rosea*	*Lumbricus castaneus*	*Lumbricus rubellus*	*Lumbricus terrestris*	*Satchellius mammalis*	# spp
1		+++		+++	+++		+			4
2	+	+	+	+++	+++		+			6
3	++	++	+	+++	+++		+			6
4		+++	++	+++	+++		+			5
5		++	++	+++	+++		+		+	6
6		+++	++	+++	++		+	+	++	7
7		+++	++	+++	+++		+		++	6
8		+++	+	+++	+		+	+	+	7
9		+++		+++	+++		+	++		5
10		++		+++	+++		+			4
11	+	++		++	++	+	+			6
12		+++		++	+	+	+			5

\+ 1 to 5 individuals per sample of 0.125 m^2

++ 5 to 10 individuals per sample of 0.125 m^2

+++ more than 10 individuals per sample of 0.125 m^2

no significant correlation between islet size and specific richness (n=12, r=0.14, p > 0.05).

Three islets were immediately discarded from the definitive sampling: islet 1 because it was the only one to be trampled (partially), and islets 11 and 12 because they were concerned with civil engineering.

Of the remaining islets, we retained islets 3 and 5, because some data were already available for them (PIZL & JOSENS, 1995a); furthermore, islet 3 contained an abundant population of *Allolobophora chlorotica*, absent or rare elsewhere, and islet 5 sheltered the highest community density.

Islets 8 and 10 were selected because they contained the highest and lowest specific richness, respectively.

Islet 9 was retained because it was the only one to contain, in addition to the four species common to all islets, a population of *Lumbricus terrestris* that seemed dense (however, this was not confirmed by the definitive sampling).

The five selected islets contained eight out of the nine species present in the avenue.

Homogeneity of sampling conditions

Since it was rarely possible to drive the electrodes completely into the soil, a heterogeneity of extraction efficiency might have been expected, but an analysis of variance on this parameter did not reveal any significant differences between islets (F=0.89, p=0.47).

Composition of communities.

After a complete sampling year, the specific richness determined by the preliminary sampling (two samples per islet) was confirmed for islets 3 and 5 and only one extra species was recorded in each of the three other islets (n° 8, 9 and 10). The five islets studied contained an average of 6.20 ± 1.10 species (Table 3).

TABLE 3

Dominance (D) and relative species frequency (F) of the earthworm species in the lawns of Roosevelt avenue (Brussels, Belgium)

	Islet 3 (n = 51)		**islet 5 (n = 50)**		**islet 8 (n = 53)**		**islet 9 (n = 52)**		**islet 10 (n = 52)**		**The 5 islets**	
	D	F	D	F	D	F	D	F	D	F	D	F
Allolobophora chlorotica	0.13	0.42			0.004	0.06					0.03	0.09
Aporrectodea caliginosa	0.10	0.5	0.15	0.77	0.23	0.68	0.31	0.64	0.34	0.48	0.20	0.61
Aporrectodea icterica	0.17	0.58	0.20	0.73	0.09	0.43	0.03	0.125	0.02	0.14	0.13	0.4
Aporrectodea longa	0.27	0.59	0.26	0.68	0.26	0.6	0.36	0.58	0.35	0.58	0.29	0.6
Aporrectodea rosea	0.33	0.53	0.19	0.63	0.15	0.46	0.23	0.41	0.30	0.42	0.23	0.49
Lumbricus rubellus	0.004	0.08	0.06	0.63	0.16	0.8	0.06	0.41	0.02	0.14	0.06	0.41
Lumbricus terrestris					0.03	0.28	0.005	0.04			0.007	0.06
Satchellius mammalis			0.14	0.65	0.08	0.52					0.06	0.23

– *Allolobophora chlorotica* Savigny, 1826, is the 6th most common earthworm species in Belgium (BOUCHÉ, 1978), and its presence was confirmed in islet 3 (frequency = 0.42). It was also occasionally found in islet 8 (frequency=0.06). All individuals found belong to the green morph.

– *Aporrectodea caliginosa* Savigny, 1826, the most common species of the Belgian earthworm fauna (BOUCHÉ, 1978), constitutes, according to the north American literature (REYNOLDS, 1977, GATES, 1972 in BOUCHÉ, 1976) and the French literature (BOUCHÉ, 1972) a complex of species and/or morphs. In this paper it is considered a single species *sensu* SIMS & GERARD (1985) but with two morphs (normal and dwarf) distinguishable from each other only at the adult stage. This species was abundant in all the islets with relative frequencies between 0.48 and 0.77; it was the second most dominant species in islets 8, 9 and 10.

– *Aporrectodea icterica* Savigny, 1826, is a relatively rare species in Belgium (BOUCHÉ, 1978) but it was abundant in Roosevelt avenue: it was found in all islets sampled, with frequencies from 0.125 (islet 9) to 0.73 (islet 5, where it was the second most dominant species in number and biomass).

– *Aporrectodea longa* Ude, 1885, is only the 10th most common earthworm species in Belgium (BOUCHÉ, 1978), but it was the dominant and most constant species in the islets of Roosevelt avenue; it represented 60 to 70% of the earthworm biomass in the five sampled islets.

– *Aporrectodea rosea* Savigny, 1826, is the 2nd most common earthworm species in Belgium (BOUCHÉ, 1978), and it was also of very regular occurrence in Roosevelt avenue with frequencies between 0.41 and 0.63; it was the dominant species in islet 3 and the second most dominant species in islets 9 and 10. However, its biomass was never very high because of its small size.

– *Lumbricus rubellus* Hoffmeister, 1843, is the 3rd most common species in Belgium (BOUCHÉ, 1978), and it was

present in all the islets although at very different frequencies (from 0.08 in islet 3 to 0.80 in islet 8). It constituted the second highest worm biomass in islets 5 and 8.

– *Lumbricus terrestris* Linné 1758, is the 5th most common earthworm species in Belgium (BOUCHÉ, 1978); however, it was rare in the avenue, with frequencies of 0.28 in islet 8 and 0.04 in islet 9 and never attained high density or biomass.

– *Lumbricus castaneus* Savigny, 1826, the 4th most common earthworm species in Belgium (BOUCHÉ, 1978), was found only in islets 11 and 12 (at low densities) during the preliminary sampling.

– *Satchellius mammalis* Savigny,1826 is a very rare species in Belgium (BOUCHÉ, 1978). It was present in islets 5 and 8 with frequencies of 0.65 and 0.52 respectively but at relatively modest densities.

Density and biomass

Tables 4 and 5 summarise average annual earthworm density and biomass data respectively.

The coefficients of variation (i.e. the standard deviations relative to the means) for global density and biomass often exceeded 100%, reflecting a high heterogeneity in our samplings.

TABLE 4

Density (individuals / m^2): annual mean ± standard deviation of earthworms in the lawns of Roosevelt avenue (Brussels, Belgium)

	Islet 3 (n = 51)	**islet 5 (n = 50)**	**islet 8 (n = 53)**	**islet 9 (n = 52)**	**islet 10 (n = 52)**
Allolobophora chlorotica	25.3 ± 49.6		0.30 ± 1.54		
Aporrectodea caliginosa	17.1 ± 28.7	40.0 ± 39.9	30.0 ± 37.6	30.6 ± 34.4	31.4 ± 43.2
Aporrectodea longa	40.2 ± 59.0	65.1 ± 70.4	34.9 ± 44.0	36.9 ± 48.8	29.5 ± 36.3
Aporrectodea icterica	30.6 ± 46.8	48.6 ± 47.3	12.1 ± 18.5	3.5 ± 12.7	2.00 ± 6.70
Aporrectodea rosea	65.4 ± 99.4	55.8 ± 65.6	24.0 ± 36.9	26.5 ± 54.7	24.9 ± 52.9
Lumbricus rubellus	0.47 ± 1.90	12.8 ± 15.0	21.1 ± 21.8	5.23 ± 7.90	1.38 ± 3.79
Lumbricus terrestris			3.17 ± 8.05	0.61 ± 2.67	
Satchellius mammalis		30.4 ± 36.4	10.9 ± 15.1		
Total	179.0 ± 237.7	252.8 ± 222.5	136.8 ± 134.3	103.4 ± 120.9	89.2 ± 113.0

TABLE 5

Fresh biomass (g/m^2): annual mean ± standard deviation of earthworms in the lawns of Roosevelt avenue (Brussels, Belgium)

	Islet 3 (n = 51)	**islet 5 (n = 50)**	**islet 8 (n = 53)**	**islet 9 (n = 52)**	**islet 10 (n = 52)**
Allolobophora chlorotica	2.71 ± 4.83		0.078 ± 0.40		
Aporrectodea caliginosa	5.57 ± 9.74	11.7 ± 12.5	9.90 ± 13.2	13.2 ± 18.6	12.4 ± 18.1
Aporrectodea icterica	12.7 ± 20.4	23.4 ± 22.9	6.47 ± 9.85	0.58 ± 1.89	0.39 ± 1.39
Aporrectodea longa	37.5 ± 57.1	101.9 ±122.3	61.6 ± 82.7	67.1 ± 100.0	33.7 ± 44.3
Aporrectodea rosea	5.20 ± 7.31	6.04 ± 6.60	2.51 ± 3.77	2.59 ± 4.77	2.37 ± 4.52
Lumbricus rubellus	0.15 ± 0.67	7.64 ± 11.05	14.3 ± 21.3	3.04 ± 7.07	0.41 ± 1.29
Lumbricus terrestris			7.27 ± 18.81	1.30 ± 5.71	
Satchellius mammalis		2.18 ± 2.66	1.10 ± 1.66		
Total	63.9 ± 83.6	153.0 ± 158.5	103.3 ± 112.6	87.8 ± 122.4	49.2 ± 62.8

ANOVA and a multiple average comparison revealed that the 5 islets were heterogeneous on the basis of density ($F=7.39$, $p<0.001$) and of biomass ($F=6.49$, $p<0.001$).

The islets ranked 5, 3, 8, 9 and 10 successively in decreasing order of density. A multiple average comparison indicated that islet 5 contained significantly more worms than any other (Tukey test, $0.04>p>0.0001$) and that islet 3 housed significantly more worms than islet 10 ($p<0.01$).

The islets ranked 5, 8, 9, 3 and 10 in decreasing order of biomass. A multiple average comparison indicated that islet 5 contained significantly more worm biomass than islets 9, 3 and 10 (Tukey test, $0.04>p>0.0001$) and that

islet 8 housed significantly more worm biomass than islet 10 ($p<0.02$).

No significant correlation could be found between worm density and the soil parameters but worm biomass was correlated positively with silt ($r=0.87$, $p=0.05$) and negatively with pH ($r=-0.89$, $p<0.05$).

DISCUSSION

Comparison of our species richness data with other earthworm inventories in the literature is complicated by taxonomic instabilities. From the whole Belgian territory, BOUCHÉ (1978) listed 30 species and subspecies that only accounted for 23 species *sensu* SIMS & GERARD (1985). Comparisons are further hampered by variation in extraction technique and effort between surveys. With the aim of comparing species richness we have used the nomenclature of SIMS & GERARD (1985).

Studies in herbaceous habitats of Belgium and the Grand Duchy of Luxemburg found that specific richness generally varied between two and ten species: six to seven species in three Ardenne meadows (SYMOENS, unpublished data), four to ten species (average=7.2) in 13 grasslands and lawns of the region of Treignes, south of Belgium (HIDVEGI, unpublished data)), nine species in a grassland near Echternach, Grand Duchy of Luxemburg (MASSARD, unpublished data), two to nine species (average=5.8) in the lawns of 19 Brussels parks (JOSENS & PIZL, unpublished data). In other studies dedicated to grasslands and meadows, the specific richness was of the same order of magnitude: six to eight species in Sweden (NORDSTRÖM & RUNDGREN, 1973), and eight to ten species in Finland (TERHIVUO, 1989).

The soil being deeply and heavily compacted below the roads, we postulate that underground population exchanges are very unlikely. Thus from the earthworm standpoint the lawns of Roosevelt avenue can be considered as a string of islands cut off from the gardens and parks of the city by the roadways of the avenue. One can then wonder if the composition of the earthworm communities of the avenue is mainly a relict of the fauna present before the construction of the avenue or that of the embankment soils, and if the theory of islands can be applied.

If the theory of islands applies, the size of the islets and their specific richness should be significantly correlated, but this was not the case. Another expected effect of insularity would be that these small islets (900 to 2000 m^2) should contain less species than larger parks. According to the preliminary sampling, the 12 islets of Roosevelt avenue contained on average 5.58 ± 1.00 species, whereas the lawns of 19 parks of Brussels studied with the same method and with the same sampling effort (JOSENS & PIZL, unpublished data) housed 5.84 ± 1.77 species. The slight difference is not significant (t test, $p=0.65$). Therefore no effect of insularity can be detected based on specific richness.

Comparison of the earthworm communities in the lawns of Roosevelt avenue with a series of 19 parks of Brussels, using the correlation coefficient of the relative frequency of species (Table 6), revealed good similarity ($r=0.69$, $p=0.04$). The five commonest species of the parks (present in at least 33% of samples) were all represented in the lawns of the avenue. However, two species of *Lumbricus* (*L. castaneus* and *L. terrestris*) were present in the islets but significantly less frequently than in parks, whereas the reverse applied for *Aporrectodea longa*.

TABLE 6

Relative species frequency of earthworms extant in the parks of Brussels and the islets of Roosevelt avenue (Brussels, Belgium).

	Parks of Brussels (n = 19)	**Roosevelt islets (n = 12)**	***Fisher test***
Allolobophora chlorotica	0.32	0.25	*ns*
Aporrectodea caliginosa	0.89	1.00	*ns*
Aporrectodea icterica	0.32	0.58	*ns*
Aporrectodea limicola	0.21	0.00	*ns*
Aporrectodea longa	0.26	1.00	*0.0001*
Aporrectodea rosea	0.95	1.00	*ns*
Dendrobaena octaedra	0.05	0.00	*ns*
Eiseniella tetraedra	0.05	0.00	*ns*
Lumbricus castaneus	0.63	0.17	*0.01*
Lumbricus rubellus	0.89	0.92	*ns*
Lumbricus terrestris	0.68	0.17	*0.006*
Octolasion cyaneum	0.16	0.00	*ns*
Octolasion lacteum	0.26	0.00	*ns*
Octolasion tyrtaeum	0.05	0.00	*ns*
Satchellius mammalis	0.10	0.33	*ns*

ns: difference not significant

It is worthwhile to point out the distribution of at least two species: *Satchellius mammalis* was present from islet 5 to 8 and absent elsewhere (Table 2), and *Aporrectodea icterica* was present from islet 2 to 8 and absent elsewhere. This kind of distribution suggests a spreading from a centre that might be in islet 6 or 7 for *S. mammalis* and in islet 4, 5 or 6 for *A. icterica*. If this is true, it means that population exchanges can occur between the islets. Actually the vehicular traffic is much more intense alongside the islets than between them, which should allow easier exchanges between islets.

Before discussing the absolute values of density and biomass we should point out that the standard deviations of these parameters are very high (Tables 3 and 4). This is a consequence of a complex set of effects: the efficiency

of the electric method and the activity of earthworms both depend on temperature and soil humidity, and our sampling was spread over a complete year including a cold winter and a dry summer.

Our figures of density and especially of biomass are fairly high in comparison with data in the literature. The latter rarely exceed 120 g/m² (NORDSTRÖM & RUNDGREN, 1973, LEE, 1985, TERHIVUO, 1989, STANDEN, 1979, DECAËNS et al., 1997) for samplings performed in the most favourable periods (spring and/or autumn), whereas they rank from 50 to 150 g/m² in Roosevelt avenue. Furthermore, our figures, based of an annual average, are obviously underestimates since the method can extract about 87% of worms only in good conditions (THIELEMANN, 1986) and efficiency is obviously much lower during cold winter weeks and dry summer periods (moreover *A. longa* is in diapause from late May till late August).

These high density and biomass figures may be explained by the abundance of food and by the rarity of predators. The grass is mown twice a month from April to October without being exported, and thus represents a regular supply of food. Moreover, PIZL & JOSENS, 1995a showed that there was no obvious impact of soil pollutants from car traffic on the communities of earthworms in the avenue. Therefore, competition and dependence on soil characteristics must be the major regulatory factors acting on those communities (SATCHELL, 1967, *in* PHILLIPSON et al., 1976).

Population biomass proved to be correlated positively with silt and negatively with pH. Although the best correlation was found with pH we doubt that this parameter, which varies in a very narrow range (from 6.9 to 7.15), can explain the biomass variation between 50 and 150 g/m², and this all the more so since this correlation is negative. This unexpected correlation can actually be explained by the presence of small amounts of carbonates in the two sandiest soils. This kind of correlation should not occur in most natural soils but is quite likely to occur in urbanised soils.

Since pH is also correlated negatively with silt and water and positively with sand, the large biomass variation probably relies on a complex set of parameters. This should be analysed factorially, but unfortunately we did not have sufficient measurements of each of the parameters to perform such an analysis. This variation is probably initiated by the soil texture: in the islets 5 and 8 with high silt (plus clay) content, water and organic matter are retained more strongly and for longer on soil particles, a situation that favours high biomass.

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Sex differentiation in the spotless starling (*Sturnus unicolor*, Temminck 1820)

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ABSTRACT. Five biometric variables (weight, and beak, tarsus, wing and middle-toe lengths) and the shape of throat feathers were analysed in spotless starlings (*Sturnus unicolor*) from Northern Spain. Sexes were correctly differentiated in 97.4% of the adults and 84.7% of the juveniles using a combination of wing and middle-toe lengths. All the adult birds and 88.3% of the juveniles were correctly sexed using only the shape of the throat feathers.

KEY WORDS: Spotless starling, *Sturnus unicolor*, biometry, sex differentiation

INTRODUCTION

Populations of starlings (*Sturnus vulgaris*, L. 1758) and spotless starlings (*Sturnus unicolor,* Temminck 1820) have increased in the Iberian Peninsula (FERRER et al., 1991; DOMINGO, 1993) and Southern France (CAMBRONY et al., 1993). Both are considered plague species since they can seriously affect agriculture and livestock (FEARE, 1989).

The control of plague species frequently involves distinguishing between sexes (MURTON et al., 1972; LACOMBE et al., 1987) especially when the species is polygamous. For this, reliable and simple sex differentiation characteristics are important, both for species management and scientific ringing (SVENSSON, 1992) and to establish intra- and inter-specific geographic differences (JAMES, 1970; BÄHRNANN, 1978).

After the post-reproductive or post-juvenile moult (July to October), spotless starling sexes are difficult to distinguish by morphology alone (PERIS, 1989; HIRALDO & HERRERA, 1974). In passerines without obvious sex dimorphism, sexes are normally differentiated by the shape of specific feathers or using biometry (SVENSSON, 1992). Some authors have used the middle-toe length to differentiate sexes in the loggerhead shrike, *Lanius ludovicianus* (COLLISTER & WICKLUM, 1996) and spotless starling (FALCETO et al., 1997). In this paper we present a sex differentiation method for the spotless starling using two biometric (wing and middle-toe length) characteristics and one morphological (throat feather shape) characteristic, which can also be applied to the starling and other passerines.

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MATERIAL AND METHODS

From October to January, 1997-1999, during a legal control trapping we captured 229 spotless starlings in mist nets in roosts near Calahorra, Northern Spain (42º19'N 1º58'W). Of these, 208 were measured (following SVENSSON, 1992) for: a) maximum wing span, with a 1 mm precision ruler; b) tarsus, by the standard measurement; c) beak, from just before the nostril to the end; d) middle-toe, from the beginning of the nail to the beginning of the toe (Fig. 1); and e) weight, with a 1 g precision balance. Only some of these variables were measured in the remaining 21 birds (eight of them adults) because of beak or tarsus deformities or feather deterioration. Tarsus, beak and middle-toe lengths were measured with a 0.1 mm precision calliper.

To analyse sex differentiation by plumage, three throat feathers were extracted in 65 adults (32 ♂♂ 33 ♀♀) and 135 young (52 ♂♂, 83 ♂♂), taking into account the feather vane. Each feather was divided into three areas: anterior, central and posterior (Fig. 2).

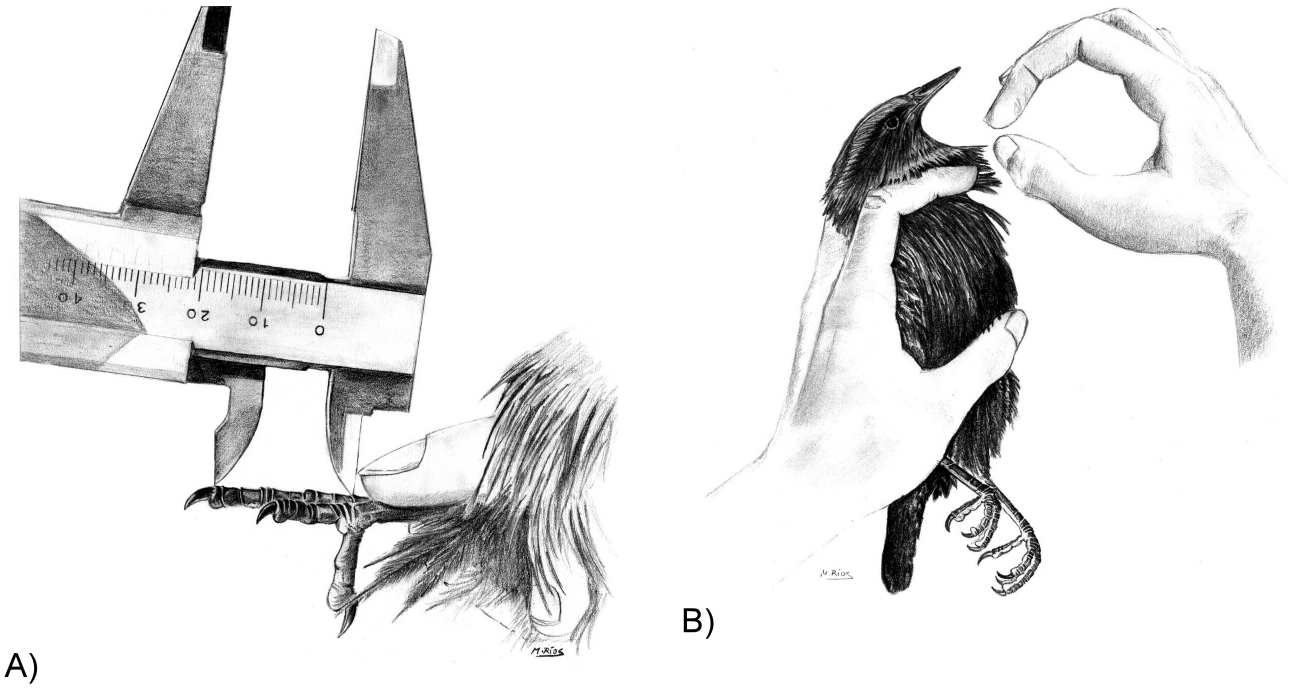

Fig. 1. – A) How to measure middle-toe length in spotless starlings. B) How to obtain throat feathers.

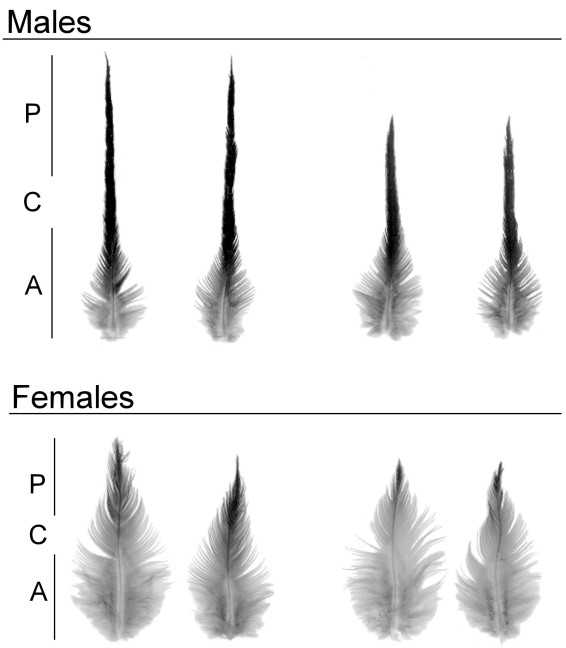

Fig. 2. – Throat feathers of male (above) and female (below) adult spotless starlings, distinguishing between the anterior (A), central (C) and posterior (P).

Bird sex was verified by dissection. The spotless starlings were grouped into two age classes: juveniles (first calendar year) and adults (second or later calendar year). All the juveniles had the relevant characteristics described by HIRALDO & HERRERA (1974).

The five measured variables followed a normal distribution, verified by the Kolmogorov-Smirnov test. The Student t-test was used to test if these values differed significantly between sexes. A discriminant analysis verified which variables more precisely segregated the sexes, using coefficients to determine the importance of each measurement (KLECKA, 1986). The more precise variables described a linear function with a limiting value, above or below which birds were either male or female, respectively.

RESULTS

TABLE 1

Averages (± SD) and ranges of the measured variable (length in cm, weight in g) in spotless starling males and females. N: sample size. t: Student t-test value (all P<0.001).

	♀♀	♂♂	t
Wing	12.78 ± 0.44	13.24 ± 0.26	9.44
Range	11.4 - 13.4	12.6 - 13.8	
N	122	107	
Middle-toe	2.13 ± 0.05	2.24 ± 0.05	15.11
Range	1.96 - 2.25	2.05 - 2.35	
N	114	9	
Beak	2.17 ± 0.09	2.25 ± 0.10	6.82
Range	1.97 - 2.39	2.07 - 2.62	
N	121	102	
Tarsus	2.84 ± 0.1	2.93 ± 0.09	6.07
Range	2.21 - 3.06	2.69 - 3.10	
N	123	105	
Weight	77.90 ± 5.40	84.40 ± 6.47	6.80
Range	64.0 - 89.0	72.0 - 105.0	
N	90	65	

Of the 229 spotless starlings captured, 144 were juveniles (61 ♂♂, 83 ♀♀) and 85 adults (45 ♂♂, 40 ♀♀). The

values of the five measured variables overlapped between sexes but were significantly higher in males (Table 1).

The discriminant analysis ordered the variables according to their importance in sex discrimination (standardised coefficients are in brackets): toe (0.889), wing (0.287), weight (0.253), beak (-0.021) and tarsus (-0.138). The first three correctly classified 87.8% of the 208 specimens, while wing and toe correctly classified 89.3%. The variable, weight, did not contribute to the discriminant capacity of the other two variables. The correlation between tarsus length and middle-toe length was relatively low ($r=0.536$, $n=207$, $P<0.01$). For this reason we chose wing length and middle-toe length for the sex differentiation analysis.

The discriminating functions between sexes using the wing and middle-toe (Table 2) helped us to correctly classify 97.4% of the adult spotless starlings (Fig. 3) and 84.7% of the juveniles.

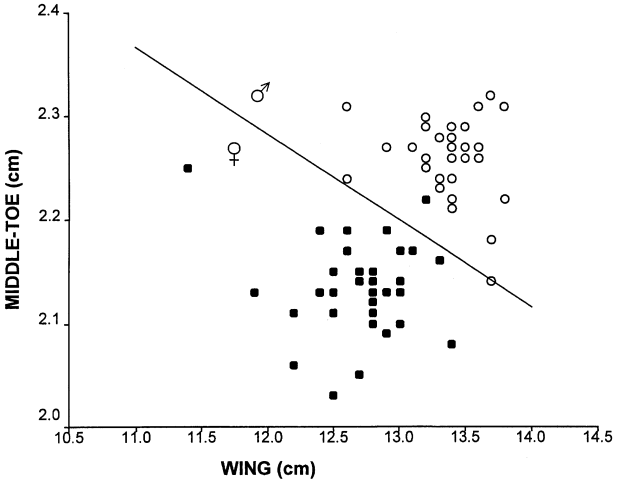

Fig. 3. – Relationship between the wing and middle-toe lengths (cm) in adult male (♂) and female (♀) spotless starlings. The line represents the discriminant function.

TABLE 2

Discriminant functions between sexes in the spotless starling (below, the simplified functions) obtained using the wing (A) and middle-toe (D) lengths (cm). Values for $y>0$ correspond to males, values $y<0$ correspond to females. Sample size in brackets.

	Discriminant function	Correctly classified birds
Juveniles	$y = 16.251\ D + 0.824\ A - 45.930$	84.7% (131)
Adults	$y = 22.222\ D + 1.870\ A - 73.174$	97.4% (77)

Throat feathers were clearly different between adult males and females (Fig. 2). All males had feathers with an abrupt narrowing of the barbs in the anterior and/or central region that continued towards the posterior end. The vane width in all the female adults gradually decreased. This sex difference was less precise for juveniles, of which we correctly classified 88.3% of the males and 100% of the females.

DISCUSSION

The spotless starling is fundamentally sedentary and moves approximately 10 km during daily displacements from the roost to foraging areas (Peris, 1991). For this reason, population overlap with starlings of a different geographic origin (or different size) would tend to be rare.

Using a combination of wing and middle-toe measurements, we could precisely sex the majority of adult spotless starlings. Only one female, whose middle-toe length was above normal (2.25 cm), was classified erroneously. For one male specimen, the value of the discriminating function was slightly higher ($y=0.07$) than the separation, and it was classified as doubtful. Thus, the reliability of our discriminating function can be considered high, and, in any case, better than other characteristics used to date. On the other hand, all our specimens could be sexed by using both morphometric and plumage data.

In juveniles this reliability was lower, doubtless because of phenological differences in hatching, since the first egg-laying bout is in April and the second in May-June (Peris, 1984).

As in other passerines, further studies are necessary to clarify why male and female spotless starlings have different middle-toe lengths. Throat feather shape clearly differentiates spotless starling adults by sex. It was also less precise in juveniles possibly because adult males use these feathers for mate attraction in the breeding season (Feare, 1986), while they are not completely developed in male juveniles. The shape of some feathers has also been used to differentiate sexes in other Passeriformes (e.g. goldcrest, *Regulus regulus*, and rose-coloured starling, *Sturnus roseus*, Svensson, 1992).

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Development of the sickle canal, an unrecognized formation in the avian blastoderm, and its spatial relationship with the first appearing blood islands, induced by Rauber's sickle

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ABSTRACT. In the present study, we demonstrate the existence in avian blastoderms of a voluminous (approximately 2-4 mm long), previously unrecognized sickle-shaped canal (termed sickle canal). It usually bulges into the subgerminal space and is localized near the caudo-lateral border of the area pellucida after approximately one day incubation. The sickle canal, which is always visible on sections, is found both in the chicken and in the quail blastoderm. It seems to function as an expansion space for lateral migration of mesoblast cells, between epiblast and endoblast. The origin and evolution of the sickle canal have been followed (using quail-chick chimeras), by apposing quail Rauber's sickle fragments on fragments of unincubated chicken blastoderms. It was seen that part of the wall of the sickle canal is formed by endoblast derived from Rauber's sickle, i.e. transitional and junctional endoblast. Very obvious, on sections through the chimeras, is the intimate contact between the V or U-shaped quail junctional endoblast and the first formed blood islands, developing from mesoblast that migrates peripherally over the sickle canal. Our study demonstrates that even in the absence of the area opaca, a sickle canal forms and blood islands start to develop from mesoblast of the area pellucida under the influence of junctional endoblast (derived from Rauber's sickle). Rauber's sickle and its derivatives seem thus to be the major organizers of the avian blastoderm. During early incubation they induce the formation of endomesoblast ingressing via the primitive streak (CALLEBAUT & VAN NUETEN, 1994), and somewhat later junctional endoblast induces the development of blood islands from the most laterally ingressed mesoderm.

KEY WORDS : avian blastoderm, Rauber's sickle, sickle canal, junctional endoblast, blood islands.

INTRODUCTION

Recent studies (CALLEBAUT, 1993a, b, c, 1994 ; CALLEBAUT & VAN NUETEN, 1994, 1995, 1996) yielded new data about the structures and developmental events in avian intra-uterine germs after bilateral symmetrization and in unincubated eggs. The terminology of the different components of an unincubated quail blastoderm and surrounding structures is represented in Fig. 1. The term Rauber's sickle (RAUBER, 1876) is used instead of Koller's sickle, since Rauber was the first to describe it (CALLEBAUT & VAN NUETEN, 1994). We used the term sickle endoblast because we have demonstrated that this part of the deep layer is directly derived from Rauber's sickle (CALLEBAUT & VAN NUETEN, 1994).

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The anti-sickle region (Fig. 1) was first described by CALLEBAUT (1993a) in gravitationally oriented quail germs. In this anti-sickle region, an irreversible disruption takes place between the future cranial part of the germ and the underlying subgerminal ooplasm at the moment of bilateral symmetrization (CALLEBAUT, 1993b, 1994). The anti-sickle itself is formed by a sickle-shaped group of loose yolk masses and cells located below the upper layer (UL) in the cranial recessus of the subgerminal space. The upper layer from the anti-sickle region of the unincubated chicken blastoderm is still uncommitted (CALLEBAUT & VAN NUETEN, 1995 ; CALLEBAUT et al., 1998a). Rauber's sickle divides the area pellucida into a peripheral caudal area marginalis and an area centralis. The area centralis contains a subgerminal space filled with liquid. By contrast, Rauber's sickle and the caudal marginal zone are directly in contact with

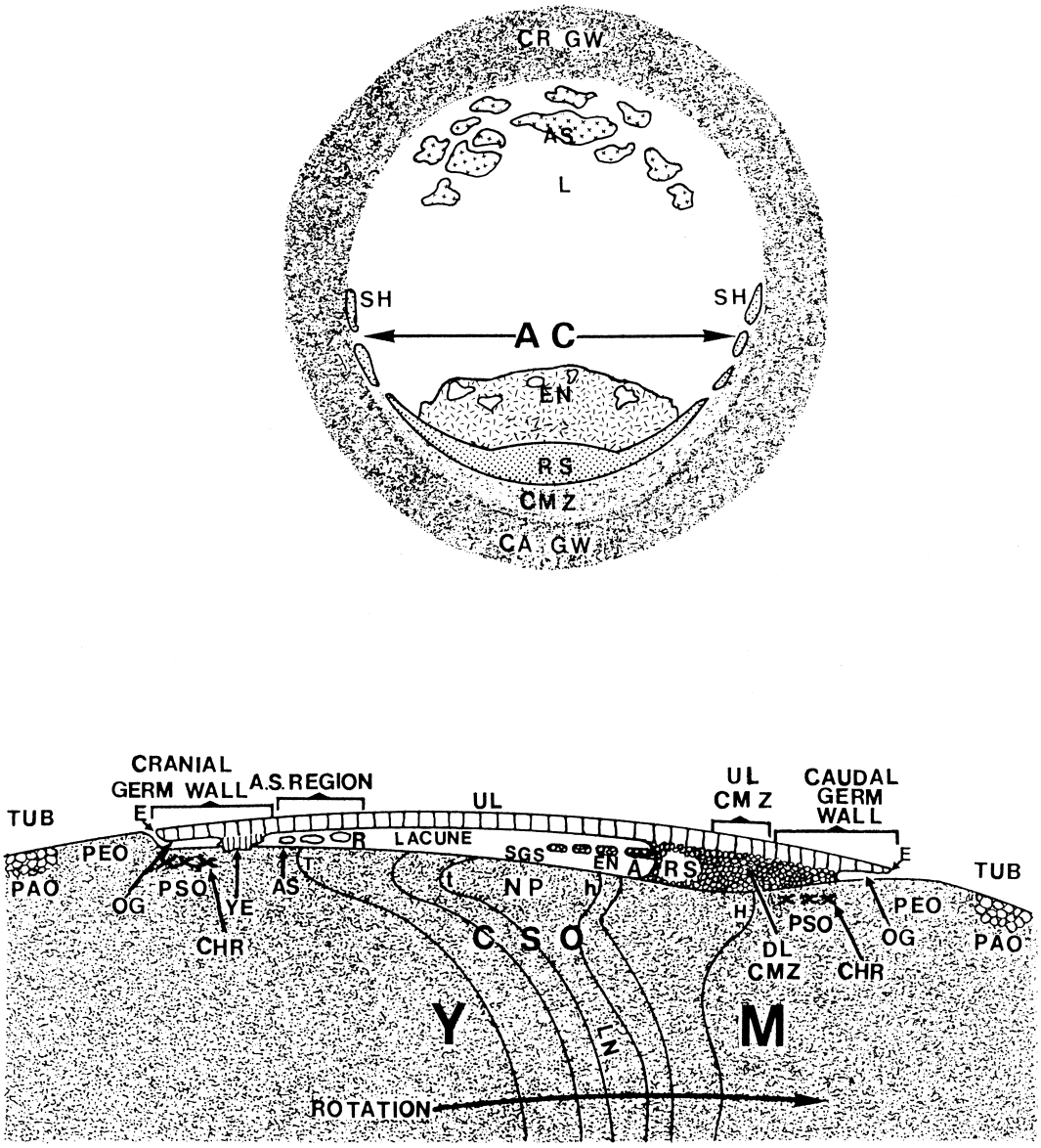


Fig. 1. – Top: Schematic representation of the components of the unincubated quail blastoderm seen from below after removal of the subgerminal ooplasm, ready for *in vitro* culture; CR GW: cranial germ wall; AS: anti-sickle region; L: lacune in the deep layer; EN: incomplete endophyll sheet; SH: fragmentary sickle horns; CMZ: caudal marginal zone more or less transparent; CA GW: caudal germ wall. AC: area centralis enclosed by Rauber's sickle (RS) and its sickle horns.

Bottom: Schematic representation of a mediosagittal section through an unincubated quail blastoderm with surrounding ooplasms after fixation *in situ* on the egg yolk ball. UL: upper layer; EN: incomplete endophyll layer; RS: Rauber's sickle; UL CMZ: upper layer from the caudal marginal zone; the caudal marginal zone being a more or less transparent part adherent to the caudal peripheral subgerminal ooplasm (PSO) via a deeper part (DL CMZ); SGS: subgerminal space forming a caudal pocket A (axilla shaped) and a cranial recess (R) in which free yolk masses or sometimes cells are found forming the anti-sickle (AS); E: edge of the blastoderm; OG: early overgrowth zone (Callebaut & Meeussen, 1988); YE: early development of the yolk endoblast, growing into the peripheral subgerminal ooplasm (PSO); CHR: chromosome clusters; PEO: perigerminal ooplasm; PAO: paragerminal ooplasm forming a tubulin-rich ring (TUB) at distance from the edge of the blastoderm (Callebaut et al., 1996b); YM: the voluminous yolk mass of the egg yolk ball in which the eccentricity of the successive yolk layers parallel with the eccentricity in the blastoderm is represented; CSO: central subgerminal ooplasm in which the central nucleus of Pander (NP) (Pander, 1817) is seen; t: toe-shaped and h: heel-shaped part of the nucleus of Pander; T: toe-shaped and H: heel-shaped part of the surrounding yolk layers as result of the rotation *in utero* (the arrow indicates the direction of rotation and compression of the yolk mass under the combined influence of gravity and egg rotation) (Callebaut, 1983, 1993a); LN: bent latebra neck. Note that by contrast to the caudal germ wall, the cranial germ wall is disrupted from the underlying peripheral subgerminal ooplasm (Callebaut, 1993, a, b, c).

the caudal underlying peripheral subgerminal ooplasm without underlying cavity. In the caudo-central region of the area centralis a more or less developed sheet of endophyll can be seen. We used the name endophyll (CELESTINO DA COSTA, 1948), and not primary hypoblast, to distinguish the endophyll from Rauber's sickle *Anlage*, which appears earlier or at the same time (CALLEBAUT, 1993a, 1993c, 1994). Previous studies (CALLEBAUT & VAN NUETEN, 1994; CALLEBAUT et al., 1996a) indicate that the main function (i.e. definitive endoderm and mesoderm induction in the upper layer) of Rauber's sickle in avian blastoderms is homologous to the function of Nieuwkoop's centre (NIEUWKOOP, 1969, 1973) in amphibian blastulas. CALLEBAUT & VAN NUETEN (1995) have shown that endophyll orients the direction of the primitive streak, starting from Rauber's sickle. Although Rauber's sickle-derived material (sickle endoblast and junctional endoblast) and endophyll have a very important and indispensible inductive function for the development of the definitive embryonic tissues (which are all derived from the upper layer) during gastrulation and neurulation, they never give rise to any definitive structure and therefore belong to the so-called extraembryonic part of the blastoderm. The junctional endoblast forms a whitish structure and can be easily seen at the surface of the living blastoderm *in situ* on the egg yolk ball of a quail egg after 11-13h incubation (Fig. 2). It forms the V or U-shaped part of the deep layer (visible through the transparent UL) in the early avian primitive streak embryo. It is derived from Rauber's sickle cells that migrate in the neighbouring ooplasm during early incubation (CALLEBAUT & VAN NUETEN, 1994). The angle formed by the junctional endoblast is bissected by the primitive streak formed in the upper layer (Figs 2, 3). Laterally from the convexity of the junctional endoblast one can distinguish the yolk endoblast, which has a less dense white aspect but which is more voluminous than the junctional endoblast. The yolk endoblast is localized in the area opaca. The junctional endoblast that forms *in situ* from the Rauber's sickle (CALLEBAUT & VAN NUETEN, 1994), has strong embryo-inducing and dominating potencies (CALLEBAUT et al., 2000a). In the present study, we observed that during avian neurogastrulation, an unidentified sickle-shaped canal (called "sickle canal") develops parallel with the V-shaped junctional endoblast by caudal fusion. Since the general shape and localization of the sickle canal strongly resemble the localization of the first appearing group of blood islands in the avian blastoderm, as described by SETTLE (1954), we tried to find out if there is a relationship between the sickle canal and early

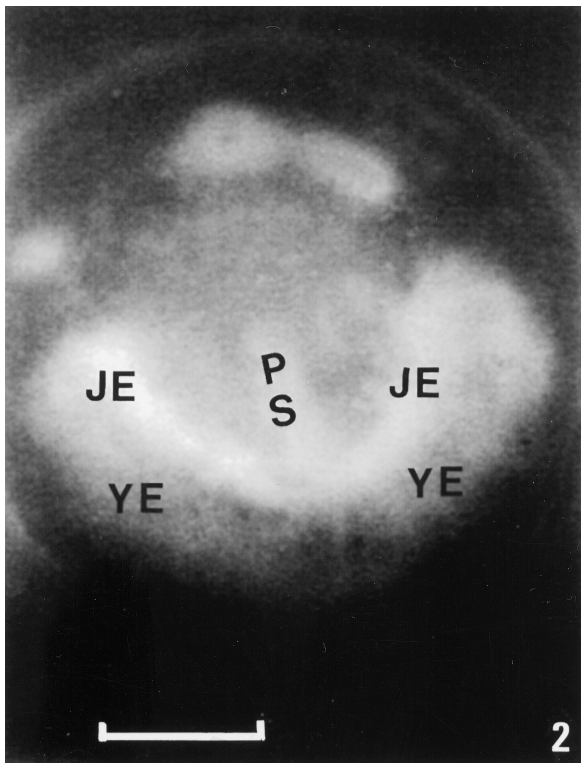


Fig. 2. – Stereomicroscopic photomicrograph of a living quail embryo (Stage HAMBURGER & HAMILTON, 1951) *in situ* on its egg yolk ball, incubated for 12h; PS primitive streak; JE: junctional endoblast; YE: yolk endoblast; bar: 1mm.

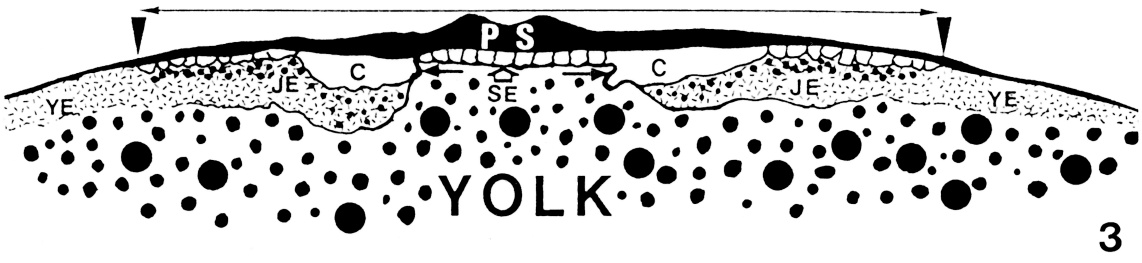


Fig. 3. – Schematic drawing of a transverse section through the primitive streak region (localized between the two vertical arrowheads as a region where the upper layer (UL) is thicker than laterally) of a stage 3+ quail embryo (HAMBURGER & HAMILTON, 1951); between the deep side of the primitive streak (PS) and the sickle endoblast (SE) indicated by a vertical hollow arrow, there are cellular extensions passing between small cavities. The horizontal arrows on the left and the right indicate the transitional endoblast (CALLEBAUT & VAN NUETEN, 1994) which connects the sickle endoblast with the junctional endoblast (JE); C: pararchenteric canals; between the lateral part of the junctional endoblast and the deep side of the primitive streak region there are numerous extensions and small cavities, whilst between the more lateral yolk endoblast (YE) and the UL, there are no cavities and no extensions.

erythropoiesis. An inductory influence of endoderm (endoblast) on the development of blood islands was suspected already at the head-process stage (revised by HAMILTON, 1965). WILT (1965) and MIURA & WILT (1969) have shown that stage 4 (HAMBURGER & HAMILTON, 1951) area opaca vasculosa (AOV) endoderm has a salutary influence on the erythropoietic differentiation of stage 4 AOV ectoderm-plus-mesoderm explants cultured on egg-white-agar medium or on plasma clots on the same or opposite sides of millipore filters. ZAGRIS (1982) has shown that multiple vascular areas (usually with a U-shaped configuration, resembling the area opaca vasculosa) are formed in unincubated or prestreak chicken blastoderms under the influence of multiple transplanted hypoblasts. PARDANAUD et al. (1996, 1999) have shown by means of quail/chick transplantation that two subsets of the mesoderm give rise to endothelial precursors: a dorsal one, the somite, produces pure angioblasts (angiopoietic potential), while a ventral one, the splanchopleural mesoderm, gives rise to progenitors with a dual endothelial and hemopoietic potential (hemangiopoietic potential). In most studies of early avian erythropoiesis, the authors describe a stimulatory influence of endoblast on the formation of blood islands. However, the source of this endoblast (originally derived from the area opaca or not?) is not mentioned or is not known. In the present study, using quail-chick chimeras, we demonstrated that the bottom of the sickle canal is formed by junctional endoblast. The first blood islands develop in the most lateral part of the mesoblast when it slides over this junctional endoblast, borderzone between area pellucida and area opaca, just peripherally from the sickle canal. This suggests an induction effect of the junctional endoblast on the mesoblast to form the first blood islands.

MATERIAL AND METHODS

Stereomicroscopic and histological observations on sections of primitive streak blastoderms of quail or chicken

Eggs from chicken or quail (*Coturnix coturnix japonica*) were incubated at 38-39°C during 13-30h.

A) Some of these blastoderms were removed from their egg yolk balls, observed and photographed in the living state. Thereafter, they were fixed in calcium-formalin or in Susa without sublimate (ROMEIS, 1948) for 1 night.

B) Other blastoderms remained *in situ* on their egg yolk balls. Some of their structures, visible from the surface, were labelled by placing charcoal particles or carmine on the surface of the vitelline membrane. The blastoderms still *in situ* on their egg yolk balls were fixed in the same fixatives as in A.

C) From other unincubated chicken or quail eggs the blastoderms were removed and cultured *in vitro* for 20-60h according to the technique of NEW (1955). Instead of Petri dishes, the culture vessels described by GAILLARD (1949) on which an optically flat glass cover was sealed with hot paraffin, were used. Fixation was performed in Susa without sublimate (ROMEIS, 1948) for 1 night (as mentioned above). After fixation, all the isolated blastoderms or blastoderms still *in situ* on their egg yolk balls were placed in tap water and progressively dehydrated in an alcohol series. The blastoderms, which were still on their egg yolk ball, were excised together with some subgerminal ooplasm in the absolute alcohol bath. After clearing in xylene, all the blastoderms were embedded in paraffin. The blastoderms were sectioned perpendicularly to their caudocephalic axis. After deparaffination, the sections were stained with Unna (after SILVERTON & ANDERSON, 1961) or with iron hematoxylin and eosin.

Study of chimeras developing under influence of quail Rauber's sickles placed on unincubated chicken blastoderm parts in culture

This was done to determine the origin and to follow the development of the sickle canal and associated structures (i.e. the embryonic blood islands). The experimental procedure used (placing quail Rauber's sickle fragments on parts of unincubated chicken blastoderms) is represented in a scheme accompanying each of the photomicrographs of the studied chimeras. The quail-chicken chimeras were cultured for 24-31h according to NEW (1955) or according to SPRATT (1947). In the latter case the culture medium was not pure egg white, but a mixture of 25ml thin egg white and a gel made of 150mg Bactoagar (Difco, Detroit, Mi) in 25ml Ringer's solution. This semi-solid medium allowed microsurgery and further culturing on the same substratum. Stereomicroscopic polaroid photographs were taken in the same direction at the beginning, during and at the end of the culture period. After fixation, dehydration and embedding in paraffin wax as described above, the chimeric blastoderms were sectioned perpendicularly to the visible or presumed axis. The deparaffinized, 8-µm-thick sections were Feulgen-stained after DEMALSY & CALLEBAUT (1967) in order to identify the origin of the nuclei, using microscopic objectives x10 or x20. This allowed us to observe the typical central or subcentral chromatin granule of the grafted quail cells (CALLEBAUT, 1968; KOSHIDA & KOSIN, 1968; LE DOUARIN & BARQ, 1969), as well as to gain an overview of the distribution of the quail cells among the chicken cells. Some sections were stained with iron hematoxylin and eosin. The photographs of sections of cultured blastoderms or chimeras were represented with the deep layer directed downwards although they were cultured with the deep layer upwards.

RESULTS

Stereomicroscopic and histological observations

After removal of the blastoderm from its egg yolk ball

Under the stereomicroscope with incident light illumination, at the lower surface of a living primitive streak blastoderm, sometimes a V-shaped transparent canal can

be seen (Fig. 4). Both sides (each approximately 1mm long) of the canal converge on the caudal midline forming a valve-like structure. The whole sickle canal, seen from the deep side, takes the aspect of a uterus bicornis. Very close to and immediately behind and lateral of the membranous part of the canal, a dense V-shaped structure can be seen. It is formed of junctional endoblast (confirmed on sections). Both sides of the sickle canal end blindly cranially, and no connections are seen with the cranial endophyllic (germinal) crescent. The cranial ends of the sickle canal do not reach the level of Hensen's node. On a low power view of a section through such a blastoderm after fixation and staining (Fig. 5), the gross morphology of the sickle canal and neighbouring structures can be observed. In a transverse section, the lumen of the sickle canal has a diameter of approximately 200µm. Laterally, the lumen extends as a narrow slit above the junctional endoblast. The bottom of the sickle canal is formed medially by the thin transitional endoblast (CALLEBAUT & VAN NUETEN, 1994) and laterally by the massive junctional endoblast. The roof of the canal is formed by mesoblast extending laterally above the junctional endoblast as a thickening (forming blood islands below the epiblast). The border zone between area opaca and area pellucida is formed by junctional endoblast (derived from Rauber's sickle material). In about half of the isolated blastoderms, no sickle canals are seen from their surface *in toto*. However after sectioning of these blastoderms, it was seen that the sickle canals were always present, sometimes with a less voluminous or asymmetric lumen, or disrupted bottom. In somewhat older blastoderms (Fig. 6), the canals still remain visible but become proportionally smaller.

After fixation in situ on the egg yolk ball

Sections through the caudal region of intermediate streak blastoderms (approximately 13h incubation, corresponding to stage 3 of HAMBURGER & HAMILTON, (1951)) invariably show pararchenteric canals, that form bilaterally an intraembryonic space between mesoblast and deep layer (Fig. 7). The bottom of these canals is first flat, but later on it usually bulges in the subgerminal cavity. The latter contains a liquid, which after fixation forms a cast adhering tightly to the thin sickle endoblast (medially) and to the junctional endoblast (laterally). On sections through the caudal part of older blastoderms (stage 7 of HAMBURGER & HAMILTON, 1951), the sickle canal composed of two asymetric pararchenteric canals can always be seen (Fig. 8). In this most caudal part a narrow connection exists (separated by an incomplete septum) between right and left (Figs 8, 9). In this caudal region, the sickle endoblast is not in direct contact with the ingressing mesoderm, as is the case in more cranial regions. The median sickle endoblast is in intimate contact with and seems tightly adherent to the underlying coagulated contents of the subgerminal cavity (Fig. 9). This tight contact is probably the reason the sickle canal is sometimes no longer visible after dissection from below in the living blastoderm. Laterally, the junctional endoblast is seen (Fig. 10) above which blood islands are forming in the mesoblast localized in the immediate neighbourhood. Also blood islands are seen above the more laterally-localized yolk endoblast in the area opaca. Immediately medial to the junctional endoblast region (at the level of the sickle canal), the blastoderm is formed only by three thin layers (Fig. 10): epiblast, mesoblast and sickle endoblast or transitional endoblast. So, the localization of the sickle canal can usually be seen and labelled from the surface of the living blastoderm as a V-shaped transparent zone. By apposing charcoal particles on it *in vivo*, the latter can be recognized on sections (Fig. 10).

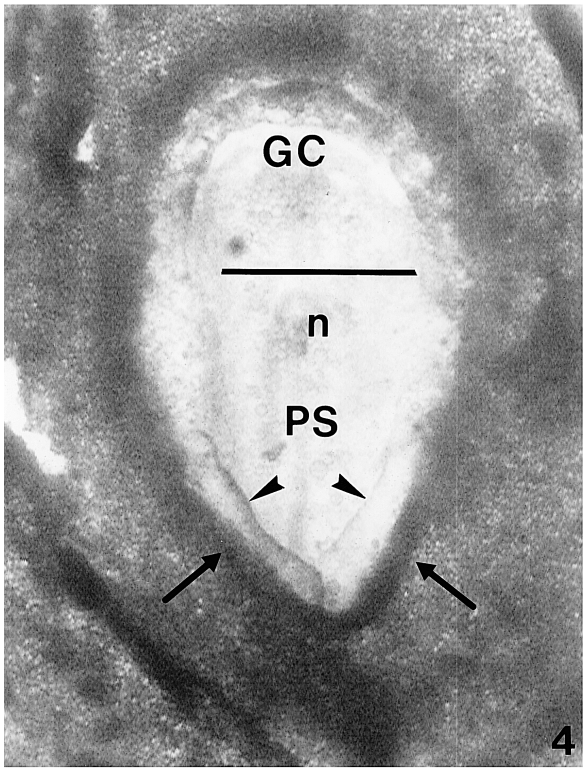
Observations on the development of the sickle canal in cultured blastoderms (starting from the unincubated stage)

After culture for 24-29h, the sickle canal is usually clearly visible (if not hidden by subgerminal contents) behind the caudal part of the embryo (Fig. 11). Frequently lateral extensions of the lumen of the sickle canal are seen. In sections, the latter are seen to extend into the area opaca. At certain stages of development the sickle canal of cultured blastoderms is visible as a broad, flat sac (Figs. 11, 12). After prolonged culture (more than two days), the sickle canal is usually still observed (Fig. 13).

Study of chimeras developing under influence of quail Rauber's sickles placed on parts of unincubated chicken blastoderms in culture

A) In a first experimental group (n=8) the caudo-lateral rim zone including the caudal marginal zone, Rauber's sickle and part of the neighbouring area centralis were removed from an unincubated chicken blastoderm (Fig. 14A). This avoids possible interference with the autochthonous chicken Rauber's sickle. Subsequently a quail Rauber's sickle fragment was placed on the anti-sickle region close to the cranial marginal zone. The stereomicrograph (Fig. 14B) shows a chimera, as represented schematically in Fig. 14A, at the onset of the culture. After 29h, a normal embryo has developed with caudocephalic axis starting from the place where the quail Rauber's sickle fragment was placed, i.e. in a diametrically opposed direction to the presumed original polarity of the chicken blastoderm (Fig. 14C). On sections through the caudal part of this chimeric embryo (Fig. 14D) the formation of a chicken blood island is seen as a swelling of the most lateral extension of the peripherally-migrating mesoblast, in the immediate neighbourhood of the quail junctional endoblast. The latter surrounds the chicken blood island from below. From medially to laterally, the chicken blood island has a denser aspect with numerous mitotic figures.

B) In a second experimental group (n=9), the central part of the area centralis of unincubated chicken blastoderms was sectioned circularly (caudally, at some dis-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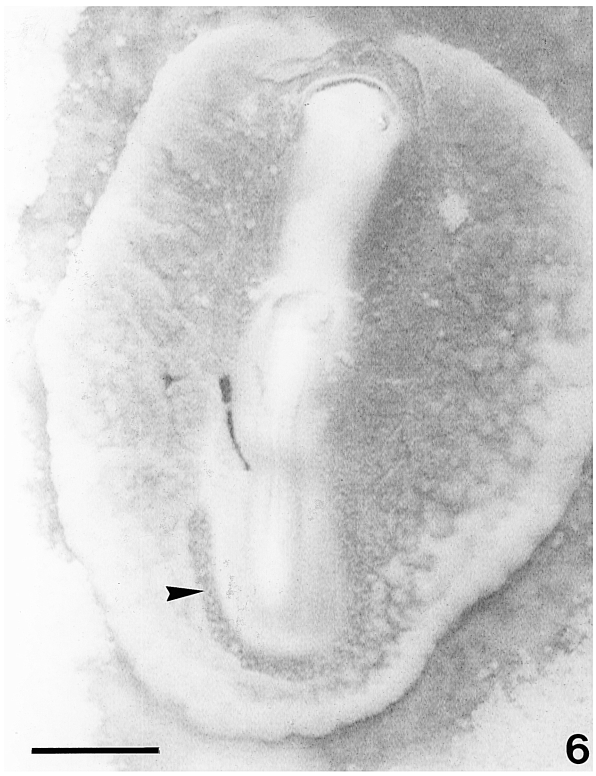
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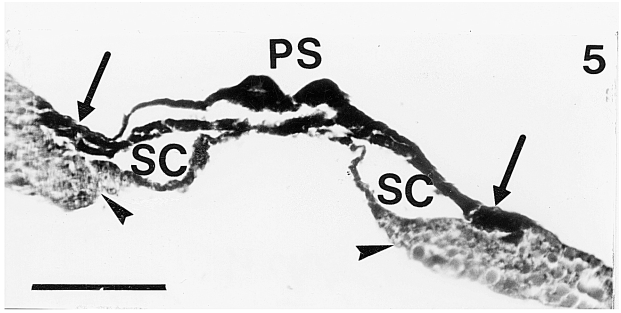
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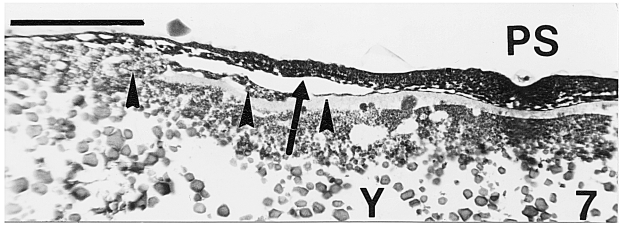
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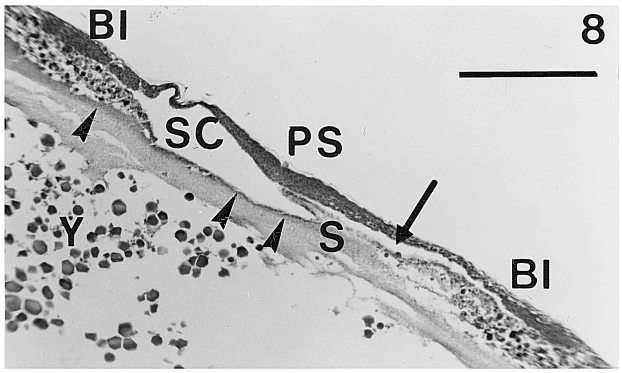
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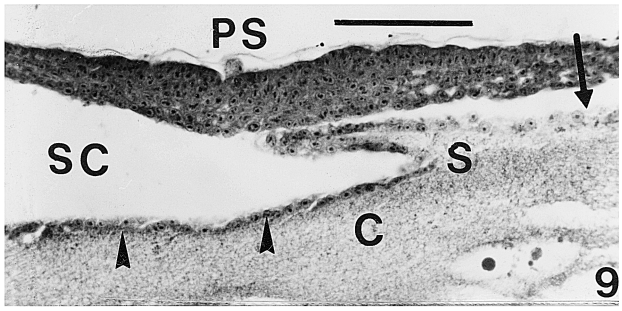
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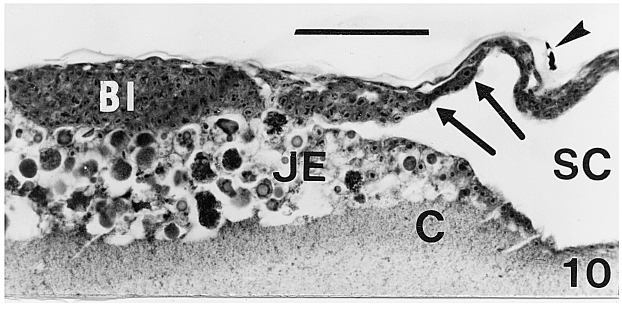
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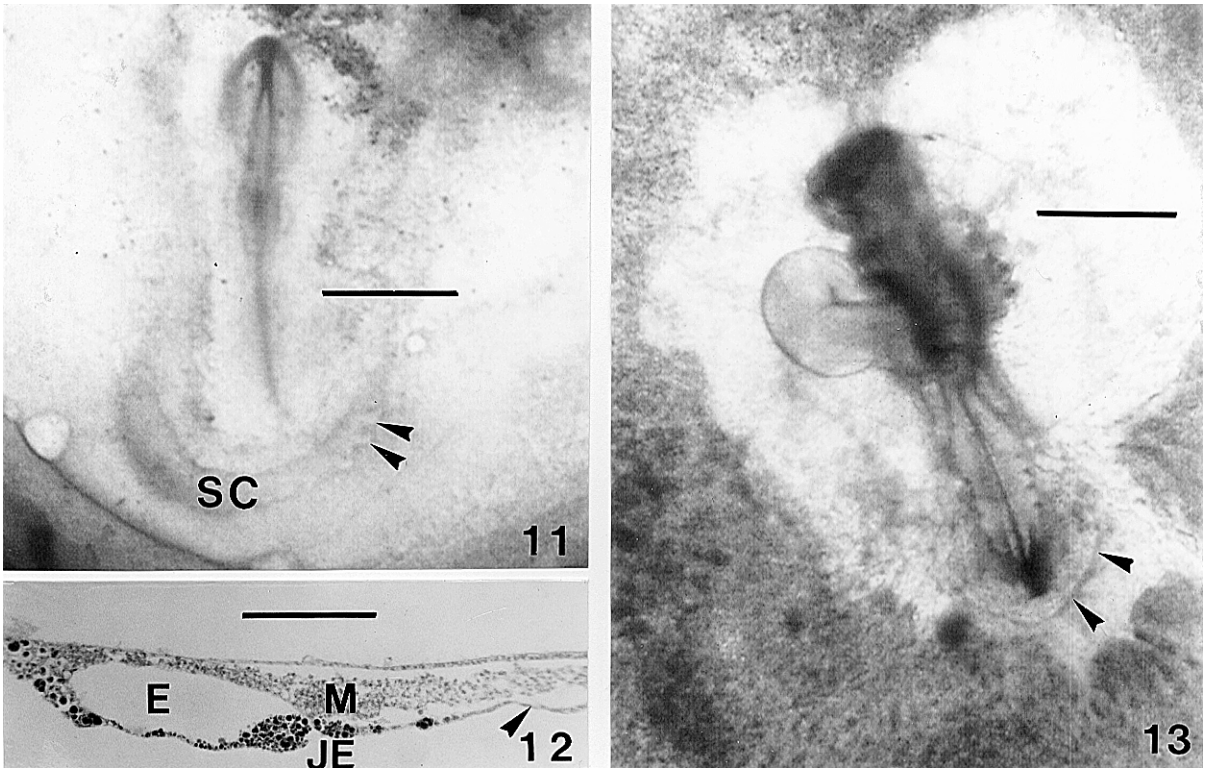


Fig. 4. – Stereomicrograph of a living quail primitive streak blastoderm incubated for 20h (corresponding with a chicken stage 5 of HAMBURGER & HAMILTON, 1951) and removed from its egg yolk ball; as seen from its deep side: the V-shaped thin-walled transparent sickle canal (arrowheads) is very obvious; arrows indicate junctional endoblast on each side; n: Hensen's node; PS: primitive streak; GC: germinal crescent formed by cranially displaced endophyll; oblique illumination; bar: 1mm.

Fig. 5. – Transversal section through a quail blastoderm of the same age as represented in Fig. 4, after fixation in calcium-formalin and staining with Unna; note the presence of the sickle canal (SC) on both sides; PS: primitive streak region; the arrowheads indicate junctional endoblast; the arrows indicate early development of blood islands from mesoblast that migrates peripherally over the junctional endoblast and below the epiblast. Note that one sickle canal would be hidden by the junctional endoblast when observed from below; bar: 200µm.

Fig. 6. – Deep side of fixed quail blastoderm after removal from its egg yolk ball after 31h incubation (corresponding with a chicken stage 7 of HAMBURGER & HAMILTON, 1951); sickle canal indicated by arrowhead; oblique illumination; bar: 1mm.

Fig. 7. – Section through the caudal region of a quail intermediate streak blastoderm (corresponding to stage 3 of HAMBURGER & HAMILTON (1951) in the chicken) fixed *in situ* on its egg yolk ball; PS: primitive streak region; the arrow indicates the lumen of the pararchenteric canal; the arrowheads indicate, from medially to laterally: sickle endoblast, transitional endoblast and junctional endoblast; Y: yolk mass; iron hematoxylin and eosin staining; bar: 200µm.

Fig. 8. – Section through the caudal part of a quail embryo after 23h incubation, (corresponding to a chicken stage 6 of HAMBURGER & HAMILTON, 1951) fixed *in situ* on its egg yolk ball (Y); PS: caudal part of the primitive streak (plate); SC: the lumen of one side of the sickle canal is very wide, whilst the lumen of the other side (indicated by arrow) is narrow; both lumina are separated by an oblique incomplete septum (S); BI: onset of formation of blood islands below the epiblast; the subgerminal space contains a coagulate, which is tigthly fixed to the sickle endoblast or junctional endoblast (arrowheads): iron hematoxylin and eosin staining; bar: 300µm.

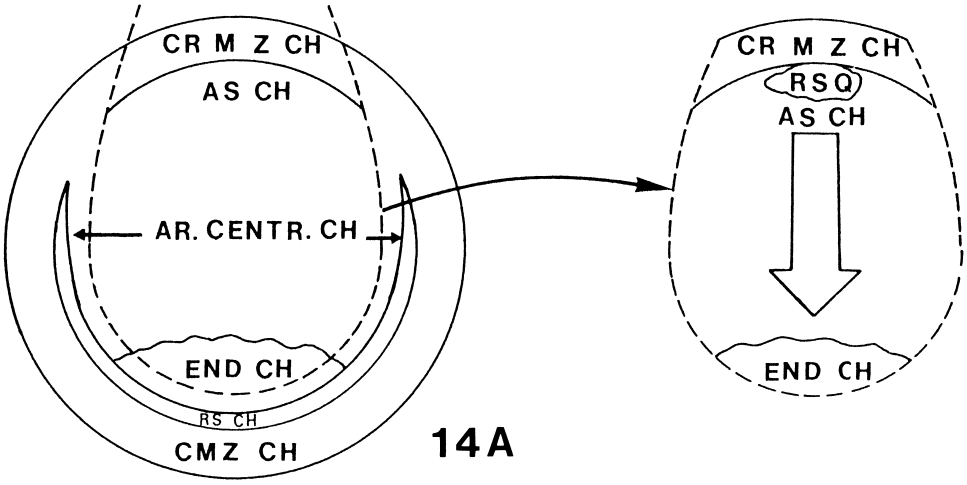
Fig. 9. – Higher magnification of the medial part of Fig. 8; same indications as for Fig. 8; C: coagulate in the subgerminal space, tightly adhering to the sickle endoblast; iron hematoxylin and eosin staining; bar: 100µm.

Fig. 10. – Enlarged view of the lateral part of Fig. 8; SC: lumen of sickle canal: BI: blood island; the arrows indicate mesoblast migrating over the junctional endoblast (JE), forming a blood island; the arrowhead indicates a charcoal particle that was placed *in vivo* on the transparent V-shaped zone visible from the surface; bar: 100µm.

Fig. 11. – Stereomicrograph of a living chicken blastoderm (stage 8 of HAMBURGER & HAMILTON, 1951) after 28h of culture. The sickle canal (SC) has a length of approximately 4 mm; some lateral extensions (indicated by arrowheads) are visible; bar: 2 mm.

Fig. 12. – Section through a lateral extension cavity (E) of the sickle canal seen in Fig. 11. Note the thickening of the mesoblast layer (M) above the junctional endoblast (JE); sickle endoblast is indicated by an arrowhead; Unna staining after calcium-formalin fixation; bar: 200µm.

Fig. 13. – Stereomicrograph of a living chicken embryo after 48h of culture (stage 14 of HAMBURGER and HAMILTON, 1951); the sickle canal (indicated by arrowheads) is still visible; bar: 1mm.



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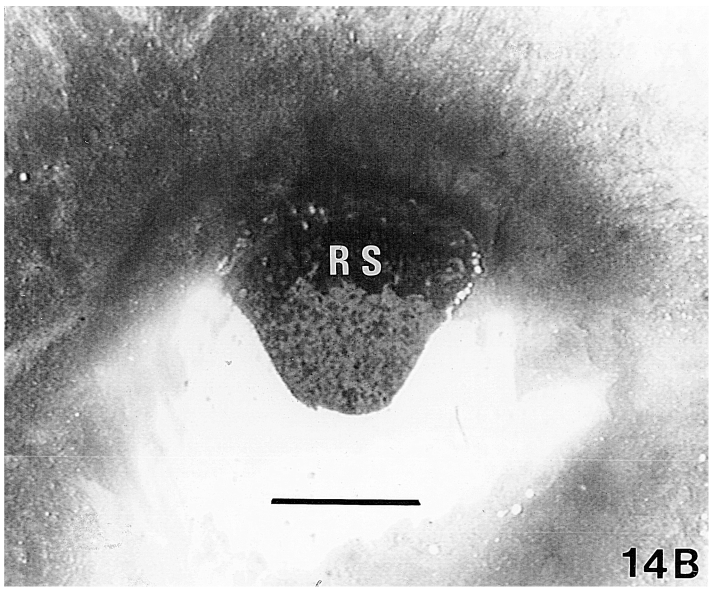

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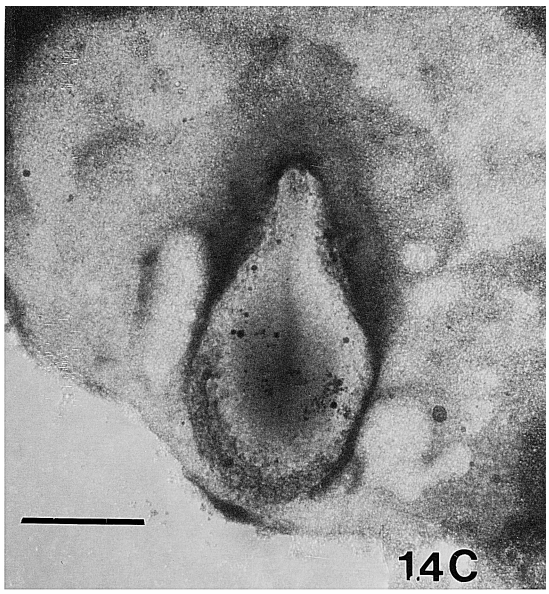

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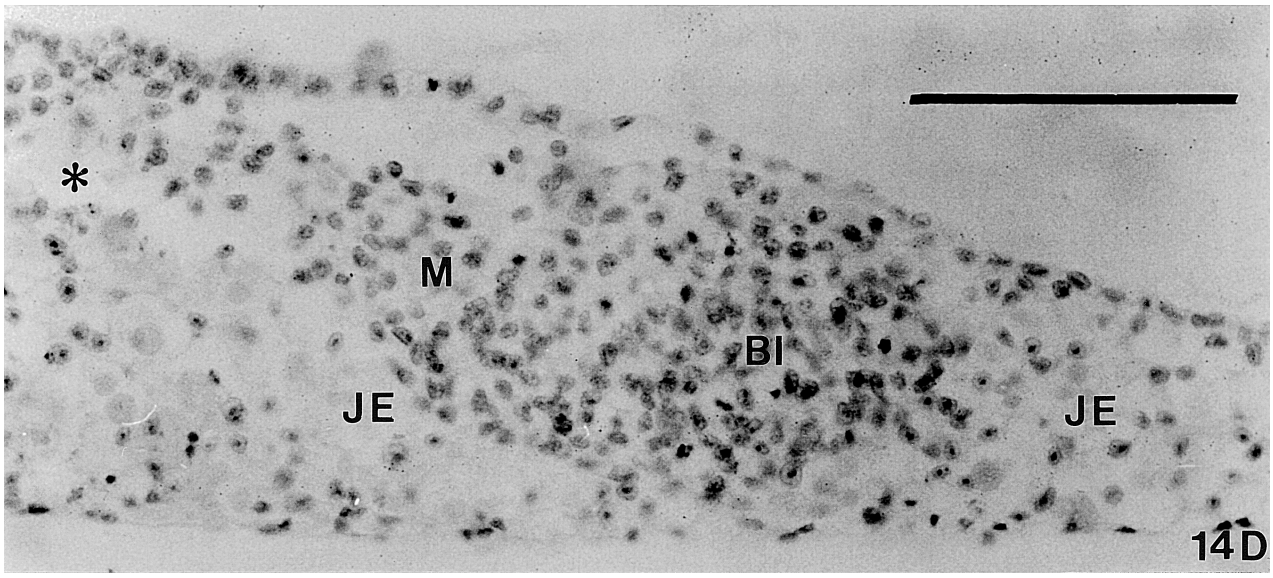


14D

tance from and parallel to Rauber's sickle) (Fig. 15A). On the cranial zone of this central part of the chicken area centralis, which caudally contains some endophyll, a fragment of a quail Rauber's sickle was placed. The photomicrograph (Fig. 15B) shows a chimera, as represented schematically in Fig. 15A, at the start of the culture. After 25h of culture, an embryo has developed (Fig. 15C), again with a caudo-cephalic axis starting from the place where the quail Rauber's sickle was placed, i.e. in a diametrically opposed direction to that originally programmed, had the autochthonous Rauber's sickle been left in place. Fig. 15D, shows a section through the caudal region of the embryo of Fig. 15C. A blood island is forming above the sickle canal. It originates from the most lateral part of the peripherally migrating chicken mesoblast where it comes in contact with the quail junctional endoblast. All the blood islands (formed from chicken cells) seen in these embryos are in close relationship with the quail junctional endoblast. This demonstrates that even in the total absence of area opaca but in the presence of a Rauber's sickle, a sickle canal and blood islands can develop in the area pellucida.

Legends to the figures (see opposite page)

Fig. 14A. – On the left: Schematic drawing representing the incision (indicated by a dotted line) in an unincubated chicken blastoderm, with the aim to remove its caudolateral rim zone including the caudal marginal zone, Rauber's sickle and part of the neighbouring area centralis; CR MZ CH: cranial marginal zone from chicken; AS CH: anti-sickle chicken; AR. CENTR CH: area centralis chicken; END CH: remaining endophyll chicken; RS CH: Rauber's sickle chicken; CMZ CH: caudal marginal zone chicken.
On the right: Scheme representing a quail Rauber's sickle fragment (RSQ) placed on the anti-sickle region (AS CH) of the remaining part of the unincubated chicken blastoderm as represented on the left; the large empty arrow indicates the caudo-cephalic axis of the future embryo that will develop from the apposed quail Rauber's sickle in the direction of the endophyll of the chicken.

Fig. 14B. – Stereomicrograph of a chimera, as obtained according to the procedure represented on Fig. 14A, at the onset of the culture; RS: apposed Rauber's sickle of quail; bar: 2mm.

Fig. 14C. – Stereomicrograph of the chimera of Fig. 14B after 29h of culture: a normal embryo has developed with diametrically opposed direction of the caudo-cephalic axis as could be predicted (see Fig. 14A); bar: 1mm.

Fig. 14D. – Section through the caudal region of the chimera of Fig. 14C; BI: chicken blood island developing from the most lateral part of the chicken mesoblast (M) under influence of the neighbouring quail junctional endoblast (JE); numerous mitotic figures are seen in the blood island; *: lateral extension of the sickle canal; Feulgen staining; bar: 100µm.

DISCUSSION

Neither HAMBURGER & HAMILTON (1951) nor HAMILTON (1965) describe the sickle canal.

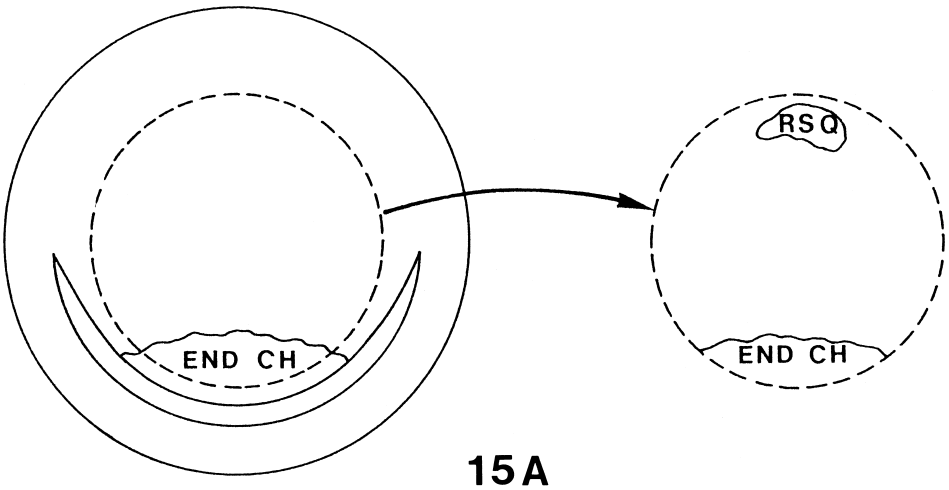
Also in more recent general descriptions of avian embryology (BELLAIRS, 1991, BELLAIRS & OSMOND, 1998), the sickle canal is not mentioned. In the present study, we show that the sickle canal is not a fixation artifact, since it can be observed in the living state. Moreover after fixation of the blastoderm, still *in situ* on its egg yolk ball, the sickle canal or the pararchenteric canals are always seen on sectioned material. After *in vivo* surface labelling with charcoal on a transparent V-shaped zone of a blastoderm of approximately one day incubation, its corresponding localization as sickle canal can be identified on the sections. The sickle canal has until now not been recognized as such for several reasons:

1) Since the endoblastic wall of the sickle canal seems to be tightly fixed to the coagulate present in the subgerminal cavity, its observation can be impaired by adhering coagulate. For the same reason, the very thin deep wall of the sickle canal can be disrupted during dissection from the lower side.

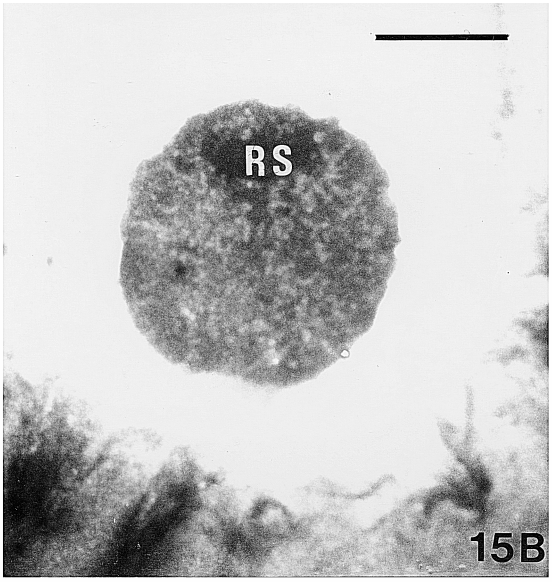
2) The sickle canal is sometimes wholly or partially obscured from its deep side by the neighbouring, also sickle-shaped junctional endoblast (see Fig. 5).

3) The sickle canal is often not clearly visible in transmitted light. By the use of oblique incident light we obtained a much better visualization of this structure.

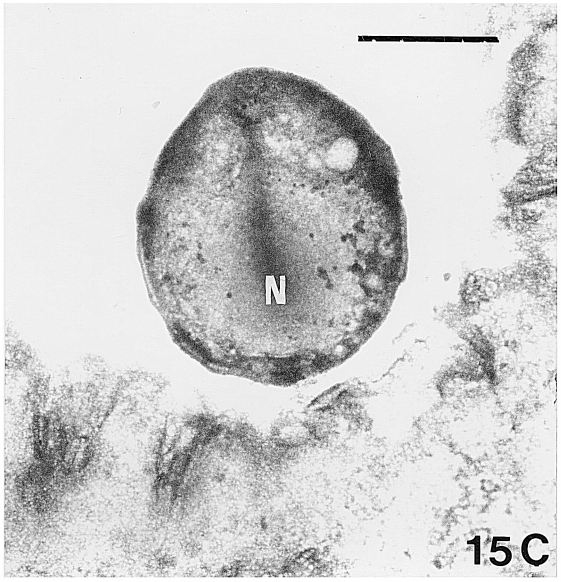
That the sickle canal is not always visible from the surface of the whole blastoderm but always observed on sectioned material can be compared with the visibility of Rauber's sickle, with which it is directly related. Indeed Rauber's sickle, already described by RAUBER in 1876, is also not always easily seen from the exterior of the egg yolk ball or after removal of the blastoderm (CALLEBAUT et al., 1998b). Therefore, for decades its existence was a matter of dispute or totally ignored. It was only in 1994 that CALLEBAUT & VAN NUETEN demonstrated its fundamental importance for the organization of the avian blastoderm during gastrulation. Moreover in the present study we demonstrated that also blood islands are formed under its inductory influence. The extraembryonic mesoderm of the chick embryo originates from the primitive streak and spreads out invasively between ectoderm and endoblast (GRODZINSKI, 1934; FLAMME, 1989). From the second day of incubation (in the chicken) onwards, the area occupied by mesoderm is called area vasculosa due to its abundant vascularization. The surrounding area that is still free of mesoderm is called area vitellina. The area vasculosa (after 2-3 days incubation) is subdivided into two concentric zones, which are named according to their optical properties: the inner transparent area pellucida vasculosa in the center of which the embryo lies, and the surrounding less transparent area opaca vasculosa, which is peripherally limited by the sinus terminalis. The different optical prop-



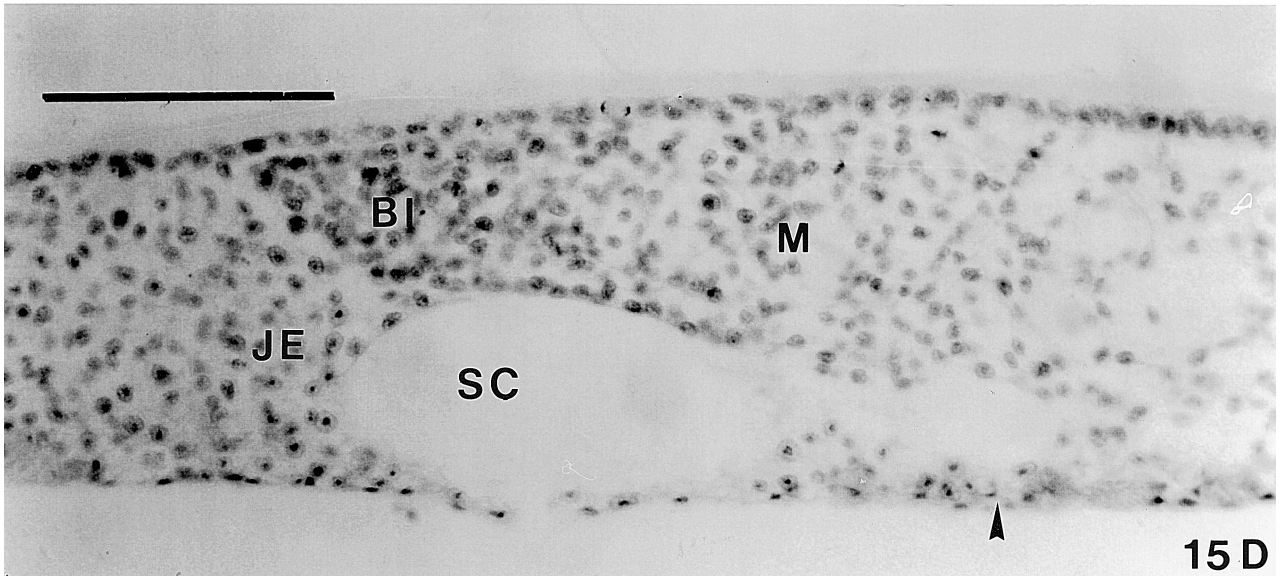
15A



15B



15C



15D

erties of these zones are due to the differing morphology of the endoblast cells: the cells of the area pellucida vasculosa endoblast are flat, while those of the area opaca vasculosa endoblast are high-prismatic and filled with large yolk vacuoles. According to FLAMME (1989) the endoblast cells of the area pellucida vasculosa and area opaca vasculosa are two different populations. The microvilli-bearing flat cells at the periphery of the area pellucida vasculosa are possibly descendants of the primary hypoblast (WAKELY & ENGLAND, 1978), lining the definitive endoderm, which invades from the upper layer through the primitive streak and which during expansion withdraws the primary hypoblast to the periphery. The origin of the erythropoietic stem cells has been analysed in avian chimeras by DIETERLEN-LIÈVRE et al (1976), DIETERLEN-LIÈVRE (1978), BEAUPAIN et al (1980) and PARDANAUD et al. (1987, 1989). These studies suggested that the first red blood cells develop in the area opaca. Therefore, the term "yolk sac" erythropoiesis was used. It encompasses red cell production by this individualized wall in which indeed big numbers of blood islands and erythrocytes appear. This suggested that the primary source of erythrocytes was localized in the area opaca, containing yolk endoblast. However, the experiments of these authors were performed by grafting the central part of the area pellucida of a quail blastoderm into the area opaca of a chick blastoderm around 30h of incubation, according to the technique of MARTIN (1972), or in still older stages. This is later than the moment when the first blood islands become visible (18-22h of incubation) at the head fold stage (HAMILTON, 1965). So, in their experiments, already a migration of the most peripheral mesoblast of the chicken area pellucida into the chicken area opaca had taken place, which could explain why they found only chicken erythrocytes in the circulation of the chimeras during the first days of embryonic life. Our experiments with quail-chick chimeras were, however, performed much earlier, starting with parts of unincubated blastoderms. After placing a quail Rauber's sickle fragment on the central region of the area centralis of an unincubated chicken blastoderm and culture, we observed the formation of blood islands in the total absence of area opaca material. All the chick blood islands, in the formed chimeric embryos, developed in close association with quail junctional endoblast. SETTLE (1954), in a series of experiments with circular pre-streak blastoderm fragments (incubated for several hours and not unincubated as in our experiments) placed in culture, clearly revealed that erythrocytes are never formed from culture of the area opaca only. When a circular cut is made inside the boundary between the area opaca and the area pellucida (where in our study Rauber's sickle or junctional endoblast is present), he was able to demonstrate the appearance of hemoglobin in both the inner and outer pieces. However, as the ring of incision approaches the center of the blastoderm, hemoglobin appears less frequently in the central piece. After culture, no hemoglobin is formed in the isolated cranial third of a prestreak embryo with visible embryonic shield (SETTLE, 1954). So, the results of SETTLE (1954) can be explained by at least a starting inductory influence of junctional endoblast on the formation of blood islands or by the absence of junctional endoblast in the cranial and/or central regions of the blastoderm. In a study of the differentiation of the yolk sac endoderm of the chicken embryo, in which cysteine lyase was observed to be a marker of differentiation, BENNETT et al. (1972) and BENNETT (1973) suggested that the mesoderm exerts an influence on the yolk sac endoderm, resulting in the differentiation of this tissue and the appearance of cysteine lyase. However, a relationship between the appearance of cysteine lyase in the yolk sac endoderm of the chicken embryo, and the presence of the blood-forming, mesodermal component, i.e. the area vasculosa, in the extraembryonic area could not be confirmed (VAN ROELEN & VAKAET, 1983). Also the assumption of a primary mesodermal subdivision into area opaca vasculosa and area pellucida vasculosa is inconsistent with the observation that a zonal subdivision exists in the endoblast long before it is overgrown by the mesoderm migrating outward from the primitive streak (PS) (BELLAIRS, 1963; ENGLAND & WAKELY, 1977). The earlier emergence of blood islands at the border of the area pellucida, earlier than shown in previous studies does not challenge the observation that the first erythrocytes emerge in the yolk sac: indeed, the migration of the mesoderm, laterally, also involves the migration of blood islands, laterally in the yolk sac before the onset of the circulation. Our

Legends to the figures (see opposite page)

Fig. 15A. – On the left: Schematic representation of the isolation of the central part of the area centralis of an unincubated chicken blastoderm, by a circular incision (dotted line) at some distance from the internal border of Rauber's sickle. Thereafter, the whole area marginalis, Rauber's sickle and the peripheral part of the area centralis were discarded.
On the right: On this isolated central part of the area centralis (which is composed of upper layer with some chicken endophyll (END CH), (only localized in the original caudal part of the blastoderm), a quail Rauber's sickle fragment (RSQ) is placed, near the anti-sickle region.

Fig. 15B. – A chimera as represented schematically in Fig. 15A, at the beginning of the culture; RS: Rauber's sickle fragment from quail; bar: 1mm.

Fig. 15C. – The same chimera as in Fig. 15B after 25h of culture (before fixation); an embryo has developed with a straight primitive streak starting from the region where the quail Rauber's sickle fragment was placed: a neural plate (N) is seen in the region where the chicken endophyll was localized. Note that the orginal caudocranial orientation of the blastoderm is completely reversed under influence of the apposed quail Rauber's sickle fragment; bar: 1mm.

Fig. 15D. – Section through the caudal region of the chimera of Fig. 15C; SC: sickle canal; JE: junctional endoblast from quail; the arrowhead indicates transitional and/or sickle endoblast from quail; BI: chicken blood island in the lateral prolongation of chicken mesoblast (M); Feulgen staining; bar: 100μm.

observations are in agreement with the postulation of FLAMME (1989) that there exists an early morphogenetic endoblastic influence on extraembryonic mesoderm differentiation. However since the culture of quail-chick chimeras is limited in time we could not follow the final evolution of the endoblastic structures in the yolk sac. In the mouse, precursors of the extraembryonic mesoderm seem to be located in the caudolateral and caudal upper layer and are the earliest mesoderm to migrate through the streak (LAWSON et al., 1991). A surprising observation, in the mouse embryo, is that the erythropoeitic precursors of the yolk sac emerge earlier than the bulk of the vitelline endothelium, which is formed continuously throughout gastrula development (KINDER et al., 1999). Since, in the chicken embryo the mesoblast cells destined to become blood islands under influence of the junctional endoblast are localized most laterally in the area pellucida, we may presume that they also have ingressed through the primitive streak during early gastrulation. Indeed, through the most caudal part of the primitive streak (caudal node, containing part of the three germ layers) there occurs ingression of extraembryonic mesoderm (VAKAET, 1973). This occurs also in the middle region of the PS, according to GALLERA & NICOLET (1969). More cranial parts of the PS (without Hensen's node) give rise less frequently to extraembryonic mesoderm. In quail blastoderms developing on inverted egg yolk balls, cultured in egg white, the peripheral deep layer components (junctional endoblast and yolk endoblast) become locally necrotic or disappear wholly (CALLEBAUT et al., 2000b). This results in large defects in the formation of the area vasculosa and finally death of the embryo. In *Xenopus laevis* many models depict induction from the Spemann organizer (from which the descendants are notochord and head mesoderm) as a gradient of dorsalizing factors that diffuse across the marginal zone. The distance of a marginal zone cell from the Spemann organizer at gastrulation would then determine its dorsoventral identity. Thus the ventral blood islands, which were proposed to arise from tissue furthest away from the Spemann organizer were thought to be specified by the absence of organizer signaling. Indeed in explanted blastula-stage marginal zones a distance pattern develops with a restricted ventral blood island-forming region at the vegetal pole, that is independent of the patterning activity of Spemann's organizer (KUMANO et al., 1999). In molecular terms, dorsoventral patterning of the mesoderm is thought to result from antagonistic interaction between ventral mesoderm inducers as bone morphogenetic proteins (BMP) and their inhibitory binding proteins, including chordin, noggin and follistatin, which are produced by the Spemann organizer (GRAFF, 1997; THOMSEN, 1997). In the chicken blastoderm the blood islands also form in marginal tissue, furthest away from cranial structures such as notochord and head mesoderm. If we compare the localization of the blood-forming regions in the revised *Xenopus* blastula fate map (LANE & SMITH, 1999) with the localization of the first blood islands in the chicken blastoderm we see that they appear at the vegetal limit in the leading-edge mesoderm in both species.

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SHORT NOTES

First occurrence of the Pontocaspian invader *Hemimysis anomala* (Sars, 1907) in Belgium (Crustacea: Mysidacea)

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KEY WORDS: Hemimysis anomala, Mysidacea, first occurrence, Galgenweel

Hemimysis anomala (Sars, 1907), initially only known from the Caspian and the Black Sea (1, 2, 3), was introduced into several water bodies in the former Soviet Union to improve fish production. These populations spread until they reached the Baltic. In 1992, this species was found in the coastal waters of Finland (4) and in 1997 it was first observed in the river Rhine, Germany (5). Recently, *H. anomala* was also observed in the Netherlands (6, 7) and it is expected that *H. anomala* will establish populations in other brackish waters along the coasts of Europe (6, 8).

On 12 October 1999, *H. anomala* was retrieved from handnet samples taken from the brackish pond 'Galgenweel'. This pond is situated on the left bank of the Westerschelde estuary, about 20 km upstream of the Dutch-Belgian border, near the harbour of Antwerp. The Galgenweel is a remainder pond which was established after a breach in the dike. In the 1970s it was deepened and its banks were raised (SOSELISA, unpublished data). Now the Galgenweel is a 13 m deep pond with steep banks that is used for recreation. The pond is connected with the Westerschelde estuary through a sluice, which is only used as an overflow when the water level in the pond is too high.

The north-west bank of the Galgenweel was sampled with a handnet during daytime. The net had a mesh size of 0.5 mm and was mounted on a metal frame of 29.0 x 18.5 cm. The net was pushed over the bottom along a 10 m stretch several times. About 50 adult individuals of *H. anomala* were collected. Temperature (13°C), salinity (3.5 PSU) and dissolved oxygen content (10 mg/l) were recorded. *H. anomala* was not found in the Galgenweel during other samplings at the south-west bank (8 October 1999 and 16 November 1999). This may be because this mysid species is restricted to substrata in which holes and crevices are present (5, 6). The north-west bank of the Galgenweel provides such a habitat, whereas the south-west bank does not.

The genus *Hemimysis* belongs to the family *Mysidae*, subfamily *Mysinae*, and consists of only six species (9, 10). *H. anomala* (Fig. 1A) can be distinguished from other species in the genus by the following characteristics: the 3rd, 4th and 5th male pleopod are well developed, the 4th male pleopod is elongated with a long exopodite and a reduced endopodite, the antennal scale is oblong with long plumose setae on the proximal portion of the outer margin and has no spines (1). The quadrately-truncated telson of *H. anomala* shows no trace of an apical cleft and has two long distal spines on both posterior corners and short spines all along the outer margins (Fig. 1B).

H. anomala is an omnivorous feeder, but with a strong feeding preference for cladocerans over copepods. Their invasion could therefore have dramatic effects on the zooplankton composition and abundance (7, 8). While little is known of the competitive ability of *H. anomala*, it seems that their establishment was not prevented by the resident mysid population of *Neomysis integer* (Leach, 1814). During the period of 1992-1997 *N. integer* was retrieved abundantly from the Galgenweel (200-1000 N/m^2) during handnet sampling. *H. anomala* was never found in this period (MEES & FOCKEDEY, personal communication). Recent samplings of the Galgenweel showed a decline in the abundance of the mysid *N. integer*, but further research is needed to clarify the role of *H. anomala* in this decline (VERSLYCKE, unpublished data).

Our observations reveal the occurrence of a new mysid species in Belgium. The presence of *H. anomala* in Belgian waters confirms the possibility that this species may be present in other brackish regions along the coasts of Europe. The hidden life-style of this species makes it difficult to assess its geographic distribution. Further research is needed to elicit the impact of this neozoon on local ecosystems.

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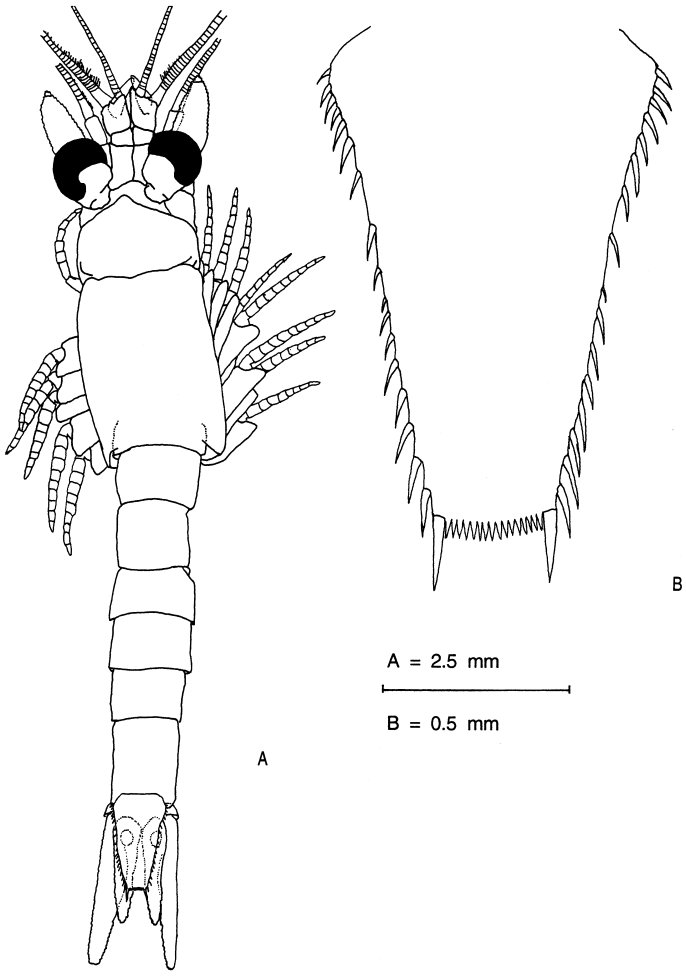

Fig. 1. – *Hemimysis anomala*. A. male; B. telson (FAASSE, 1998).

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A novel exocrine gland in the antennal scape of the army ant Eciton burchelli

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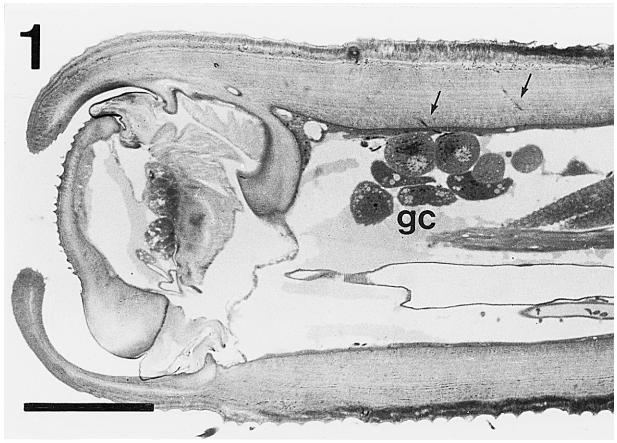
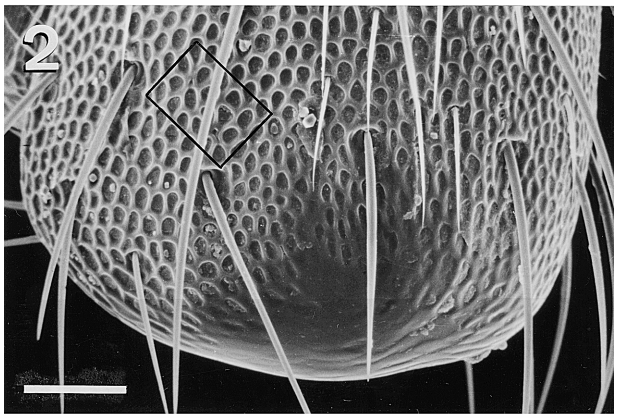
Ants are often regarded as miniaturized walking glandular factories because of their extremely well developed exocrine system (1). More than 60 different glands can be distinguished in this hymenopteran family, with for a majority of the glands a clear function in the social organization and communication system of the ant colony (2). Most glands are situated in the head, thorax and abdomen, but also the legs have recently been found to contain several glands, such as metatibial glands (3,4) and glands in the various tarsal segments (5-8). Other remarkable locations where glands have been found are inside the mandibles (9), inside the sting (10), and inside the gemmae of *Diacamma*, which represent vestigial wing buds (11).

The antennae of ants, however, have generally been overlooked as a possible location for exocrine glands. JANET already in 1894 reported on the existence of 'antennal glands' in *Myrmica rubra* Linnaeus, 1758, but these occur near the antennal base within the head capsule (12), and therefore cannot be considered as real intra-antennal glands. In dacetonine ants, mention was made very recently of a gland in the antennal scape, although its description, without histological observations, was restricted to *"a circular, oval or more elongated patch of pale tissue on the ventral surface of the scape, close to its apex but proximal of the articulation with the first funicular segment"* (BOLTON, 1999: p.1665) (13). When observ-





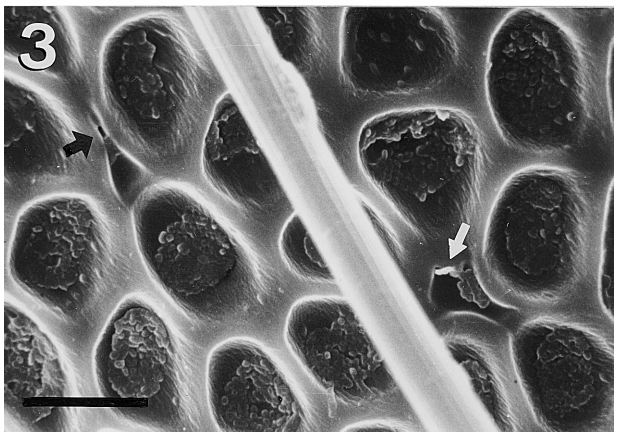


Fig. 1. – Semithin section through distal part of antennal scape of a worker of *Eciton burchelli*, showing cluster of glandular cells (gc). Note narrow ducts penetrating tegumental cuticle (arrows). Scale bar 100 µm.

Fig. 2. – Scanning micrograph of dorsodistal portion of antennal scape. Framed part, under slightly different angle, is shown in Fig. 3 (scale bar 50 µm).

Fig. 3. – Detail of dorsodistal surface of antennal scape, showing two duct openings (arrows). Note whitish secretory material oozing out of pore at right side (white arrow). Scale bar 10 µm.

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ing semithin sections of the antennae of the army ant *Eciton burchelli* (Westwood, 1842), I found a novel antennal scape gland. While the present manuscript was in preparation, an independent study bringing a detailed first description of exocrine glands in the distal antennomeres in fire ants was equally in press (14).

Material for the present study was obtained from foraging workers of *Eciton burchelli* that were collected from a natural raiding column in Manaus, Brasil. Their antennae were cut off near the scape base and fixed in 2% glutaraldehyde in Na-cacodylate buffer. After postfixation in 2% osmium tetroxide and dehydration in a graded acetone series, the antennae were embedded in Araldite. Semithin 1 µm sections were stained with methylene blue and thionin. Air dried material for scanning microscopy was examined in a Philips XL30 ESEM microscope.

The distal part of the antennal scape of *Eciton burchelli* workers contains, in its dorsal region, a cluster of approx. 10 rounded glandular cells (diameter around 25 µm, Fig. 1). Each glandular cell is associated with a corresponding duct cell. The ducts penetrate the scape's tegumental lining under an oblique angle and open on its external surface as very small pores with a diameter of approx. 0.3 µm. Whereas the outer cuticle of the scape shows a more or less regular reticular sculpturing, it displays local changes in the region of pore openings, which occur in between two more parallel cuticular ridges (Figs 2-3). Occasionally, a secretory substance can be seen oozing out of a pore like toothpaste (Fig. 3).

The antennal scape gland is a novel exocrine gland in ants. It is not homologous to the possibly glandular structure, reported for Dacetini (13), that occurs in a ventral position and the external appearance of which does not correspond with the gland we found in *Eciton*. The dacetine structure that is visible as a pale area may correspond with an underlying glandular epithelium in analogy to the metatibial gland (3,4), whereas the *Eciton* gland is formed by bicellular units (2,15). The function of the antennal scape gland remains unknown at present. It probably does not serve as a source of lubricants, as for such function an association and opening through the articulation membrane would be expected. The paste-like nature of the secretion may be indicative for the production of substances that need to be distributed by smearing or rubbing. In an independent study, Isidoro et al. (14) described an exocrine gland in the 9th and 10th antennomeres of the fire ant *Solenopsis invicta* Buren, 1972. This gland is equally formed by bicellular units, of which the duct cell openings appear as small pores that occur as a ring circling the proximal part of the antennomere. The function of these antennomere glands is also still unknown. Similar antennomere glands in parasitoid Hymenoptera were reported to be involved in the production of sex recognition substances in males or spatial information cues in females (14, and references therein). Whether the antennal scape gland as described here also exists in other ants is not yet known and needs further examination.

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