

## New crustacean invaders in the Schelde estuary (Belgium)

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**ABSTRACT.** This paper reports on the first records of three non-indigenous crustacean species in the Zeeschelde estuary (Belgium). All three species are native to the Western Pacific Ocean and are probably transported in ballast water or hull fouling. The isopod *Synidotea laevidorsalis* has been recorded earlier in Europe from two locations, in the south of France and in Spain. The present records constitute a range extension of more than 1000 kilometres to the north. The crab *Hemigrapsus takanoi* and the prawn *Palaemon macrodactylus* were found for the first time in the brackish reaches of the Schelde estuary. The finding of these species demonstrates the vulnerability of the estuarine system to the invasion of exotic species.

**KEY WORDS:** Schelde estuary, *Synidotea laevidorsalis*, *Hemigrapsus takanoi*, *Palaemon macrodactylus*, invasive species.

## INTRODUCTION

Invasions of non-indigenous species are occurring throughout the world. However, the magnitude of the phenomenon is still underestimated (RUIZ et al., 1997). This is partly because exotic species are frequently misidentified during the first years after their arrival in the new habitat. Regions with harbours and shellfish culture are especially vulnerable to introductions of alien species. Brackish harbour regions in Western Europe are particularly susceptible to introductions (RUIZ et al., 1997). In low saline regions the diversity of native species is low (REMANE, 1971) and the import rate of new species is high. The port of Antwerp (Belgium) is a large international port, mainly focussed on container transport and receiving ships from over 800 locations worldwide. The main port activities are situated in the brackish part of the Schelde estuary, between the Dutch-Belgian border and the city of Antwerp. The high loads of ballast water and the recent improvement of the water quality make the system vulnerable to introductions of non-indigenous species (YSEBAERT et al., 1997, STEVENS et al., 2004; AZÉMAR et al., 2007). In the mesohaline part of the Schelde estuary, where the port of Antwerp is situated, the invertebrate fauna contains a high proportion of alien species. The invasion rate in the ecosystem has never been higher than during the last 20 years (KERCKHOF et al., 2007).

The present paper reports on the first records of three crustacean species in the Zeeschelde, the Belgian part of the Schelde estuary. For the isopod *Synidotea laevidorsalis* (Miers, 1881) (Fig. 1) this is the first record of this species in north-west Europe, while the crab *Hemigrapsus takanoi* (Asakura & Watanabe, 2005) (Fig. 2) and the prawn *Palaemon macrodactylus* (Rathbun, 1902) (Fig. 3) were already known from Belgian coastal waters and

from the Dutch part of the Schelde estuary (Wester-schelde).

*S. laevidorsalis* is a large isopod from the Western Pacific Ocean (China, Japan, and the east coast of Russia). It has spread during the past 100 years to an almost cosmopolitan distribution in temperate regions (CHAPMAN & CARLTON, 1991; CHAPMAN & CARLTON, 1994; BUSHEK & BOYD, 2006). Before the records in the present note, the genus *Synidotea* had only been recorded in Europe from the Gironde estuary in France (MEES & FOCKEY, 1993) and the Guadalquivir estuary in southern Spain (CUESTA et al., 1996). The species invaded the Gironde, more than 1000km south of the Schelde estuary, prior to 1975. The species was recorded for the first time in the Guadalquivir estuary in the early 1990's (CUESTA et al., 1996).

*P. macrodactylus* is native to the north-west Pacific, but has been introduced already to the west coast of North and South America, Australia and Europe (GONZÁLEZ-ORTEGÓN & CUESTA, 2006). This species was first recorded in Belgium in June 2004 (DE BLAUWE, 2006) and was found in the Zeeschelde near Doel in 2005 (D'UDEKEM D'ACCOZ et al., 2005). However, the earliest specimen we found came from a sample from autumn 2003, which makes it the first observation of this species in Belgium.

The crab *H. takanoi* has much the same native distribution as *P. macrodactylus* (Western Pacific). The species invaded north-west Europe in the early 1990's, The Netherlands in 2000 (D'UDEKEM D'ACCOZ & FAASSE, 2002) and the Belgian coast in 2003 (DUMOULIN, 2004). This species was found already in 2003 in the Westerschelde near Baalhoek, but despite a search in the same period, it could not be found in the Zeeschelde (DUMOULIN, 2004).

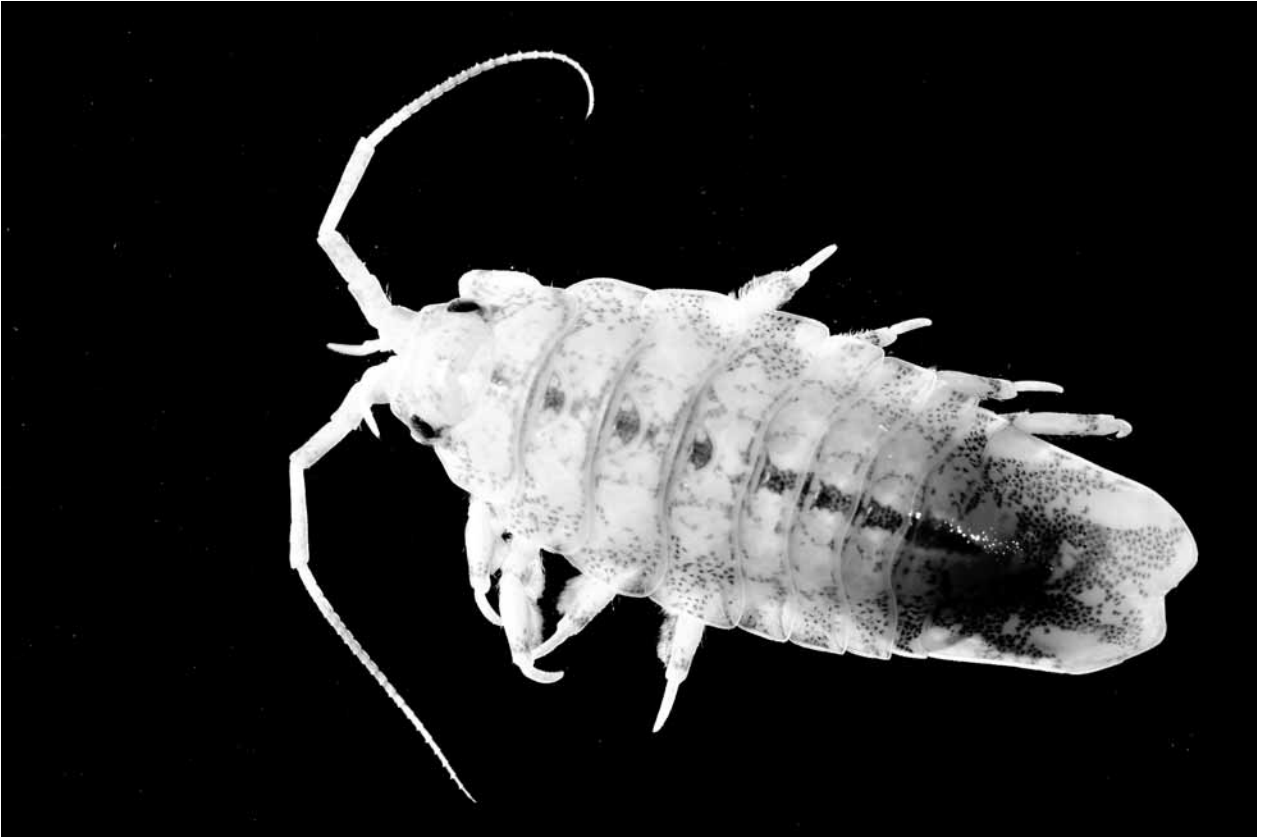


Fig. 1. – *Synidotea laevidorsalis* (Vilda/ Yves Adams)



Fig. 2. – *Hemigrapsus takanoi* (Vilda/ Yves Adams)

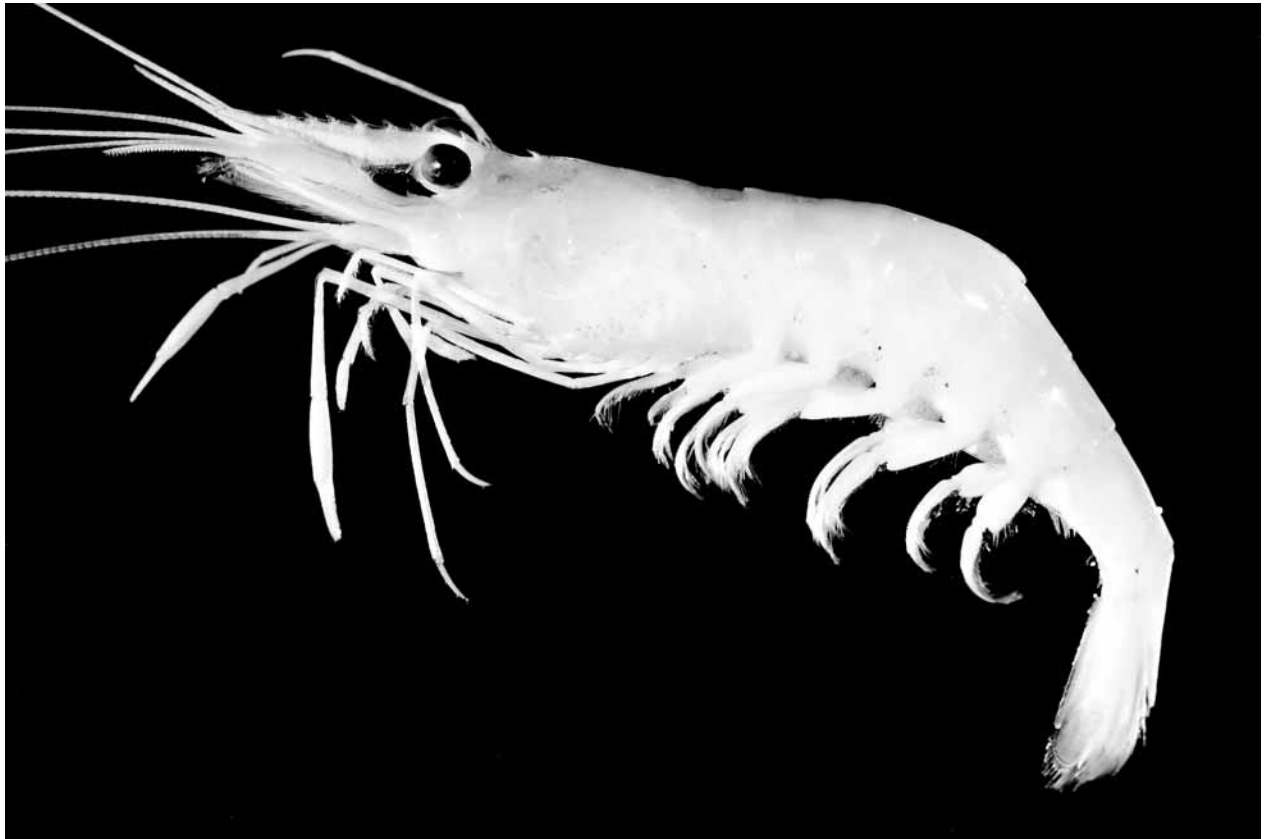


Fig. 3. – *Palaemon macrodactylus* (Vilda/ Yves Adams)

## MATERIALS AND METHODS

### Study area

The river Schelde rises in the north of France and flows into the North Sea near Vlissingen (The Netherlands). It is a lowland river with a total length of 355km and a fall of 100m at most. Its catchment area, approximately 21000km<sup>2</sup>, has around ten million inhabitants. This study focuses on the estuary, which extends to Gent, 160km from the mouth, where tidal influence is stopped by sluices. The tributaries are also under tidal influence and are considered as a part of the estuary. The Dutch part of the estuary (Westerschelde) is characterised by flood and ebb channels, separated by intertidal sand- and mudflats. Upstream from the Dutch-Belgian border, it changes into a one channel system. The study area is situated in the mesohaline part of the estuary (Beneden-Zeeschelde), between the Dutch-Belgian border and Antwerp (Fig. 4). This part of the estuary is characterized by a steep salinity gradient, ranging between 17psu in summer near the border and 0.5psu in winter near Antwerp (Table 1). The oxygen concentration may be low near Antwerp in summer when river discharge is low and temperature high. Near the border

the oxygen concentration is generally higher and varies between 3.5 and 10mg/L.

### Data collection

The species were collected by various sampling techniques including sieving of cooling water, benthic soil samples, hand netting and analysis of fish stomach contents. Regular fish monitoring in the brackish part of the Schelde estuary started in 1991 using monthly cooling water samples from the nuclear power plant Doel (NPP Doel). The power plant is situated on the west bank of the Schelde estuary between Antwerp (Belgium) and the Dutch-Belgian border (Fig. 4). The main purpose of these surveys was to study seasonal trends in the estuarine fish and crustacean communities. Fish were collected from the intake screens of the NPP Doel. Cooling water is withdrawn through five intake apertures (25m<sup>3</sup>/s) and filtered by two vertically-travelling screens with a mesh size of 4mm to prevent larger organisms and debris from obstructing the condensers. A full description of the sampling site and the methodology is given in MAES et al. (2001). Former catches of isopods in Doel were probably misidentified as the native harbour isopod (*Ligia oceanica*). In 2007, some isopods were taken to the laboratory and could be identified as *S. laevidorsalis*.

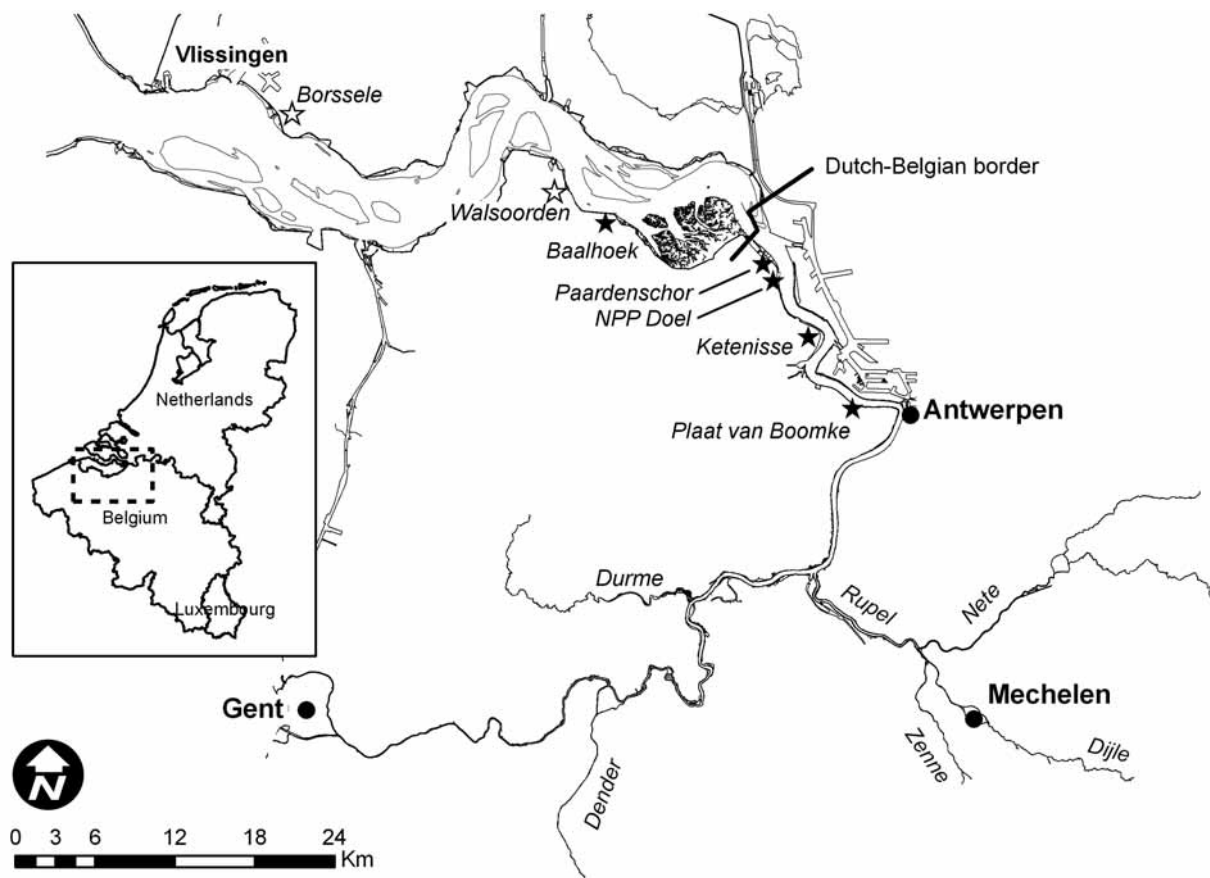


Fig. 4. – Map of the Schelde estuary. The lower part of the estuary (Westerschelde – downstream of the Dutch-Belgian border) consists of multiple channels and the upper part (Zeeschelde – upstream of the border) consists of a single channel.

TABLE 1

Water quality parameters in the study area (2002-2007).  
Data were obtained from the Centre for Estuarine and Marine Ecology (CEME - NIOO).

	Salinity (psu)		Temperature (°C)		Dissolved oxygen (mg/L)	
	Border	Antwerp	Border	Antwerp	Border	Antwerp
average	9.0	4.6	13.6	13.5	6.9	4.5
max	16.8	11.3	24.0	23.6	10.0	9.1
min	1.2	0.4	4.2	2.9	3.3	0.3

The subtidal macrobenthic community of the mesohaline part of the Schelde is sampled three-yearly (autumn) since 1996. In 2003 an additional monitoring campaign was set up to evaluate the impact of dredging activities. On each location one Van Veen grab (0.105m<sup>2</sup>) was taken. The samples were sieved through a 1-mm mesh in the field and preserved in neutralized formalin (8%). In the laboratory the samples were sorted after staining with 0.02% Rose Bengal. When possible, the organisms were

identified to species level and counted. With this method hyperbenthic organisms (such as *Synidotea* spec. and *Palaeomon* spec.) are sampled only accidentally. A few additional samples have been included in this study, i.e. two van Veen grab samples containing specimens of *S. laeviodorsalis*, two samples from fykes containing *H. takanoi* originating from a local fisherman, stomach contents of fish caught near Doel and two samples collected under boulders in the littoral zone (Table 2).

TABLE 2  
Catch results and site characteristics.

	Location	Date	Habitat	#
<i>S. laevidorsalis</i>	Near NPP Doel (Van Veen grab)	27/09/2005	Subtidal, >10m TAW	2
	D-B border near Prosperhaven (Van Veen grab)	27/09/2005	Subtidal, >10m TAW	1
	NPP Doel	17/08/2007	Water intake	>100
	NPP Doel	11/12/2007	Water intake	23
	<i>P. macrodactylus</i>	Antwerp (Plaat van Boomke)	25/09/2003	Subtidal, soft
Paardenschor		17/07/2007	Mudflat (Fish stomachs)	2
NPP Doel		17/08/2007	Water intake	19
<i>H. takanoi</i>	Ketenisse	28/07/2007	Mudflat (fyke)	1
	NPP Doel	17/08/2007	Water intake	4
	Ketenisse	25/10/2007	Under boulders	2
	Ketenisse	12/11/2007(*)	Under boulders	1
	NPP Doel	11/12/2007	Water intake	2

(\*) On 12/11/2007 together with *H. takanoi* the alien (American) amphipod *Crangonyx pseudogracilis* and the native amphipod *Corophium lacustrum* were collected.

## RESULTS

An overview of all specimens collected is given in Table 2. Since the isopods and prawns at the NPP Doel were picked out randomly, higher numbers of *S. laevidorsalis* were probably present. In the fish stomach samples of 17 July 2007, six specimens of the native prawn *Palaemon longirostris* (H. Milne Edwards, 1837) were present as well. In the August samples of Doel, species of Pacific origin predominated. A single specimen of the native prawn *Palaemon adspersus* (Rathke, 1837) (Fig. 5) was collected on the same date, constituting its first record from the Belgian part of the Schelde. On 11 December 2007 the crab *Rhithropanopeus harrissii* (Gould, 1841), another alien crustacean, originating from the north-west Atlantic and recorded from the Schelde since 1985 (MAES et al., 1998; YSEBAERT, 2000; WOUTERS, 2002; WOLFF, 2005) was found in the samples. Native species collected on the same date were *Palaemon longirostris* and *Crangon crangon* (Linnaeus, 1758). Pontoon scrapings and hand net catches near the low water mark in the mesohaline part of the Dutch Westerschelde did not yield any specimens of *S. laevidorsalis*. Also inspection of the cooling water inlet of the power plant in Borssele in the polyhaline part of the Westerschelde was not successful in detecting this species.

## DISCUSSION

*S. laevidorsalis* is an invasive species that should be expected to arrive in several more temperate estuarine areas worldwide. It has been misidentified and/or re-described erroneously in different parts of the world where it has been introduced (CHAPMAN & CARLTON, 1991;

CHAPMAN & CARLTON, 1994). Ballast water or hull fouling is the most likely vector for the introduction of *S. laevidorsalis* in the Gironde, Guadalquivir and Schelde estuaries. All three estuaries have international ports and receive large amounts of ballast water (NIIMI, 2004). The tendency of *S. laevidorsalis* to cling to floating objects (CHAPMAN & CARLTON, 1994; BUSHEK & BOYD, 2006) increases the chance of it being introduced with ships.

*S. laevidorsalis* is mostly recorded from intertidal pilings, rock jetties or floats and buoys, among dense masses of hydroids or bryozoans in shallow brackish and marine waters (CHAPMAN & CARLTON, 1991). According to MEES & FOCKEY (1993) however, the species was never collected during the extensive macrobenthos surveys in the Gironde. They found the species by trawling the bottom of the Gironde with a hyperbenthic sledge in the main subtidal channel at a sampling depth between 6 and 14m. In the Gironde the species is found in salinities ranging from 0.1 to 24g/L with highest densities around 3g/L. The same authors mention that Hydrozoa and Bryozoa have not been reported to occur in important numbers in the brackish part of the estuary. They suggest that the animals crawl over the bottom and that at least a part of the Gironde population migrates into the water column, even during daytime. *S. laevidorsalis* is a typical estuarine species. The European finds are situated in three of the biggest European estuaries. The optimal salinity of 3g/L for *S. laevidorsalis* found in the Gironde corresponds well with the salinity range of the records in the Schelde (Table 1). In the Guadalquivir estuary, the species was found commonly in a salinity ranging from 16g/L to 24g/L (CUESTA et al., 1996). This makes it very likely that the species could also occur in the mesohaline part of the Westerschelde (The Netherlands). However, this could not yet be demonstrated.



Fig. 5. – *Palaemon adspersus* (Vidal/ Yves Adams)

The stations where this species is found correspond well with its ecological preferences. Since the current monitoring program is focussed on benthic organisms, we assume that the species is more common than the few observations suggest. Alternatively, it is possible that this species takes advantage of the locally higher water temperatures near the outflow of the NPP Doel.

*P. macrodactylus* tolerates a broad range of ecological conditions. It is known to occur in protected harbours, bays, ponds and tidal creeks (GONZALEZ ORTEGON & CUESTA, 2006). In the UK, this species has also been collected from the water intake of a power station (WORSFOLD & ASHELBY, 2006). The first observations of *P. macrodactylus* in (coastal) Belgium were in 2004 (DE BLAUWE, 2006). However, our own observation of two specimens in 2003 indicates that this species was already present in the Zeeschelde before that date. This is not very surprising considering that the first observation in the Westerschelde was in 1999 in Walsoorden (D'UDEKEM D'ACOEZ et al., 2005). The Belgian observations show that this species has a broad salinity-tolerance. The species seems to be quite common and coexists with at least two other native *Palaemon* species. In the Thames, where the presence of this species has been demonstrated since 1992, it has become a common species, but has not out-competed the native species *P. longirostris* (WORSFOLD & ASHELBY, 2006). BEGUER et al. (2007) consider it very likely that there is a significant competition for food between *P. macrodactylus* and native species such as *P. longirostris* and even *Crangon crangon*. In California, RICKETS et al. (1968) considered the species as responsible for the disappearance of a native *Crangon* species.

*H. takanoi* reached the Belgian part of the Schelde and can be considered as quite common on the stony banks of the brackish part of the Schelde. Since the species was already present close to the Belgian border in 2003, it is very likely that this species did arrive much earlier. No targeted research was undertaken between 2003 and 2007, when the species was accidentally found in the NPP Doel. In contrast with the other two exotic species, *H. takanoi* was not found in Gironde and Guadalquivir estuaries. *H. takanoi* often hides under boulders in intertidal areas of estuaries, lagoons, boulder beaches and wave sheltered rocky shores (ASAKURA & WATANABE, 2005), but occurs also on stony mediolittoral river banks or in association with mussels or oysters such as the Japanese oyster *Crassostrea gigas* (Thunberg, 1873) (DUMOULIN, 2004). This author found the species most numerous in sheltered places such as marinas and harbours, canals and coastal bays. The larvae of this crab are pelagic which means that it can easily colonize new areas. This species is a possible competitor with other crabs in the estuary such as the native *Carcinus maenas*, which has a similar habitat preference and which reaches its lower salinity limit in the estuary near the Dutch-Belgian border. Also competition with *Eriocheir sinensis* and *Rhithropanopeus harrisi* cannot be ruled out.

The coexistence of *S. laevidorsalis*, *P. macrodactylus* and *H. takanoi* in the basin of the NPP Doel may be explained by their habitat preferences. All three species are regarded as euryhaline and prefer shallow, often calm and protected waters with stones or other hard substrates such as harbour walls and pontoon floats. All three species take advantage of the human introduction of hard substrates in the Schelde-estuary where soft sediments

naturally prevail. It's very likely that *S. laevidorsalis*, *P. macrodactylus* and *H. takanoi* were already present in the Zeeschelde for several years in the period before 2003-2005. However, they were never found and/or identified as such, because a consistent monitoring programme for hyperbenthos in the Zeeschelde is lacking. The current benthos monitoring in the Schelde estuary is focussed on intertidal and subtidal sampling of sediments. As a result, hyperbenthic organisms are sampled only occasionally. Walk-over-surveys could make a difference for *H. takanoi*, since this species can be easily found under stones in the intertidal.

## CONCLUSIONS

This article mentions three exotic crustacean species that have been collected in the Zeeschelde-estuary recently. *Synidotea laevidorsalis* is new to northwestern Europe. *Hemigrapsus takanoi* had never been found before in the Zeeschelde. *Palaemon macrodactylus* was present, but had not been recorded, as early as 2003 from Belgium. The presence of several large ports in the estuary makes the Zeeschelde very vulnerable to invaders. The transformation of the estuary by introduction of hard substrates has provided new niches that are now occupied by new, often exotic and opportunistic species. In addition, recent improvement of the water quality in the freshwater part of the estuary may facilitate the introduction and establishment of non-native species. Further research should focus on the impact of introduced species on the ecological functioning of the estuary. An adjusted monitoring programme for the hyperbenthos may reveal the presence of more exotic species.

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## Stratégies de reproduction de *Marcusenius ussheri* (Pisces, Mormyridae) en fonction des conditions hydrologiques créées par la construction du barrage hydroélectrique d'Ayamé I sur le cours principal de la rivière Bia (Côte d'Ivoire)

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**RÉSUMÉ.** La biologie de la reproduction de *Marcusenius ussheri* a été étudiée au cours de 12 mois d'échantillonnage dans le bassin de la rivière Bia. Le cours d'eau est divisé en trois zones écologiques différentes nées de la présence d'un barrage hydroélectrique sur son lit principal (amont, lac et aval). Les poissons ont été pêchés essentiellement aux filets maillants. La taille de première maturité sexuelle présente une variation le long du cours d'eau (amont, lac et aval) pour les deux sexes. Elle varie également en fonction du sexe, elle est légèrement plus faible chez les femelles que chez les mâles de l'espèce. Les données relatives à la maturation des gonades, au rapport gonado-somatique révèlent que la période de reproduction varie en fonction des conditions hydrologiques et de la saison. Le diamètre de l'ovocyte à la ponte est de 1.8mm; la fécondité relative atteint 31834 ovocytes par kg de poids corporel. Les conditions environnementales qui prévalent dans l'habitat anthropique (lac) étudié, semblent plus favorables à la reproduction de l'espèce que les parties fluviales.

**MOTS CLÉS:** Mormyridae, *Marcusenius ussheri*, Reproduction, rivière Bia, Côte d'Ivoire.

## Reproductive strategy of *Marcusenius ussheri* (pisces, Mormyridae) in the fluvio-lacustrine system of the Bia River (Ivory Coast)

**ABSTRACT.** A study of the reproductive biology of *Marcusenius ussheri* has been carried out during 12 sampling months in the Bia River catchments' area. Fishes have been caught with gill nets. A dam has been built on the stream of the river. This dam has created three different ecological zones. The size at first sexual maturity presents variations along the river (upstream, the lake and downstream). Females are slightly smaller than males. Data of gonadal maturation and gonado-somatic index both indicate that breeding varies according to the hydrological and seasonal conditions. The oocyte diameter at spawning is 1.8mm; relative fecundity reaches 31834 oocytes per kg body weight. The environmental conditions which exist in the lake seem more favorable to *M. ussheri* reproduction than those in the stream parts.

**KEY WORDS:** Mormyridae, *Marcusenius ussheri*, reproduction, Bia River, Ivory Coast

### INTRODUCTION

Chez les poissons comme pour tous les autres animaux, le succès de la reproduction par opposition à la croissance et au maintien dépend de la quantité de ressources qui lui sont allouées, de la période ainsi que de l'endroit de la ponte (LÉVÊQUE, 1997). De plus, la reproduction a lieu dans le milieu aquatique où œufs et sperme sont déversés. Par ailleurs, LÉVÊQUE & PAUGY (1999) indiquent que la saison de ponte des poissons change lorsque les conditions du milieu changent aussi et notamment lorsque l'on construit un barrage sur une rivière. En effet, lorsqu'un cours d'eau est barré pour créer une retenue, on provoque de nombreuses perturbations de l'habitat et des populations de poissons par suite de la fragmentation de l'éco-

système initial (JACKSON et al., 1988). Dans ces conditions, les menaces potentielles venant de tels travaux d'aménagement, de pollutions d'origines diverses, de surexploitation due à la pêche ou encore de divers prédateurs, peuvent mettre en danger les populations de poissons.

Face à ces différents périls, un individu peut développer des tactiques qui sont en réalité des variations par rapport au schéma de reproduction type de l'espèce de manière à répondre avec succès à des modifications des facteurs de l'environnement. Il s'agit dans ce cas d'un comportement adaptatif à des conditions écologiques particulières qui ont pour objectif d'assurer la survie de l'espèce (LÉVÊQUE, 1999a), par conséquent de sa conservation.

Ces tactiques qui associent différents traits comprenant notamment la taille de première maturité sexuelle, la fécondité, le diamètre de l'ovocyte à la ponte constituent ce que plusieurs auteurs appellent la stratégie de reproduction (BENECH & QUENSIERE, 1985; LÉVÉQUE, 1999b). Selon HOPKINS (1981), il y a une relation entre la stratégie de reproduction du poisson et la communication intra-spécifique. Or l'un des traits caractéristiques des poissons de la famille des Mormyridae dont *M. ussheri*, est la présence d'un organe électrique situé au niveau du pédoncule caudal et servant à la communication entre individus de la même espèce. Relativement à ces poissons, si de nombreuses études portant sur leur système de communication ont été réalisées (FESSARD, 1975; CRAWFORD et al., 1986; HOPKINS, 1986), les connaissances acquises sur la biologie de la reproduction, la capacité d'adaptation de ces poissons endémiques en Afrique, restent encore au stade d'observations préliminaires, fragmentaires et basées sur des échantillons réduits ou parfois même inexistantes. Par ailleurs, aucune étude n'avait été entreprise sur la biologie de reproduction des poissons de la rivière Bia avant la construction du barrage. Aussi, ce travail vise à la fois à combler ce vide et expliquer les différentes stratégies de reproduction de *Marcusenius ussheri*, dans les différents biotopes créés par cet aménagement.

## MATERIEL ET MÉTHODES

### Milieu d'étude

Ce travail a été réalisé dans le bassin de la rivière Bia, situé dans le sud de la Côte d'Ivoire et soumis au climat de

type tropical humide. La Bia prend sa source au Ghana, longe l'Est de la Côte d'Ivoire et se jette au sud dans la lagune Aby. Son débit moyen à l'embouchure est de  $83\text{m}^3.\text{s}^{-1}$ . La superficie totale de son bassin versant est d'environ  $9300\text{km}^2$ . Ayamé I est le premier barrage hydroélectrique du pays construit en 1959. Il est à l'origine du lac artificiel d'une superficie de  $90\text{km}^2$  (OUATTARA et al., 2006) sur cette rivière. Selon DURAND & CHANTRAINE (1982), la Bia est soumise à l'influence du climat équatorial fait de quatre saisons de durées inégales: deux saisons de pluies dont la plus grande part d'avril à juin et la petite qui se situe entre septembre et novembre. Les deux saisons sèches se rencontrent entre décembre et mars pour la plus grande, et entre juillet et août pour la plus petite.

### Echantillonnage

L'échantillonnage a été réalisé entre août 1996 et juillet 1997, soit une fois par mois, pendant 12 mois dans 4 stations: Bianouan ( $6^{\circ} 01' \text{ N}$  et  $3^{\circ} 00' \text{ W}$ ) en amont, Ayamé ( $5^{\circ} 36' \text{ N}$  et  $3^{\circ} 10' \text{ W}$ ) et Bakro ( $5^{\circ} 33' \text{ N}$  et  $3^{\circ} 15' \text{ W}$ ) dans le lac et Aboisso ( $5^{\circ} 28' \text{ N}$  et  $3^{\circ} 12' \text{ W}$ ) en aval (Fig. 1). Selon KOUAMELAN (1999), l'amont de la Bia est caractérisé par une canopée assez importante (environ 70%), un substrat sableux parsemé de débris végétaux (feuilles et tiges) et l'existence d'îlots rocheux sur son lit principal. Le lac est un écosystème totalement ouvert, avec une profondeur maximale de 30m et un fond morphologiquement vaseux. L'aval de la rivière est fait d'un substrat argilo-sableux parsemé de rochers. Son régime hydrologique dépendant de la fermeture et de l'ouverture du lac.

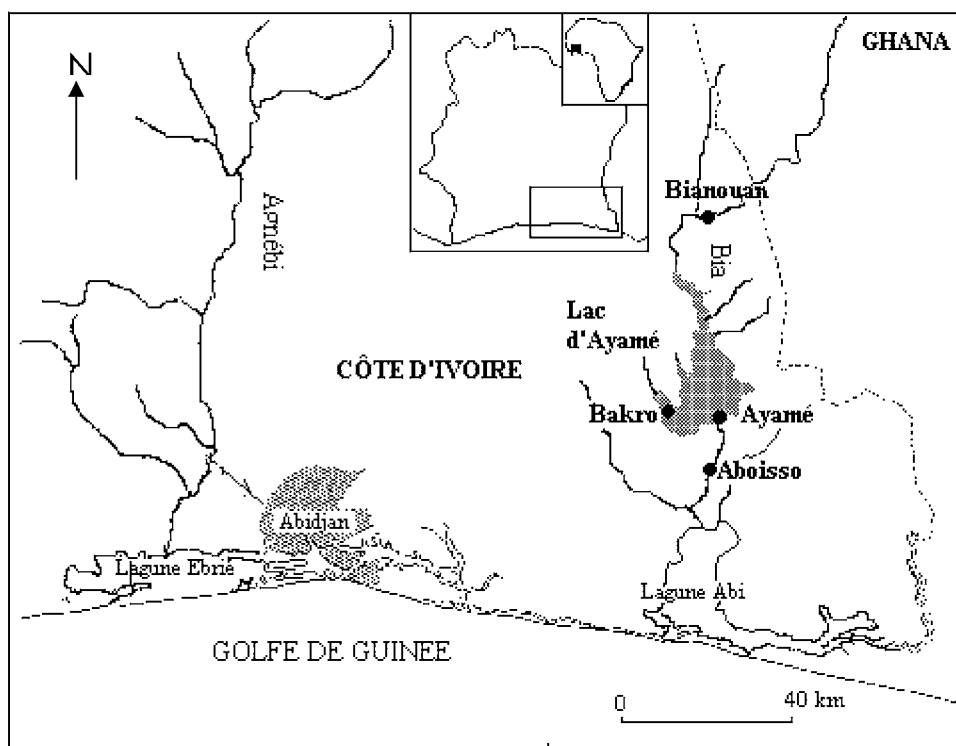


Fig. 1. – Situation géographique des sites d'échantillonnage sur la rivière Bia (Gourene et al., 1999).  
● : station.

Au niveau de chaque station, une pêche de nuit et une de jour ont été effectuées. Deux batteries de filets mailants de différentes mailles (10; 12; 15; 20; 25; 30; 35; 40; 50mm) ont servi à la capture des poissons. Secondairement, des poissons ont été achetés aux pêcheurs locaux. Chaque poisson a été identifié selon les clés de détermination de LÉVÊQUE et al. (1992) et de GOURÈNE et al. (1995). Ils ont été mesurés (longueur standard) au millimètre près, puis pesés au gramme près. L'échelle de maturité (5 niveaux) est celle utilisée par LEGENDRE & ÉCOUTIN (1996) et reprise par DUPONCHELLE & LEGENDRE (2000) mais également par CHIKOU et al., (2007). Le stade 1 comprend les individus immatures, le stade 2 représente le début de la maturité sexuelle, le stade 3 indique les individus matures, le stade 4 caractérise les individus qui se reproduisent (ponte ou spermiation) et le stade 5, la post-ponte pour les femelles. L'évolution saisonnière de l'activité sexuelle a été déterminée à partir des pourcentages mensuels des différents stades de la maturation sexuelle. Le rapport gonado-somatique mensuel moyen (RGS moyen) des individus matures (stade 3 au moins) a été calculé comme suit:  $RGS = (\text{poids des gonades/poids corporel éviscéré}) \times 100$ . Ces deux paramètres ont permis de déterminer la période de reproduction. La taille de première maturité sexuelle a été établie à partir des spécimens dont la gonade avait atteint un stade supérieur ou égal à 3. Les gonades contenant des ovocytes en vitellogénèse avancée ont été pesées et conservées dans le formaldéhyde 5% (10 jours maximum) en vue de l'établissement de la fécondité relative et de la mesure du diamètre des ovocytes (30 ovocytes par femelle).

#### Mesure des paramètres physico-chimiques

Les paramètres abiotiques ont été mesurés mensuellement. La température et l'oxygène dissous ont été déterminés à l'aide d'un oxymètre modèle OXI 96, le pH, à l'aide d'un pHmètre modèle WTW 95, le taux de solides dissous et la conductivité avec un conductimètre modèle 44600 et la transparence de l'eau à l'aide d'un disque de Secchi.

#### Tests statistiques

Les tests statistiques de Kruskal-Wallis et de Mann Witney ont été utilisés pour comparer les valeurs des paramètres physico-chimiques biotiques (croissance et reproduction) de *M. ussheri* afin d'identifier d'éventuelles variations spatiales et ou temporelles. Au préalable, un test de normalité de Kolmogorov-Smirnov effectué a montré que la distribution des données n'était pas normale. Le test de Kruskal-Wallis a été utilisé pour évaluer la significativité des différences constatées pour chaque paramètre suivant les milieux. Le test de Mann-Witney a été réalisé pour comparer les stations deux à deux pour un paramètre donné. Ces tests ont été réalisés à l'aide des logiciels R (IHAKA & GENTLEMAN, 1996) pour les paramètres biotiques et Statistica 7.1 (paramètres abiotiques).

## RÉSULTATS

#### Caractéristiques physico-chimiques des milieux étudiés

Le test de Kruskal-wallis effectué sur la matrice des paramètres physico-chimiques a montré qu'entre les milieux écologiques étudiés, il existe des différences significatives au niveau de la transparence, de la température, du taux de solides dissous et de la conductivité ( $p < 0.05$ ). En comparant deux à deux les stations (test de Mann-Witney,  $p < 0.05$ ), on constate que la transparence est significativement plus élevée dans le lac que dans les deux autres milieux (amont et aval) (Fig. 3a). En revanche, entre ces deux derniers, la différence n'est pas significative. Concernant la température, les valeurs relevées en amont sont significativement plus faibles que celles du lac et de l'aval (Fig. 3b). Quant au taux de solides dissous (Fig. 3c) et à la conductivité (Fig. 3d), les plus fortes valeurs ont été observées en amont. En revanche, les valeurs les plus faibles ont été enregistrées dans le lac.

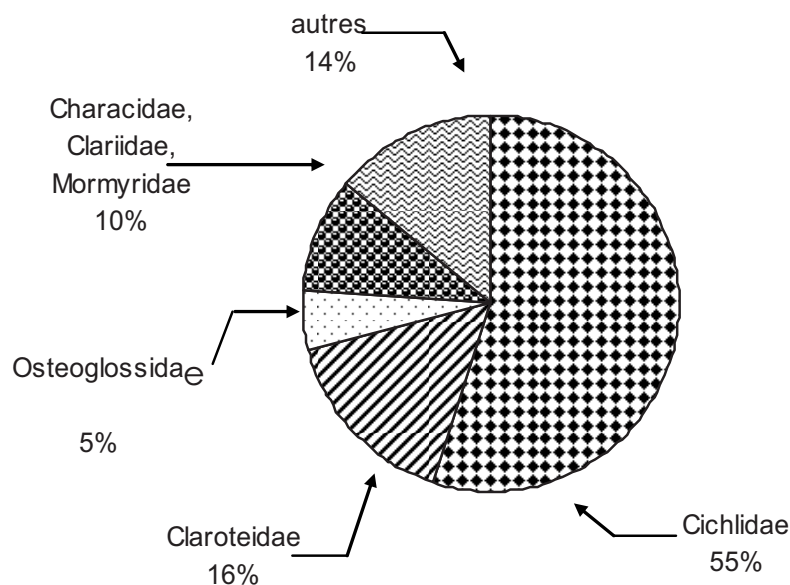


Fig. 2. – Composition par famille des poissons provenant du lac d'Ayamé.

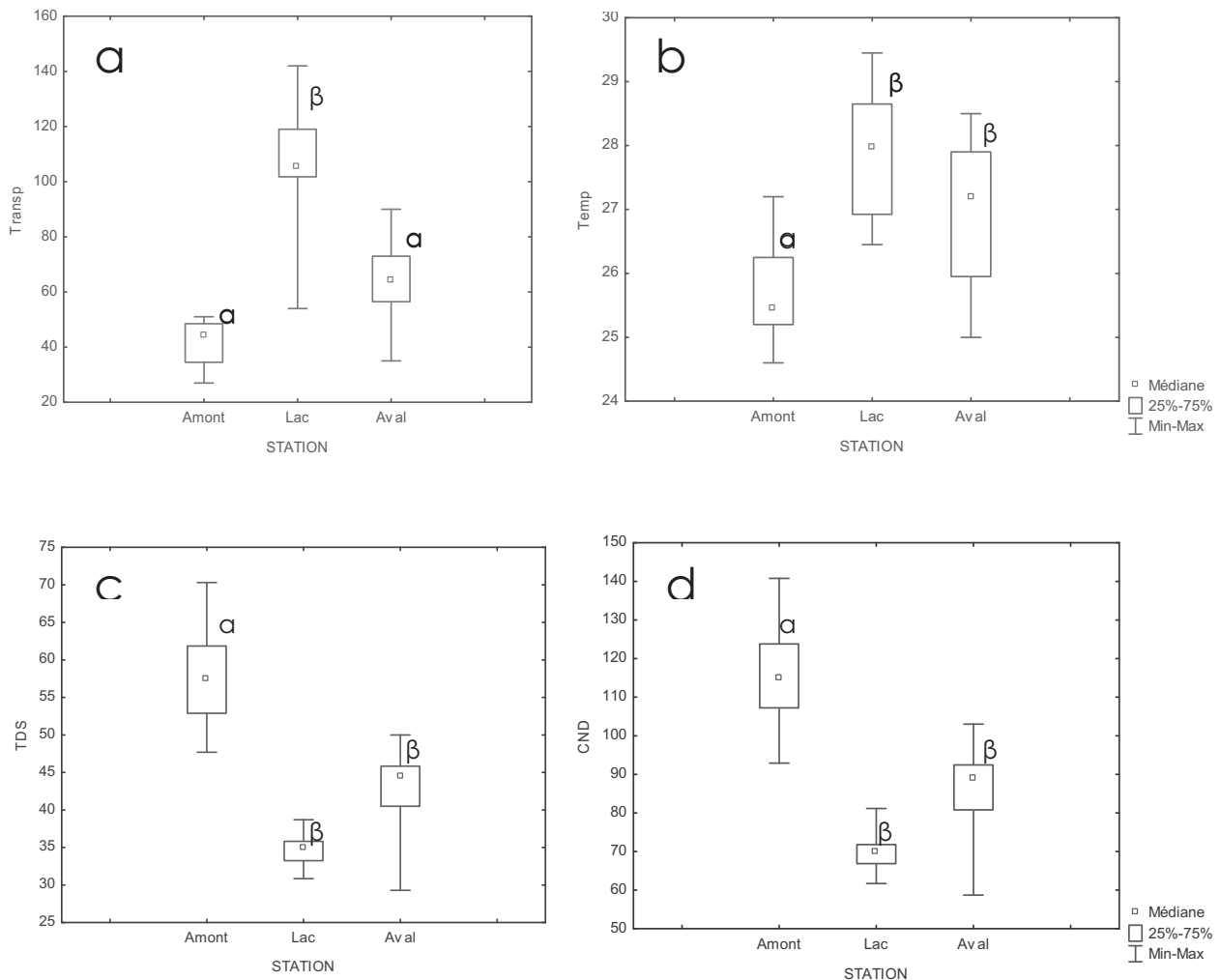


Fig. 3. – Box-plots affichant les variations de certains paramètres physico-chimiques (a=transparence (Transp), b=température (Temp), c=taux de solide dissous (TDS) et d=conductivité (CND)) en fonction des zones écologiques. Les box-plots portant des lettres grecques différentes ont des valeurs significativement différentes.

### Abondance et sex-ratio

Relativement rares, les Mormyridae ont constitué moins de 10% des poissons échantillonnés (Fig. 2). Mille quatre cent vingt et cinq (1425) spécimens de *Marcusenius ussheri* ont été capturés. Quatre cent quatre-vingt dix huit (498) étaient immatures contre 927 spécimens qui présentaient une différenciation sexuelle. La répartition entre sexes est de 425 femelles contre 502 mâles. Ces individus matures se répartissent comme suit: 79 en amont; 593 dans le lac et 255 en aval. Les proportions relatives entre mâles et femelles (mâles: femelles) sont comme suit: 1:2.5 (amont), 1:1.35 (lac) et 1.38:1 (aval). Dans la population des immatures, on rencontre 287 en amont; 73 dans le lac et 138 en aval.

### Taille de première maturité sexuelle

La taille de première reproduction n'a pu être calculée en amont par insuffisance d'individus matures. Dans le lac, la maturité sexuelle est atteinte à 168mm pour les femelles contre 173mm chez les mâles. Dans ce milieu,

64% des femelles présentent une taille égale ou supérieure à celle de première reproduction. Les 36% restants mesurent entre 100 et 165mm. Concernant les mâles, 65% des individus ont atteint la maturité sexuelle, 35% mesurent entre 118 et 165mm. En aval, les femelles matures mesurent au moins 135mm et les mâles 175mm. Ce sont 12% des femelles capturés qui sont parvenues à la maturité sexuelle, les autres ont une taille allant de 98 à 120mm.

### Evolution spatiale des tailles et des poids

La figure 4 présente la variation du poids et de la taille le long du cours d'eau par zone écologique ainsi que par sexe. Concernant les zones, le poids des mâles (Fig. 4a) pêchés dans le lac est significativement plus élevé (test de Mann-Witney,  $p < 0,05$ ) que celui des mâles capturés aussi bien en amont qu'en aval. Le poids des spécimens mâles de l'aval à son tour est plus élevé (test de Mann-Witney,  $p < 0,05$ ) que celui des poissons de l'amont. La variation

de la taille des mâles (Fig. 4b) provenant des différentes zones évolue exactement de la même façon que celle du poids. Les femelles capturées dans le lac présentent un poids plus élevé que celui des femelles provenant de l'aval et de l'amont (Fig. 4a). De même, le poids des femelles enregistré en aval est significativement supérieur à celui des femelles issues de l'amont ( $p < 0,05$ ).

La taille des femelles (Fig. 4b) est significativement plus élevée dans le lac qu'en amont et en aval, les poissons de l'aval étant comme pour le poids, plus longs que ceux de l'amont. Pour une même zone écologique, en dehors de l'amont (pour le poids), les paramètres biologiques (poids et longueur) des mâles sont significativement plus élevés (test de Mann-Witney,  $p < 0,05$ ) que celui des femelles (Fig. 5a et 5b).

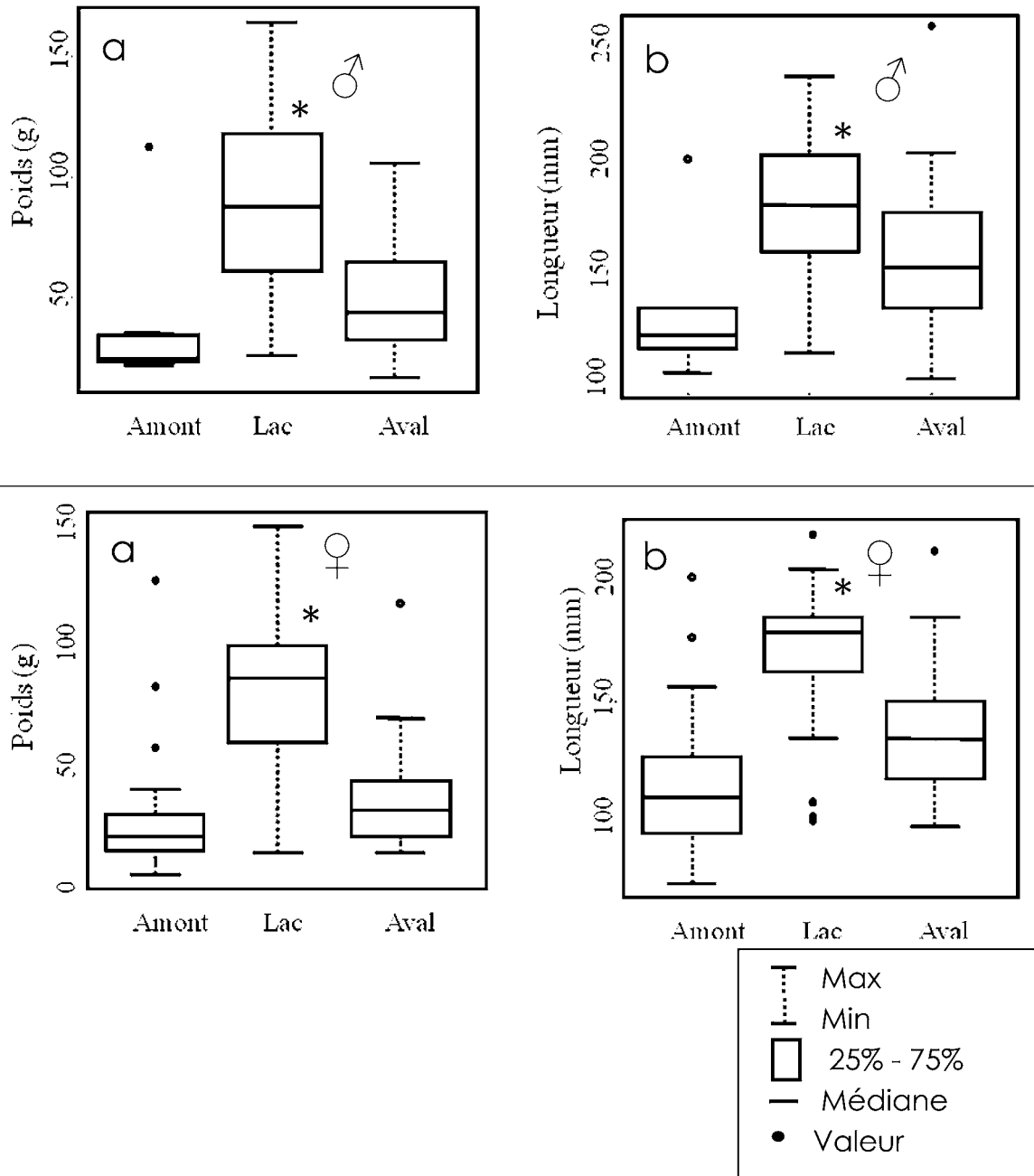


Fig. 4. – Box-Plot montrant la variation du poids (a) et de la longueur (b) des spécimens mâles et femelles de *Marcusenius ussheri* en fonction des zones écologiques définies.  
\*: valeur significativement plus élevée.

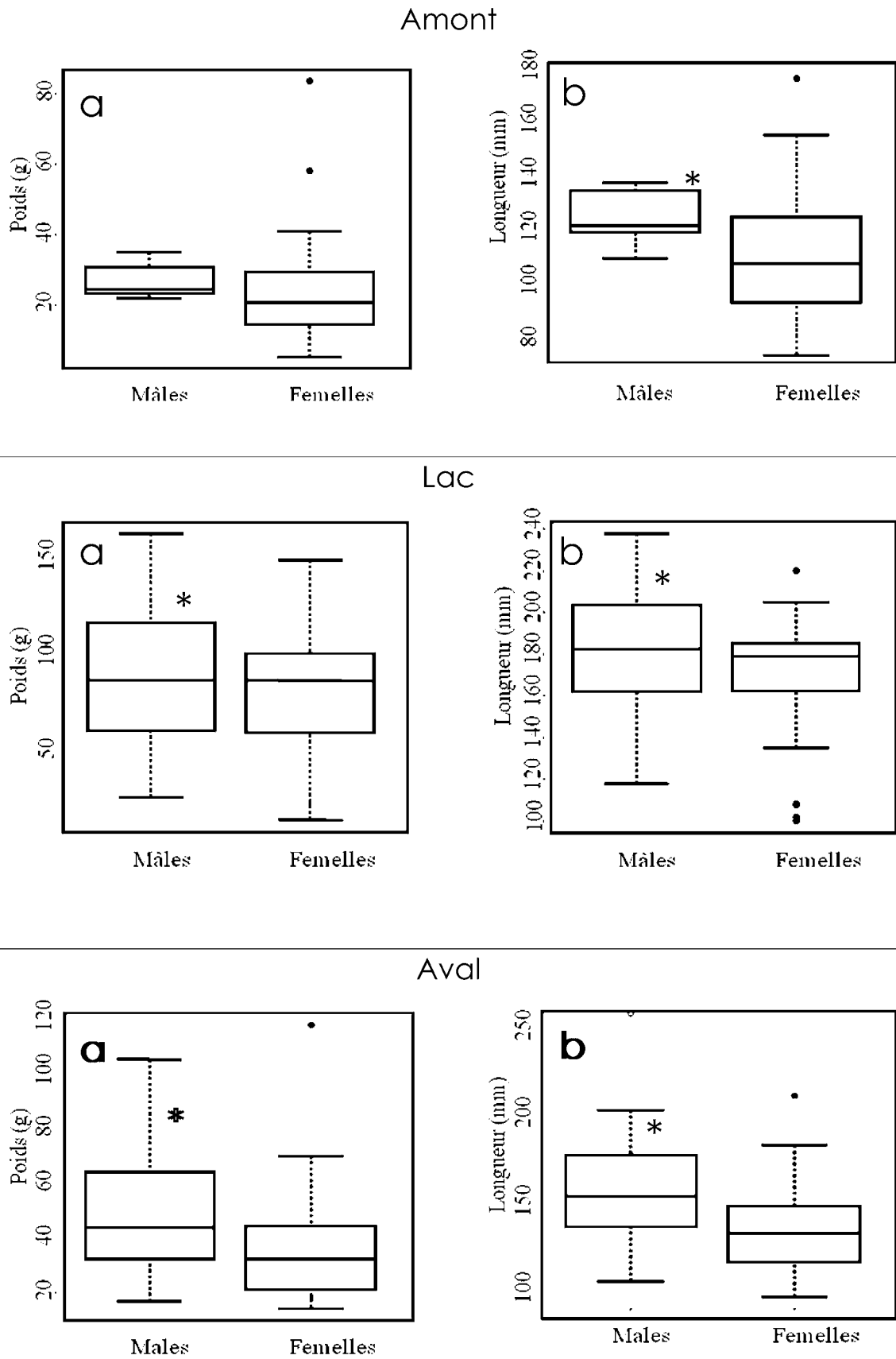


Fig. 5. – Box-Plot montrant la variation du poids (a) et de la longueur (b) des spécimens mâles et femelles de *Marcuseinius ussheri* en amont, dans le lac et à l’aval de la rivière Bia.

\*: valeur significativement plus élevée.

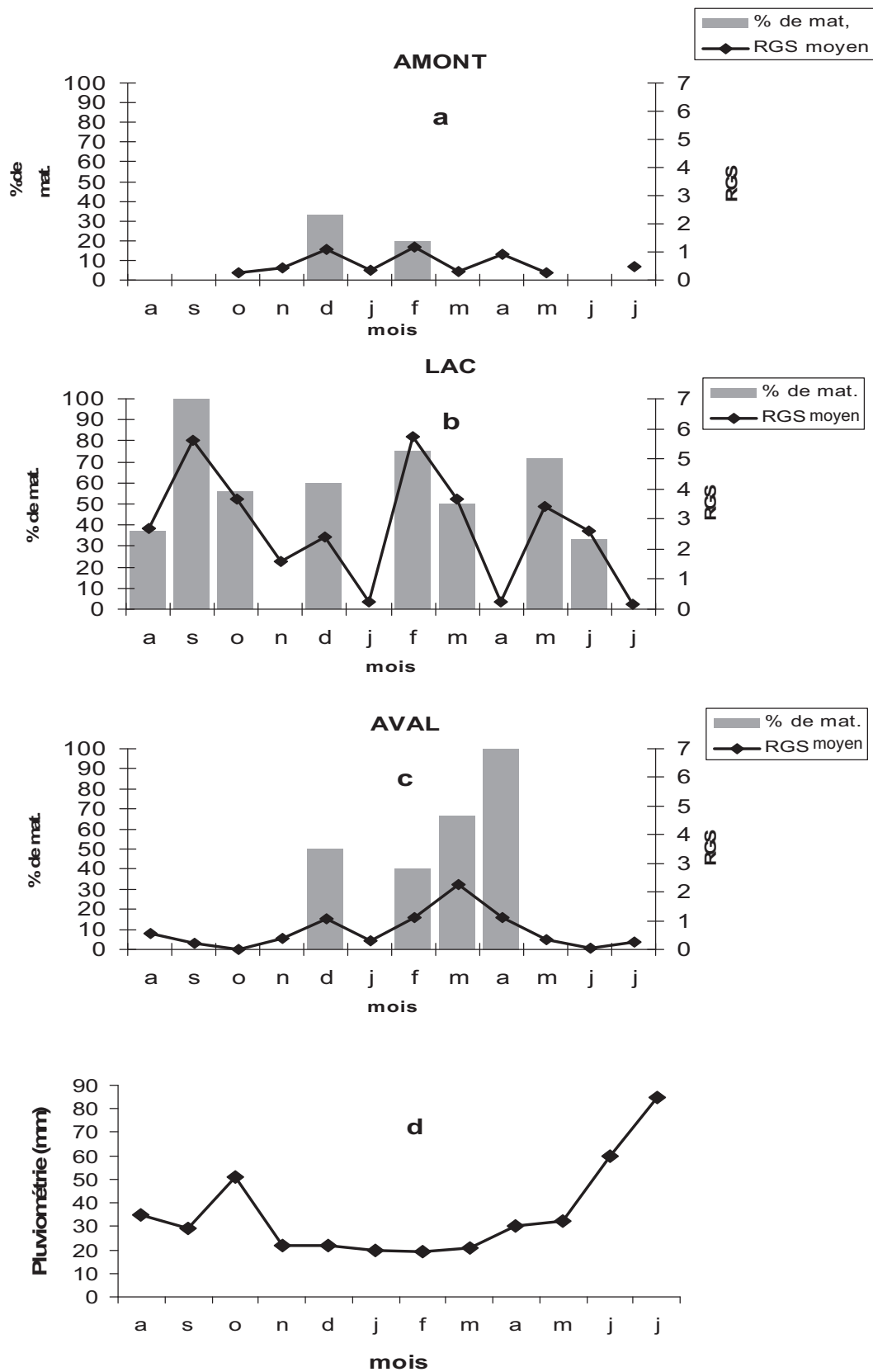


Fig. 6. – Pourcentage des femelles portant des gonades matures et Rapport Gonado-somatique (RGS) mensuels en amont (a), dans le lac (b) et à l’aval (c). La figure (6d) fait référence à la pluviométrie.

### Evolution saisonnière de l'activité sexuelle femelle

L'activité sexuelle des individus femelles a été analysée au niveau des trois milieux (Fig. 6). En amont (Fig. 6a), trois maxima de RGS moyen sont présents respectivement en décembre (1.08%), février (1.19%) et avril (0.93%). Le pourcentage de femelles matures rencontrées est de 33% en décembre et 20% en février. Le graphique correspondant au lac (Fig. 6b) montre quatre pics principaux de RGS moyen: le premier se situe en septembre (5.6%), le second en décembre (2.39%), le troisième est centré au mois de février (5.73%) et le dernier en mai (3.42%). Des individus matures se rencontrent presque toute l'année, en particulier en septembre (100%), décembre (60%), février (73%) et mai (71%). En aval de la Bia (Fig. 6c), le RGS maximum présente deux pics localisés en décembre et en mars. Les valeurs respectives associées sont de 0.2 et 1.2. Les femelles matures se rencontrent en décembre puis en février et constituent respectivement 33 et 20% de la population.

### Diamètre des ovocytes et fécondité

Trente-deux ovaires de stade 4 et trente ovocytes mesurés par ovaire ont été examinés, soit au total 960 ovocytes. Le diamètre moyen obtenu est de 1.81mm. La fécondité relative moyenne s'élève à 3183 par kg de poids corporel. Le maximum atteint 53897 ovocytes.

## DISCUSSION

L'analyse des valeurs relatives à la taille de première reproduction de *Marcusenius ussheri*, montre que ce paramètre varie en fonction du milieu. Ainsi, ALBARET (1982) indique à propos de la même espèce qu'à 130mm, 50% des femelles atteignent la maturité sexuelle dans le bassin de la Comoé, autre bassin de la Côte d'Ivoire, contre 168mm dans ce travail. Par ailleurs, ALBARET (1994) attribue ces variations de taille de première reproduction en fonction des bassins ou des secteurs considérés, au fait que les poissons développent des stratégies différentes dans des milieux différents en vue sans doute d'une meilleure adaptation. Selon ce même auteur, ces stratégies peuvent être d'ordre alimentaire ou de la reproduction ou les deux à la fois dans la mesure où la première a une influence sur l'autre. La taille de première maturité varie également en fonction du sexe. Elle est en effet plus faible chez les femelles que chez les mâles. Les femelles seraient donc sur le plan de la maturité sexuelle, beaucoup plus précoces que les mâles.

Au niveau de la population (mâle et femelle), c'est en amont que l'on enregistre aussi bien au niveau de la taille, du poids corporel et du pourcentage des individus matures, les valeurs les plus faibles. Il en est de même du RGS. Tous ces éléments tendent à suggérer une présence majoritaire de juvéniles en ce lieu. En revanche, le lac abrite la grande majorité des individus matures avec les plus grandes valeurs des paramètres biologiques. De plus, l'écoulement unidirectionnel des eaux rend le lac solidaire de l'amont et en fait une zone d'inondation favorable à la reproduction des poissons (MERONA, 2005). C'est ce que semble montrer la valeur du sex-ratio dans le lac. En

effet, plus ce rapport est proche de l'équilibre 1:1, plus la reproduction est meilleure comme l'indique LEGENDRE (1991) chez le poisson *Sarotherodon melanotheron*. Plus encore, le niveau d'eau élevé et la baisse de la conductivité dans le lac (par rapport à l'amont et l'aval), sont, selon KIRSCHBAUM (1987), des facteurs qui induisent la reproduction chez les Mormyridae. Quant à l'aval de la rivière, il est séparé du lac par le barrage (sauf en période d'ouverture). Ainsi, les populations amont et lac seraient les mêmes et les résultats énoncés indiquent l'existence en leur sein, d'une stratégie particulière, celle d'une migration des individus matures du lac (zone lentique) pour aller pondre en amont. Un tel comportement reproducteur a été évoqué par BENECH & QUENSIERE (1985) à propos des Mormyridae dans le lac Tchad. Diverses espèces de ce groupe sont en effet, capables d'effectuer des migrations de faibles, moyennes et grandes amplitudes pour se reproduire. L'amont de la rivière, zone lotique est plus riche en oxygène à cause de l'écoulement de l'eau. De plus, son substrat sableux parsemé de débris végétaux (feuilles et tiges) et l'existence d'îlots rocheux sur son lit principal se prêtent parfaitement à la fraie des poissons. L'aval de la rivière héberge une quantité relativement importante d'individus matures (31%) à côté d'une assez grande cohorte de juvéniles. Isolé mécaniquement du reste de la rivière, l'aval, par les résultats obtenus, semble être une zone écologique différente où la reproduction de *Marcusenius ussheri* pourrait se dérouler *in situ*. En appui à cette répartition faite en relation avec la reproduction, KOUAMELAN (1999), indique que 21% des organismes-proies constituant le régime alimentaire de *Marcusienues ussheri* se rencontrent en amont, contre 53% dans le lac et 26% en aval. Ces résultats vont dans le même sens que ceux relatifs au potentiel reproducteur de l'espèce dans les différents milieux écologiques étudiés. Ils sont tous en accord avec les indications de ALBARET (1994) selon lequel, il y a une influence positive entre régime alimentaire et reproduction. Par ailleurs, en prenant en compte les périodes de reproduction en fonction des saisons, on constate qu'en amont, la reproduction se déroule pendant une petite partie de la grande saison sèche. Dans le lac, l'activité reproductrice se déroule pratiquement toute l'année aussi bien en saison sèche qu'en saison des pluies. En aval, le poisson se reproduit pendant toute la grande saison sèche. Ces différentes stratégies de reproduction sont en accord avec les observations de BLAKE (1976) qui note que l'espèce peut se reproduire en une seule fois (ponte unique), en plusieurs fois (ponte fractionnée) en fonction des conditions environnementales.

## CONCLUSION

Le Mormyridae *Marcusenius ussheri* présente des tactiques de reproduction variables en fonction du milieu. La taille de première maturité sexuelle est plus grande dans le lac (milieu lentique) qu'en aval où l'eau est courante. De même, le taux de maturation des gonades ainsi que l'indice gonadique sont d'autant plus importants que le courant d'eau diminue. La taille et le poids des spécimens évoluent dans le même sens. Ainsi, le lac apparaît comme un préférendum pour *M. ussheri*.



## REMERCIEMENTS

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## Artificial hard substrata from the Belgian part of the North Sea and their influence on the distributional range of species

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**ABSTRACT.** A comprehensive list of the macrospecies recorded on ten shipwrecks from the Belgian Part of the North Sea is presented. The historical records of these species in the area are also reviewed to address the potential role of shipwrecks in providing habitats for species typically associated with hard substrata. The current pooled species richness for Belgian shipwrecks consists of 224 spp., including 12 fish species. Among these species, 46 are new records to the Belgian fauna. Species on shipwrecks fulfil a gap in the regional distribution of sessile and mobile epibenthos. The records of several species are interrupted in the Southern Bight of the North Sea, simply because their habitats, natural hard substrata, are rare in this area and poorly studied. Another consequence of the presence of hard substrata offered by artificial sources is the increase in the distributional range of several species. Due to the presence of artificial substrata, a total of 12 southern species presented an extension of their known geographical range to the North.

**KEY WORDS:** Epifauna, shipwreck, North Sea, distribution of species

### INTRODUCTION

In the Southern Bight of the North Sea, the seabed is largely dominated by fine sediments (HOUBOLT, 1968). At the southern tip of this area, the seabed of the Dover Strait is the last large area comprising hard substrata (rocky outcrops, gravel and pebble fields) (CABIOCH & GLAÇON, 1975). North-East of the Dover Strait, the increasing opening between continental Europe and UK results in decreased current velocity, allowing sedimentation of finer particles. Consequently, soft sediments dominate the seabed of the Belgian part of the North Sea (BPNS). Natural hard substrata such as rocky bottoms are absent and pebbles are rare, only occurring locally in the swales between sandbanks (LANCKNEUS et al., 2001). Further to the North, areas of gravel become even smaller and more widely separated (VEENSTRA, 1969; KUHNE & RACHOR, 1996; LANCKNEUS et al., 2001). A strong correlation exists between substrate type and associated taxa (DUINVELD et al., 1991; VAN HOEY et al., 2004; VAN HOEY et al., 2005). Hard substrata favour development of assemblages dominated by large epifaunal species, which in turn allow settlement of secondary colonizers. In comparison, the third dimension offered by fine sediment substratum promotes the establishment of infaunal species (FRASCHETTI et al., 2003). In the BPNS lie a large number of artificial structures comprising mainly shipwrecks (MASSIN et al., 2002; ZINTZEN et al., 2006). Even if the shipwrecks were not planned as artificial reefs, they provide a habitat for species typically associated with hard substrata that may not be encountered otherwise in the area (HISCOCK, 1980; FORTEATH et al., 1982; PICKEN, 1986; LEEWIS et al., 2000; ZINTZEN et al., 2007; ZINTZEN et al., 2008). *In fine*, they are a source of hard substrata, which facilitate the development of an epifaunal community, locally increasing the pool of species. In a context of local population of hard substrate epifaunal species that

are widely and sparsely dispersed over an area such as the Southern Bight of the North Sea, the existence of shipwrecks may have important implications on the repartition, dissemination and genetic variability of these species.

In this paper, we present a comprehensive list of the macrospecies recorded on ten shipwrecks from the BPNS, complemented by a review of past records of these species in the North Sea to address the potential role of shipwrecks in providing habitat for species typically associated with hard substrata.

### MATERIALS AND METHODS

Ten shipwreck sites from the BPNS (8-37m depth) were investigated between 2001 and 2005 using SCUBA divers. The ten vessels (Table 1, Fig. 1) had been sunk for at least 40 years and consequently, it was considered that their communities had reached a mature state (VAN MOORSEL et al., 1991; LEEWIS et al., 2000). In total, 108 samples were collected. Quantitative sampling, during daylight, was done by scraping off all the living fauna within frames of 25x25cm on randomly selected surfaces (both vertically and horizontally orientated). On board, animals were relaxed in a 3.5% MgCl<sub>2</sub> solution during two hours and transferred to a buffered formalin solution (final concentration 4%, pH 8.2-8.4). Later, specimens were transferred to 70% buffered alcohol for permanent storage. The samples were sorted using a binocular microscope, and the macrofauna (>1mm) was identified to the lowest possible taxonomic level and counted. The material collected was deposited in the collections of the Royal Belgian Institute of Natural Sciences under the General Inventory number 29462. Most samples were taken between March and September as weather conditions during late autumn and winter generally did not

TABLE 1  
Studied shipwreck sites.

SHIPWRECK SITES	WGS-84 COORDINATES	DEPTH (MLLWS) (m)	DATE OF SUNK	SAMPLING YEARS (20xx)
1 - Birkenfels	N 51°38',989 E 02°32',268	37	1966	01-02-03-04-05
2 - Callisto	N 51°41',950 E 02°37',330	28	1959	04-05
3 - Garden City	N 51°29',170 E 02°18',320	26	1969	05
4 - John Mahn	N 51°28',930 E 02°41',350	29	1942	05
5 - Duc de Normandie	N 51°25',524 E 02°36',345	29	1942	05
6 - LCT 457	N 51°24',670 E 02°43',720	21	1944	05
7 - Kilmore	N 51°23',730 E 02°29',790	30	1906	03-04-05
8 - Bourrasque	N 51°14',964 E 02°33',026	18	1940	02-03-04-05
9 - LST 420	N 51°15',510 E 02°40',830	8	1944	05
10 - Sperrbrecher	N 51°16',650 E 02°49',780	9	1942	04

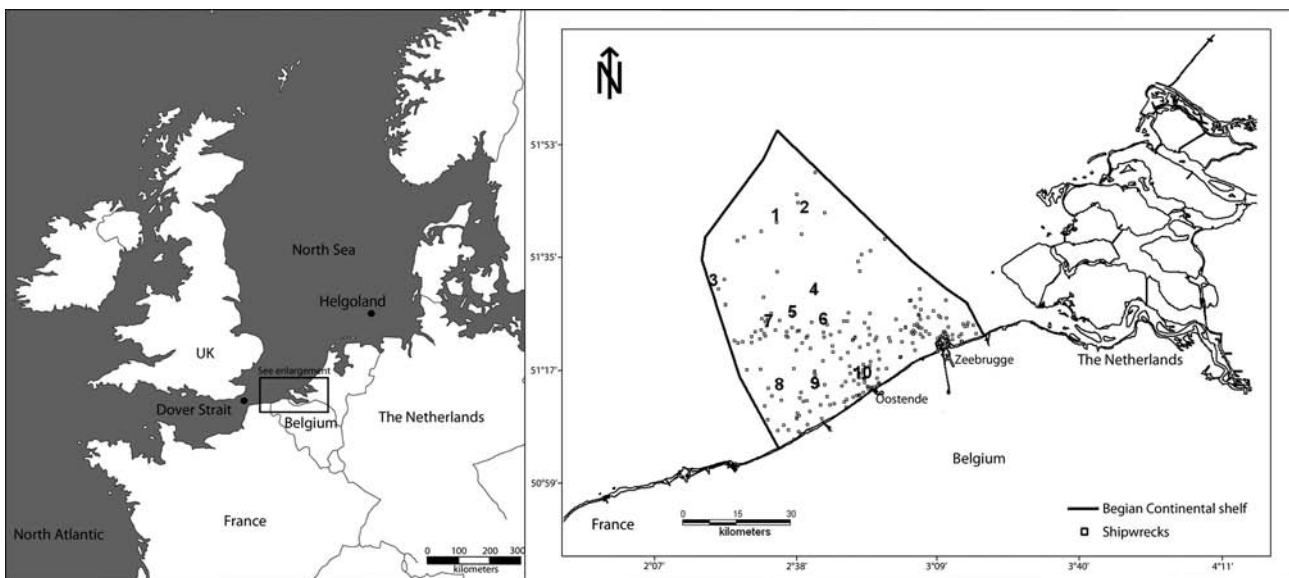


Fig. 1. – Localization of the studied shipwrecks. 1: Birkenfels, 2: Callisto, 3: Garden City, 4: John Mahn, 5: Duc de Normandie, 6: LCT 457, 7: Kilmore, 8: Bourrasque, 9: LST 420, 10: Sperrbrecher. Source: Afdeling Waterwegen Kust, Belgium.

allow sampling. Species only recorded between October and February are presented in a separate table. Some species were also recorded as *in situ* observations by divers and digital picture analysis. They are also presented in a separate table. Rare species are defined as those mentioned once or twice in past studies.

In addition, we reviewed the historical records of these species in the BPNS by screening regional faunal listings (GOVAERE, 1978; CATRIJSSE & VINCX, 2001; DAUVIN et al., 2003; DEGRAER et al., 2006) and the Zoological Record for the last 30 years. For records considered new to the area, we went through the literature to compile distributional ranges at the scale of the North Sea and surrounding area. Species were defined either as Northern species, if their distributional range was restricted to the Northern part of the North Sea, or as Southern species if they were only recorded from the Lusitanian Province. The distributional range was further refined as 'Southern North Sea' if the species was recorded as far North as Helgoland, or 'Northern North Sea' if the range extended South to this point. Species are defined as cosmopolitan if they have been previously recorded outside the present

area of study. From this information, the implications of shipwrecks from the BPNS were defined as either (1) filling a gap in the distributional range of the species if the species was recorded North and South of the BPNS or (2) permitting an extension of the distributional range to the North or to the South.

## RESULTS

The species richness and average density ranges for the different shipwrecks between March and September (2001-2005) are presented in Table 2. The species richness for the period March-September totals 193 species. Another nine species were only sampled between October and February (Table 3). An additional 22 species were observed *in situ* or after examination of digital pictures (Table 4). Consequently, the pooled species richness for Belgian shipwrecks is 224 spp. Of these species, 46 can be considered new to the Belgian fauna (Table 5). Their actual distributions in the North Sea are given in this table. Most of the species mentioned in our study have

been recorded from north and south of the BPNS, although ten species have a meridional distribution, with shipwrecks extending their distribution to the north (*Actinothoe sphyrodeta*, *Eulalia aurea*, *Lysidice ninetta*, *Marphysa sanguinea*, *Polydora hoplura*, *Sphaerosyllis bulbosa*, *Thelepus setosus*, *Tritonia manicata*, *Acasta*

*spongites*, *Lysianassa ceratina*). Some species rare for the area have also been recorded (Table 6). Although our results are focussed on the fauna of shipwrecks, we note that no macroalgae were either seen or sampled by divers at any time.

TABLE 2

Distribution of the epifaunal species on the ten investigated shipwrecks. Only the data collected between April and September are used. Sites are ordered from offshore to coastal zone. BRK: Birkenfels (N=25 samples of 25x25cm), CAL: Callisto (N=7), GAR: Garden City (N=3), JON: John Mahn (N=3), DUC: Duc de Normandie (N=3) LCT: LCT 457 (N=3), KLM: Kilmore (N=32), BRQ: Bourrasque (N=23), LST: LST 420 (N=3), SPR: Sperrbrecher (N=6). A simplified scale of dominance was attributed to the colonial species: ● present, ●● abundant, ●●● dominant.

	BRK	CAL	GAR	JON	DUC	LCT	KLM	BRQ	LST	SPR
<b>PORIFERA</b>										
<i>Dysidea fragilis</i> (Montagu, 1818)	●●	●●			●●		●●			
<i>Esperiopsis fucorum</i> (Esper, 1794)				●	●●		●●			
<i>Halichondria cf panicea</i> (Pallas, 1766)					●●		●●			
<i>Haliclona oculata</i> (Pallas, 1766)					●		●●			
<i>Haliclona</i> sp.	●						●●	●		
<i>Hymeniacion perlevis</i> (Montagu, 1818)		●					●●			
<i>Leucosolenia</i> sp.		●	●	●		●	●●			
<i>Myxilla rosacea</i> (Lieberkühn, 1859)					●●		●●			
<i>Phorbas plumosus</i> (Montagu, 1818)							●●			
<i>Sycon ciliatum</i> (Fabricius, 1780)	●	●●	●	●●	●●	●●	●●			
<b>CNIDARIA</b>										
<b>Hydrozoa</b>										
<i>Bougainvillia muscus</i> (Allman, 1863)								●●		
<i>Campanularia volubilis</i> (Linnaeus, 1758)	●							●●		
<i>Clytia gracilis</i> (Sars, 1850)								●●		
<i>Clytia hemisphaerica</i> (Linnaeus, 1767)	●						●	●		
<i>Halecium</i> sp.							●			
<i>Hydractinia echinata</i> (Flemming, 1828)	●							●●	●	
<i>Hydrallmania falcata</i> (Linnaeus, 1758)								●●	●	
<i>Laomedea flexuosa</i> Alder 1857								●●	●	
<i>Nemertesia antennina</i> (Linnaeus, 1758)							●	●		
<i>Obelia bidentata</i> Clarke, 1875	●						●	●	●	
<i>Sarsia eximia</i> (Allman, 1859)		●								
<i>Sertularia cupressina</i> Linnaeus, 1758								●●		
<i>Tubularia indivisa</i> Linnaeus, 1758	●●●	●●●	●●●	●●●	●●●	●●●	●●●	●●	●●●	
<i>Tubularia larynx</i> Ellis & Solander, 1786	●●					●●	●●	●●		
<b>ANTHOZOA</b>										
<b>Actiniaria</b>										
<i>Alcyonium digitatum</i> Linnaeus, 1758										
<i>Diadumene cincta</i> Stephenson, 1925										
<i>Metridium senile</i> (Linnaeus, 1767)										
<b>PLATYHELMINTHES</b>										
<i>Eurylepta cornuta</i> (O.F. Müller, 1776)										
<i>Turbellaria</i>										
<b>NEMERTEA</b>										
<i>Baseodiscus delineatus</i> (Delle Chiaje, 1825)										
<i>Nemertinata</i> sp.										
<i>Oerstedtia dorsalis</i> (Abildgaard, 1806)										
<b>ANNELIDA</b>										
<b>POLYCHAETA</b>										
<i>Alentia gelatinosa</i> (M. Sars, 1835)										
<i>Autolytus</i> sp.										
<b>Cirratulidae</b>										
<i>Cirratulus cirratus</i> (O.F. Müller, 1776)										
<i>Cirratulus filiformis</i> Keferstein, 1862										
<i>Cirratulus</i> sp.										
<i>Cirriformia tentaculata</i> (Montagu, 1808)										
<i>Dipolydora coeca</i> (Oersted, 1843)										
<i>Eteone longa</i> (Fabricius, 1780)										
<i>Eteone picta</i> Quatrefages, 1865										

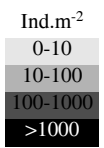


TABLE 2

Distribution of the epifaunal species on the ten investigated shipwrecks. Only the data collected between April and September are used. Sites are ordered from offshore to coastal zone. BRK: Birkenfels (N=25 samples of 25x25cm), CAL: Callisto (N=7), GAR: Garden City (N=3), JON: John Mahn (N=3), DUC: Duc de Normandie (N=3) LCT: LCT 457 (N=3), KLM: Kilmore (N=32), BRQ: Bourrasque (N=23), LST: LST 420 (N=3), SPR: Sperrbrecher (N=6). A simplified scale of dominance was attributed to the colonial species: ● present, ●● abundant, ●●● dominant.

	BRK	CAL	GAR	JON	DUC	LCT	KLM	BRQ	LST	SPR
<i>Eulalia aurea</i> Gravier, 1896										
<i>Eulalia</i> sp.										
<i>Eulalia viridis</i> (Linnaeus, 1768)										
<i>Eumida</i> sp.										
<i>Eupolymnia nebulosa</i> (Montagu, 1818)										
<i>Eupolymnia nesidensis</i> (Delle Chiaje, 1828)										
<i>Eusyllis blomstrandii</i> Malmgren, 1867										
<i>Gattyana cirrhosa</i> (Pallas, 1766)										
<i>Harmothoe</i> sp.										
<i>Kefersteinia cirrata</i> (Keferstein, 1862)										
<i>Lanice conchilega</i> (Pallas, 1766)										
<i>Lepidonotus squamatus</i> (Linnaeus, 1758)										
<i>Lumbrineris latreilli</i> Audouin & Milne-Ed., 1834										
<i>Lumbrineris</i> sp.										
<i>Marphysa sanguinea</i> (Montagu, 1815)										
<i>Neoamphitrite figulus</i> (Dallyell, 1853)										
<i>Nereimyra punctata</i> (O.F. Müller, 1788)										
<i>Nereis pelagica</i> Linnaeus, 1758										
<i>Nicolea venustula</i> (Montagu, 1818)										
<i>Nicomache</i> sp.										
<i>Ophelia</i> sp.										
<i>Owenia fusiformis</i> Delle Chiaje, 1842										
<i>Pectinaria koreni</i> (Malmgren, 1866)										
<i>Pholoe inornata</i> Johnston, 1839										
<i>Phyllodoce longipes</i> Kinberg, 1866										
<i>Phyllodoce mucosa</i> Oersted, 1843										
<i>Phyllodoce</i> sp.										
<i>Pista cristata</i> (O.F. Müller, 1776)										
<i>Poecilochaetus serpens</i> Allen, 1904										
<i>Polycirrus</i> sp.										
<i>Polydora hoplura</i> Claparède, 1870										
<i>Polydora</i> sp.										
<i>Pomatoceros triquetus</i> (Linnaeus, 1758)										
<i>Proceraea</i> sp.										
<i>Procerastea halleziana</i> Mallaquin, 1893										
<i>Procerastea nematodes</i> Langerhans, 1884										
<i>Sabellaria spinulosa</i> Leuckart, 1849										
<i>Scoloplos armiger</i> (Allman, 1859)										
<i>Sphaerodoropsis flavum</i> Oersted, 1843										
<i>Sphaerosyllis bulbosa</i> Southern, 1914										
<i>Sphaerosyllis</i> sp.										
<i>Sthenelais boa</i> (Johnston, 1833)										
<i>Subadyte pellucida</i> (Ehlers, 1864)										
Syllidae										
<i>Syllis armillaris</i> (O.F. Müller, 1776)										
<i>Syllis gracilis</i> Grube, 1840										
Terebellidae										
<i>Thelepus cincinnatus</i> (Fabricius, 1780)										
<i>Thelepus setosus</i> (Quatrefages, 1865)										
<b>MOLLUSCA</b>										
<b>GASTROPODA</b>										
Aeolidiidae										
<i>Archidoris pseudoargus</i> (Rapp, 1827)										
<i>Catriona gymnota</i> (Couthouy, 1838)										
<i>Cerithiopsis tubercularis</i> (Montagu, 1803)										
<i>Crepidula fornicata</i> (Linnaeus, 1758)										
<i>Cuthona amoena</i> (Alder & Hancock, 1845)										
<i>Cuthona concinna</i> (Alder & Hancock, 1843)										
<i>Cuthona</i> sp.										
<i>Dendronotus frondosus</i> (Ascanius, 1774)										

Ind.m<sup>-2</sup>  
 0-10  
 10-100  
 100-1000  
 >1000

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	BRK	CAL	GAR	JON	DUC	LCT	KLM	BRQ	LST	SPR
<i>Doto pinnatifida</i> (Montagu, 1804)							●●			
<i>Epitonium clathratulum</i> (Kanmacher, 1798)	●●	●●	●●					●●		
<i>Eubranchus pallidus</i> (Alder & Hancock, 1842)							●●			
<i>Eubranchus</i> sp.	●●						●●		●●	
<i>Euspira pulchella</i> (Risso, 1826)	●●	●●						●●		
<i>Facelina bostoniensis</i> (Couthouy, 1838)	●●					●●				
<i>Lamellaria latens</i> (O.F. Müller, 1776)						●●	●●			
<i>Nassarius incrassatus</i> (Ström, 1768)	●●	●●	●●		●●		●●			●●
<i>Nassarius reticulatus</i> (Linnaeus, 1758)	●●						●●	●●		
<i>Raphitoma linearis</i> (Montagu, 1803)	●●						●●			
Rissoidae	●●						●●			
<i>Tergipes tergipes</i> (Forskål, 1775)	●●						●●			
<i>Tritonia cf. manicata</i> Deshayes, 1853							●●			
<i>Tritonia plebeia</i> Johnston, 1828		●●								
<i>Trivia monacha</i> (da Costa, 1778)							●●			
<b>BIVALVIA</b>										
<i>Aequipecten opercularis</i> (Linnaeus, 1758)	●●	●●	●●	●●			●●	●●		
<i>Heteranomia squamula</i> (Linnaeus, 1758)	●●	●●	●●	●●	●●		●●	●●		
<i>Musculus</i> sp.	●●	●●	●●	●●	●●		●●	●●		
<i>Mysella bidentata</i> (Montagu, 1803)	●●	●●	●●	●●	●●		●●	●●		
<i>Mytilus edulis</i> Linnaeus, 1758	●●	●●	●●	●●	●●		●●	●●	●●	●●
Ostreidae							●●			
<i>Venerupis geographica</i> (Gmelin, 1791)	●●						●●			
<i>Venerupis</i> sp.	●●	●●					●●	●●		
<b>SIPUNCULA</b>										
Golfingiida							●●			
<b>CRUSTACEA</b>										
<b>COPEPODA</b>										
Copepoda	●●						●●	●●		
<b>CIRRIPEDIA</b>										
<i>Acasta spongites</i> (Poli, 1795)							●●			
<i>Balanus crenatus</i> Bruguière, 1789	●●				●●		●●	●●		●●
<i>Verruca stroemia</i> O.F. Müller, 1776							●●			
<b>CUMACEA</b>										
Cumacea	●●							●●		
<b>ISOPODA</b>										
<i>Janira maculosa</i> Leach, 1814							●●			
<i>Pleurocrypta porcellanae</i> Hesse, 1876							●●			
<b>AMPHIPODA</b>										
<i>Abludomelita obtusata</i> (Montagu, 1813)								●●	●●	
<i>Atylus swammerdami</i> (Milne-Edwards, 1830)				●●				●●	●●	
<i>Caprella linearis</i> (Linnaeus, 1767)	●●	●●	●●	●●	●●		●●	●●	●●	●●
<i>Caprella tuberculata</i> Guérin, 1836	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●
<i>Iphimedia nexa</i> Myers & McGrath, 1987	●●	●●	●●	●●	●●		●●	●●	●●	●●
<i>Jassa herdmani</i> (Walker, 1893)	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●
<i>Lysianassa ceratina</i> (Walker, 1889)	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●
<i>Melita hergensis</i> Reid, 1939	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●
<i>Metopa alderi</i> (Bate, 1857)	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●
<i>Monocorophium acherusicum</i> (Costa, 1851)	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●
<i>Monocorophium sextonae</i> (Crawford, 1937)	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●
<i>Pariambus typicus</i> (Kroyer, 1844)	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●
<i>Phtisica marina</i> Slabber, 1769	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●
<i>Pseudoprotella phasma</i> (Montagu, 1804)	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●
<i>Stenothoe marina</i> (Bate, 1856)	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●
<i>Stenothoe monoculoides</i> (Montagu, 1815)	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●
<i>Stenothoe</i> sp.	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●
<i>Stenothoe valida</i> Dana, 1855	●●	●●	●●	●●	●●	●●	●●	●●	●●	●●
<b>DECAPODA</b>										
<i>Anapagurus chiroacanthus</i> (Lilljeborg, 1856)	●●									
<i>Atelecyclus rotundatus</i> (Olivieri, 1792)	●●									

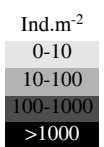


TABLE 2

Distribution of the epifaunal species on the ten investigated shipwrecks. Only the data collected between April and September are used. Sites are ordered from offshore to coastal zone. BRK: Birkenfels (N=25 samples of 25x25cm), CAL: Callisto (N=7), GAR: Garden City (N=3), JON: John Mahn (N=3), DUC: Duc de Normandie (N=3) LCT: LCT 457 (N=3), KLM: Kilmore (N=32), BRQ: Bourrasque (N=23), LST: LST 420 (N=3), SPR: Sperrbrecher (N=6). A simplified scale of dominance was attributed to the colonial species: ● present, ●● abundant, ●●● dominant.

	BRK	CAL	GAR	JON	DUC	LCT	KLM	BRQ	LST	SPR
<i>Ebalia tumefacta</i> (Montagu, 1808)							0-10			
<i>Hyas araneus</i> (Linnaeus, 1758)	0-10									
<i>Inachus phalangium</i> (Fabricius, 1775)							0-10			
<i>Liocarcinus arcuatus</i> (Leach, 1814)							0-10			
<i>Liocarcinus holsatus</i> (Fabricius, 1798)							0-10	0-10		
<i>Liocarcinus</i> sp.	0-10						0-10	0-10		0-10
<i>Macropodia parva</i> Van Noort & Adema, 1985							0-10		0-10	
<i>Macropodia rostrata</i> (Linnaeus, 1761)	0-10									
<i>Macropodia</i> sp.							0-10			
<i>Necora puber</i> (Linnaeus, 1767)	0-10									
Paguridae				0-10				0-10		
<i>Pilumnus hirtellus</i> (Linnaeus, 1761)	0-10	0-10	0-10	0-10	0-10	0-10	0-10	0-10	0-10	0-10
<i>Pisidia longicornis</i> (Linnaeus, 1767)	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
<i>Thorulus cranchii</i> (Leach, 1817)							0-10			
<b>CHELICERATA</b>										
<b>PYCONOGONIDA</b>										
<i>Achelia</i> sp.			0-10				0-10			
<i>Callipallene emacinata</i> (Dohrn, 1881)							0-10			
<i>Nymphon rubrum</i> Hodge, 1865							0-10			
<i>Pycnogonum littorale</i> (Strom, 1762)			0-10					0-10		
<b>BRYOZOA</b>										
<b>CYCLOSTOMATIDA</b>										
<i>Crisia aculeata</i> Hassall, 1841							●			
<i>Disporella hispida</i> (Fleming, 1828)	●									
<i>Plagioecia patina</i> (Lamarck, 1816)	●						●			
<b>CHEILOSTOMATIDA</b>										
<i>Bicelliariella ciliata</i> (Linnaeus, 1758)							●●			
<i>Conopeum reticulum</i> (Linnaeus, 1767)								0-10		
<i>Electra pilosa</i> (Linnaeus, 1767)	●	●●	●●	●●	●●	●●	●●	●	●	
<i>Scruparia chelata</i> (Linnaeus, 1758)	●									
<i>Scrupocellaria scruposa</i> (Linnaeus, 1758)	●	●					●●			
<b>CTENOSTOMATIDA</b>										
<i>Alcyonidium hydrocoailitum</i> Porter, 2004	●						●			
<i>Panolicella nutans</i> Jebram, 1985	●									
<i>Vesicularia spinosa</i> (Linnaeus, 1967)								0-10		
<b>ECHINODERMATA</b>										
<b>ASTEROIDEA</b>										
<i>Asterias rubens</i> Linnaeus, 1758	0-10	0-10			0-10		0-10	0-10	0-10	0-10
<i>Asterias rubens</i> juv. Linnaeus, 1758		0-10	>1000	>1000	>1000	>1000	0-10	0-10	>1000	0-10
<b>ECHINOIDEA</b>										
<b>Euechinoidea</b>										
<i>Psammechinus miliaris</i> (Gmelin, 1778)	>1000	>1000	0-10	0-10	0-10	0-10	0-10	0-10	0-10	0-10
<b>OPHIUROIDEA</b>										
<i>Amphipholis squamata</i> (Delle Chiaje, 1829)	0-10	>1000	>1000	>1000	>1000	>1000	>1000	0-10	0-10	0-10
<i>Ophiothrix fragilis</i> (Abildgaard, 1789)	>1000	>1000	>1000	>1000	>1000	>1000	>1000	0-10	0-10	0-10
<i>Ophiura albida</i> Forbes, 1839	0-10									
<i>Ophiura</i> sp.							0-10			
<b>CHORDATA</b>										
<b>TUNICATA</b>										
<b>Ascidiacea</b>										
<i>Diplosoma</i> sp.		●	●●	●●	●●	●●	●●	●●		
<i>Molgula cf occulta</i> Kupffer, 1875	0-10	0-10					0-10	0-10		
<i>Polyclinum aurantium</i> Milne-Edwards, 1841		●●					●●	●●		

Ind.m<sup>-2</sup>  
0-10  
10-100  
100-1000  
>1000

TABLE 3

Distribution of the epifaunal species exclusively recorded between October and March. Sites are ordered from offshore to coastal zone. BRK: Birkenfels (N=7), KLM: Kilmore (N=14), BRQ: Bourrasque (N=12). A simplified scale of dominance was attributed to the uncountable species: ●: present, ●●: abundant, ●●●: dominant. Density never exceeded 10 individuals.m<sup>2</sup> (grey).

	BRK	KLM	BRQ
<b>PORIFERA</b>			
<i>Mycale cf macilenta</i> (Bowerbank, 1866)	●		
<i>Suberites ficus</i> (Esper, 1794)			●
<b>ANNELIDA</b>			
<b>POLYCHAETA</b>			
Eunicidae			
<i>Lysidice ninetta</i> Audouin & Milne-Ed., 1833			
<b>NERMERTEA</b>			
<i>Tubulanus</i> sp.			
<b>MOLLUSCA</b>			
<b>GASTEROPODA</b>			
<i>Tergipes tergipes</i> (Forsk., 1775)			
<b>CRUSTACEA</b>			
<b>CUMACEA</b>			
<i>Bodotria arenosa</i> Goodsir, 1843			
<b>AMPHIPODA</b>			
<i>Amphilochus manudens</i> Bate, 1862			
<b>DECAPODA</b>			
<i>Pagurus cuanensis</i> Bell, 1845			

TABLE 4

Species observed *in situ* by divers or from digital images but not sampled.

<b>CNIDARIA</b>	
<b>ANTHOZOA</b>	
<i>Urticina felina</i> (Linnaeus, 1767)	
<i>Sagartia</i> sp.	
<i>Sagartiogeton undatus</i> (O.F. Müller, 1788)	
<i>Actinothoe sphyrodeta</i> (Gosse, 1858)	
<b>CRUSTACEA</b>	
<b>DECAPODA</b>	
<i>Cancer pagurus</i> Linnaeus, 1758	
<b>MOLLUSCA</b>	
<b>CEPHALOPODA</b>	
<i>Loligo vulgaris</i> Lamarck, 1798 – eggs	
<i>Acanthodoris pilosa</i> (Abildgaard in O.F. Müller, 1789)	
<b>BRYOZOA</b>	
<b>CYCLOSTOMATIDA</b>	
<i>Bugula cf turbinata</i> Alder, 1857	
<b>CHORDATA</b>	
<b>TUNICATA</b>	
<i>Asciadiella scabra</i> (O.F. Müller, 1776)	
<i>Clavelina lepadiformis</i> O.F. Müller, 1776	
<b>PISCES</b>	
<i>Dicentrarchus labrax</i> (Linnaeus, 1758)	
<i>Gadus morrhua</i> Linnaeus, 1758	
<i>Myoxocephalus scorpius</i> (Linnaeus, 1758)	
<i>Parablennius gattorugine</i> (Linnaeus, 1758)	
<i>Pollachius pollachius</i> (Linnaeus, 1758)	
<i>Pollachius virens</i> (Linnaeus, 1758)	
<i>Pomatoschistus</i> sp.	
<i>Scomber scombrus</i> Linnaeus, 1758	
<i>Spondyliosoma cantharus</i> (Linnaeus, 1758)	
<i>Trisopterus luscus</i> (Linnaeus, 1758)	
<i>Trisopterus minutus</i> (Linnaeus, 1758)	
<i>Trachurus trachurus</i> (Linnaeus, 1758)	

TABLE 5

Species (from shipwrecks) that are new records for the Belgian fauna. SNS: Southern North Sea species, NNS: Northern North Sea species, NC: indicates the presence of the species in the neighbouring countries (fr: France, nl: The Netherlands, uk: United Kingdom), SS: Southern species, NS: Northern species, COS: cosmopolitan species, HS (Hard Substrata) CONSEQUENCES: the implication of the presence of the Belgian shipwreck for the distributional range of species. ?: information unavailable. See text for detailed explanations of the distributional ranges.

TAXON	SNS	NNS	NC	SS	NS	COS	HS CONSEQUENCES
<b>PORIFERA</b>							
<i>Dysidea fragilis</i>	+	+	fr,uk	+	+		fill in the gap
<i>Phorbas plumosus</i>	+	+	fr,uk				fill in the gap
<b>CNIDARIA</b>							
<i>Actinothoe sphyrodeta</i>	-	-	fr,uk	+	-		extension to the north
<i>Diadumene cincta</i>	+	+	fr,uk,nl			+	fill in the gap
<b>PLATYHELMINTHES</b>							
<i>Eurylepta cornuata</i>	+	+	fr,uk	+	-	+	fill in the gap
<b>NEMERTEA</b>							
<i>Oerstedia dorsalis</i>	+	+	fr,uk,nl			+	fill in the gap
<b>POLYCHAETA</b>							
<i>Alentia gelatinosa</i>	+	+	fr,uk				fill in the gap
<i>Cirratulus cirratus</i>	+	+	fr,uk,nl			+	fill in the gap
<i>Cirriformia tentaculata</i>	+	+	fr,uk			+	fill in the gap
<i>Dipolydora coeca</i>	+	+	fr,uk			+	fill in the gap
<i>Eteone picta</i>	+	-	fr,uk,nl	+	-		fill in the gap
<i>Eulalia aurea</i>	-	-	fr,uk	+			extension to the north
<i>Eupolymnia nebulosa</i>	+	+	fr,uk,nl	-	+	+	fill in the gap
<i>Eupolymnia nesidensis</i>	+	+	fr,uk			+	fill in the gap
<i>Lysidice ninetta</i>	-	-	fr,uk	+	-	+	extension to the north



TABLE 5

Species (from shipwrecks) that are new records for the Belgian fauna. SNS: Southern North Sea species, NNS: Northern North Sea species, NC: indicates the presence of the species in the neighbouring countries (fr: France, nl: The Netherlands, uk: United Kingdom), SS: Southern species, NS: Northern species, COS: cosmopolitan species, HS (Hard Substrata) CONSEQUENCES: the implication of the presence of the Belgian shipwreck for the distributional range of species.?: information unavailable. See text for detailed explanations of the distributional ranges.

TAXON	SNS	NNS	NC	SS	NS	COS	HS CONSEQUENCES
<i>Marphysa sanguinea</i>	+	-	fr,uk	+	-	+	extension to the north
<i>Neoamphitrite figulus</i>	+	+	fr,uk,nl			+	fill in the gap
<i>Nereimyra punctata</i>	+	+	fr,uk,nl	-	+		fill in the gap
<i>Phyllococe longipes</i>	+	+	fr,uk			+	fill in the gap
<i>Pista cristata</i>	-	+	?			+	fill in the gap from the north
<i>Polydora hoplura</i>	+	-	fr,uk			+	extension to the north
<i>Procerastea halleziana</i>	-	+	fr,uk				fill in the gap
<i>Procerastea nematodes</i>	-	+	fr,uk	+	-		fill in the gap
<i>Sphaerodoropsis flavum</i>	+	+	?			+	fill in the gap
<i>Sphaerosyllis bulbosa</i>	+	-	fr,uk	+	-		extension to the north
<i>Subadyte pellucida</i>	+	+	fr,uk,nl			+	fill in the gap
<i>Thelepus cincinnatus</i>	+	+	fr,uk,nl			+	fill in the gap
<i>Thelepus setosus</i>	-	-	fr,uk	+	-	+	extension to the north
<b>GASTROPODA</b>							
<i>Cerithiopsis tubercularis</i>	-	+	fr,uk				fill in the gap
<i>Cuthona amoena</i>	+	+	fr,uk,nl	+	-		fill in the gap
<i>Cuthona concinna</i>	+	+	fr,uk,nl	-	+	+	fill in the gap from the north
<i>Raphitoma linearis</i>	-	+	fr,uk				fill in the gap
<i>Tritonia cf manicata</i>	-	-	uk	+	-		extension to the north
<b>CRUSTACEA</b>							
<i>Acasta spongites</i>	-	-	fr,uk	+	-		extension to the north
<i>Caprella tuberculata</i>	+	+	fr,uk	+	-		fill in the gap
<i>Lysianassa ceratina</i>	-	-	fr,uk				extension to the north
<i>Pleurocrypta porcellanae</i>	+	-	fr,uk,nl	+	-		fill in the gap
<i>Pseudoprotella phasma</i>	+	+	fr,uk				fill in the gap
<i>Anapagurus chiroacanthus</i>	-	+	fr,uk				fill in the gap
<i>Macropodia parva</i>	+	+	fr,uk,nl		+		fill in the gap
<b>PYGNOGONIDE</b>							
<i>Callipallene emacinata</i>	-	-	fr,uk	+			?
<b>BRYOZOA</b>							
<i>Crisia aculeata</i>	+	+	fr,uk,nl				fill in the gap
<i>Alcyonidium cellarioides</i>	+	-	fr,uk,nl	+	-		fill in the gap and in extension to the north
<i>Nolella pusilla</i>	+	-	fr,uk,nl	+	-		fill in the gap and in extension to north
<b>TUNICATA</b>							
<i>Clavelina lepadiformis</i>	+	+	fr,uk,nl				fill in the gap
<i>Polyclinum aurantium</i>	+	+	fr,uk,nl				fill in the gap

TABLE 6

Species (from Belgian shipwrecks) that could be considered rare for the Belgian fauna (mentioned only one or two times in past studies).

<b>PORIFERA</b>	
	<i>Hymeniacion perlevis</i>
	<i>Esperiopsis fucorum</i>
<b>CNIDARIA</b>	
	<i>Sagartiogeton undatus</i>
<b>CRUSTACEA</b>	
	<i>Iphimedia nexa</i>
	<i>Atelecyclus rotundatus</i>
	<i>Inachus phalangium</i>
<b>PYGNOGONIDE</b>	
	<i>Pycnogonum littorale</i>
<b>Tunicata</b>	
	<i>Ascidia scabra</i>

## DISCUSSION

The species richness of the shipwreck macrofauna from our studies is 224, including 12 fish species observed *in situ* by divers. This is in the range of the species richness observed for soft sediment macrofauna of the BPNS where DEGRAER et al. (2006) identified a total of 265 species from 771 Van Veen grab samples. Taking into account that we collected only one-sixth the number of samples as did DEGRAER et al. (2006) and that shipwrecks represent a relatively small area compared to the continental shelf, we hypothesize that these artificial hard substrata concentrate species richness. In this respect, they can be considered as hot spots of species richness.

The number of species recorded as new to the Belgian fauna is high (46 spp.). It appears that shipwrecks fulfil a gap in the regional distribution of sessile and mobile epibenthos for these species. Shipwrecks provide a habitat suitable for typically hard substrate-associated species found further South and extend the range of populations

previously found restricted to the Dover Strait (DAVOULT, 1990). The distribution of several species is interrupted in the Southern Bight of the North Sea, simply because their habitat, natural hard substrata, is rare in this area and has been poorly studied. The Paguridae *Anapagurus chiroacanthus* is a nice example of such a distribution (GARCIA-GOMEZ, 1994). It is present from Norway to the Azores, but is not recorded in the Southern North Sea. Its presence on shipwrecks of the BPNS fills the gap between populations living along the coast of France and Germany. For these species, it is possible that shipwrecks favour transfer by stepping-stone effect between local populations.

Other species such as the sea anemone *Actinotheroe sphyrodeta*, have their northern limit extended by shipwrecks (see WILLIAMS, 1997). The sponge-inhabiting barnacle *Acasta spongites* was also described for the first time during this study as inhabiting the Southern Bight of the North Sea (ZINTZEN & KERCKHOF, 2009). In these examples, the hard substrata offered by artificial sources extend the range of these species. Table 2 shows 12 species presenting another extension of geographical range from South to North. In this study, we did not detect any potentially invasive species, except juveniles of *Crepidula fornicata* which have been found in small densities. However, this network of artificial hard substrata may also facilitate the dispersal of invasive species that need hard substrata to complete their life cycle. In a context of global warming, the presence of artificial hard substrate spots, which include shipwrecks, may significantly reduce the physical barriers, such as large areas of soft sediments, preventing the northward spreading of warm-water species.

No algal species were identified on subtidal Belgian habitats, either on soft sediments (VAN HOEY et al., 2004) or shipwrecks. In contrast, intertidal zones in harbours and groynes host 78 macroalgae identified in a previous study (VOLCKAERT et al., 2002). The absence of Chlorophyta and Phaeophyta needing substantial light intensities may be related to the low quantity of light reaching the seabed due to the high turbidity close to the coast and the depth offshore. The absence of Rodophyta is puzzling as some of these algae are able to live with low light intensity at more than 70m depth (VERGÉS & RODRIGUEZ-PIETO, 2006). Encrusting red algae such as *Lithotamnium* spp. are present along the Atlantic coast of France up to the Dover Strait (FOVEAU, 2005) and of England (MALLINSON et al., 1999), but they do not penetrate into the Southern Bight of the North Sea. This could be linked to turbid conditions, abrasion of sand close to the bottom or competition with fast-growing sessile invertebrates such as *Tubularia* spp. and *Metridium senile*, which are the dominant species on the shipwrecks from the BPNS (ZINTZEN et al., 2007). Any algae establishing on the shipwreck could be removed by grazing echinids (*Psammechius miliaris*) present in large number on many shipwrecks.

The fauna of shipwrecks from the BPNS has affinities with the fauna found on gravel beds of the Dover Strait area (DAVOULT & RICHARD, 1988; PRYGIEL et al., 1988; DAVOULT, 1990; DEWARUMEZ et al., 1992; MIGNÉ & DAVOULT, 1997; FOVEAU, 2005; ALIZIER, 2005). The dominant mobile species of the pebble community from the Dover Strait are *Ophiothrix fragilis* and *Pisidia longicornis*, two species also very abundant on the Belgian shipwrecks (this study and ZINTZEN et al., 2007; ZINTZEN et al., 2008).

However, the dominant sessile species are different. In Dover Strait pebble beds, the sessile fauna is mostly dominated by bryozoan species, the hydrozoan *Alcyonium digitatum* and the anthozoan *Urticina felina*, while the largest fraction of the sessile epifauna of wrecks consists of cnidarians (*Tubularia* spp. and *Metridium senile*) (ZINTZEN et al., 2007; ZINTZEN et al., 2008). The causes for such a pattern could be abiotic factors that control the recruitment and development of these assemblages. Another factor that potentially separates pebble beds and shipwrecks is the stability of the substratum. Particularly strong currents or storm events may have a more profound effect on pebbles than on large rigid structures (POSEY et al., 1996). Pebbles can be moved and the epifauna damaged, promoting the dominance of species tolerant to physical disturbance. On shipwrecks, large storm events can lead to collapse of a part of the superstructure but frequent small scale perturbation events are unlikely to occur. Passive suspension feeding seems to be the dominant feeding mode on both habitats. The rate of particle filtration by a specimen is a function of particle density and current velocity. This last parameter could be enhanced on shipwrecks since current speed is higher at increasing distance from the bottom, influencing the final pattern of dominating species. Finally, human activities, such as commercial fishing, are more intense on smooth grounds such as pebbles than on shipwrecks, also leading to more frequent perturbation. These disturbances all have a strong effect on the development of epibenthic species (ENGEL & KVITEK, 1998; FRASCHETTI et al., 2001).

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## Alien macrocrustaceans in freshwater ecosystems in the eastern part of Flanders (Belgium)

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**ABSTRACT.** Biological invasions of freshwater macroinvertebrates are gaining more and more interest because the ecological and economical impact of some of these species is high. Since crustacean taxa appear to be successful groups invading new areas, an inventory of the macrocrustaceans in Flanders was made. At least 22 freshwater macrocrustacean species have been reported from Flemish water bodies. A detailed study of six canals, one small artificial watercourse and one natural river in the eastern part of Flanders revealed that invaders such as *Dikerogammarus villosus*, *Gammarus tigrinus* and *Chelicorophium curvispinum* are already quite common. Especially *D. villosus* is currently rapidly expanding and has a serious impact on native and other exotic gammarid species. Based on observations in neighbouring countries, several additional species are expected to arrive in the near future. A follow-up of the alien species together with a monitoring scheme to detect new incoming species is valuable to estimate the size of the problem and to be able to closely follow their ecological and economical impact.

**KEY WORDS:** *Chelicorophium curvispinum*, *Dikerogammarus villosus*, exotic species, *Gammarus tigrinus*, macroinvertebrates.

### INTRODUCTION

During the last century, an increasing number of alien species has been observed in watercourses worldwide. Although migration of species can be considered as a natural process, anthropogenic influences have altered the geographical scale, speed and manner of spread of invaders (ELTON, 1958; CROOKS, 2002). In Europe, different groups of exotic macroinvertebrates are found in freshwater systems, of which the majority belong to the crustaceans and the molluscs originating from the Ponto-Caspian basin (VANDEN BOSSCHE et al., 2001; BIJ DE VAATE et al., 2002; DEVIN et al., 2005b). Their intrinsic characteristics, such as a short generation time, rapid growth with early sexual maturity, high fecundity, euryhaline and omnivorous, make them extremely suitable for rapid expansion and establishment in freshwater ecosystems (BIJ DE VAATE et al., 2002). It is known that alien species can have an impact on both native and exotic biota (KINZELBACH, 1997; DICK & PLATVOET, 2000; SALA et al., 2000; VAN DER VELDE et al., 2000; BIJ DE VAATE et al., 2002; DICK et al., 2002; BOLLACHE et al., 2004; KELLY & DICK, 2005; VAN RIEL et al., 2007); change the functional diversity within macroinvertebrate communities (DEVIN et al., 2005a); cause homogenization of freshwater ecosystems (RAHEL, 2002; BOLLACHE et al., 2004) and facilitate the establishment of other invaders (RICCIARDI, 2001; DEVIN et al., 2003).

Because of the growing number of invaders and the ecological and economical consequences, the invasion

issue has received special attention. As a result, the number of publications, workshops, congresses and journals about exotic species has increased substantially (PYSEK et al., 2006). In contrast to other countries and even Wallonia, the southern region of Belgium (VANDEN BOSSCHE et al., 2001; VANDEN BOSSCHE, 2002; JOSSENS et al., 2005), Flanders has hardly been examined for the presence and spread of freshwater exotic species. WOUTERS (2002) gave an overview of the alien macrocrustaceans in the whole of Belgium. He stated that at least 13 macrocrustaceans had invaded Belgium. Other studies revealed that exotic molluscs (*Dreissena polymorpha* (Pallas, 1771), *Potamopyrgus antipodarum* (Gray, 1843), *Corbicula fluminea* (Müller, 1774), *C. fluminalis* (Müller, 1774)), annelids (*Hypania invalida* (Grube, 1860)) and flatworms (*Dendrocoelum romanodanubiale* (Codreanu, 1946) and *Girardia tigrina* (Girard, 1850)) have invaded Flemish watercourses (SWINNEN et al., 1998; NGUYEN & DE PAUW, 2002; VERCAUTEREN et al., 2005). For marine and brackish waters in Flanders, an overview of all alien taxa has been presented by KERCKHOF et al. (2007). Also for Cladocera, a group of microcrustaceans, an overview was given of the indigenous as well as non-indigenous species occurring in Flanders (LOUETTE et al., 2007). The proximity of the sea, the interconnection between different waterways, the high degree of canalisation, the boat transport and the presence of harbours make Flemish watercourses susceptible for aquatic invasions (VERCAUTEREN et al., 2005).

## MATERIALS AND METHODS

### Data and sampling locations

The Flemish Environment Agency (VMM) monitors a large number of sampling sites scattered over the different stagnant and running freshwater systems in Flanders. Annually, almost 1500 biological samples of macroinvertebrates are identified at genus or family level. Consequently, information about alien macrocrustacean species such as *Dikerogammarus villosus* (Sowinsky, 1894) or *Gammarus tigrinus* Sexton, 1939 was not available since both species belong to the same family Gammariidae as the indigenous species *G. pulex* (Linnaeus, 1758). The present study aims to make an inventory of the presence of alien freshwater macrocrustacean species in Flemish watercourses based on the samples of the VMM and the available literature. Three types of data were available.

(1) In total, 292 samples of macrocrustaceans, collected by the VMM in the eastern part of Flanders (Fig. 1) between 1991 and 2005, were identified at species level. The sampling sites were located in six large canals: the Albert canal, the Dessel-Schoten canal, the Bocholt-Herentals canal, the Dessel-Kwaadmechelen canal, the Zuid-Willemsvaart canal and the Briegden-Neerharen canal; one natural watercourse: the Kleine Nete and one small artificial watercourse: the Postelvaartje. The canals were chosen based on previous observations of several alien macrocrustaceans and their connection with the Scheldt as well as the Meuse catchment. The other watercourses were selected because of their connection with one of the canals and for comparing their status of invasion with the canals. The samples were collected at 66 different locations scattered over the different watercourses, however, those stations were not sampled annually. Table 1 gives an overview of the number of samples collected and ana-

lysed in the watercourses. For each watercourse, the data of the different localities were combined and the average relative abundance of the crustacean families was calculated (Fig. 2). Macrocrustaceans were identified at species level, based on different identification keys (GLEDHILL et al., 1993; EGGERS & MARTENS, 2001; HUWAE & RAPPÉ, 2003).

(2) In addition to these samples, a macroinvertebrate dataset of the VMM was available. This dataset contains data from families of crustaceans in Flemish watercourses between 1989 and 2005. Since some families are only represented by a single exotic species in these samples (the family Janiridae by *Jaera istri* Veuille, 1979, Cambaridae by *Orconectes limosus* (Rafinesque, 1817), Varunidae by *Eriocheir sinensis* H. Milne Edwards, 1854, Talitridae by *Orchestia cavimana* Heller, 1865 and Atyidae by *Atyaephyra desmaresti* (Millet, 1831)), the distribution of these species in other parts of Flanders could be analysed.

(3) Additional information of exotic macroinvertebrates was available from literature and observations made by colleague zoologists (personal communications).

### Sampling methodology

Depending on the depth of the watercourses, macroinvertebrates were sampled by means of a standard handnet or artificial substrates as described by GABRIELS et al. (in press). The canals were sampled with artificial substrates; the samples of the Kleine Nete and Postelvaartje were collected with a handnet. The sampling technique affects the catchability and hence the observed proportion of the densities of the different species reflects how often these species were caught and not necessarily their relative abundance in the field

TABLE 1  
Number of sampled localities in the studied watercourses per year  
(NA = not available, - = not identified; () = no crustaceans found)

Watercourse	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	Total (n=292)
Albert canal	1	2	NA	7	8	3	6	6	5	1	4	3	8	5	5	64
Dessel-Schoten canal	2	NA	2	4	4	5	2	2	3	4	2	4	3	2	2	41
Dessel-Kwaadmechelen canal	1	NA	NA	1	1	1	1	3	1	NA	NA	2	1	2	2	16
Bocholt-Herentals canal	5	2	6	5	5	5	4	6	5	4	6	3	6	3	6	71
Zuid-Willemsvaart canal	NA	NA	NA	1	4	2	1	1	1	1	1	2	NA	1	2	17
Briegden-Neerharen canal	NA	NA	NA	1	NA	NA	1	NA	NA	NA	NA	1	NA	NA	NA	3
Postelvaartje	-	-	-	NA	NA	NA	(1)	NA	2	1	NA	3	NA	5	1	13
Kleine Nete	-	-	-	1	NA	7	9	6	5	7	5	8	5	8	6	67

TABLE 2

Overview of the freshwater macrocrustaceans encountered in Flanders, with indication of their origin, the year of first detection of exotic species in Flanders with reference, the current abundance in Flanders (+ only a few records; ++ rare; +++ common; ++++ very common) and the number of sampling sites in the study area where the species was found. R.B.I.N.S. : Royal Belgian Institute of Natural Sciences.

Order	Family	Species	Origin	First occurrence in Flanders	Reference	Presence in Flanders	Sites in study area (n=66)
Amphipoda	Corophidae	<i>Chelicorophium curvispinum</i>	Ponto-Caspian	1990	Vercauteren et al., 2005	+++	40
	Crangonictidae	<i>Crangonyx pseudogracilis</i>	North America	1992	Wouters, 2002	+++	3
	Gammaridae	<i>Dikerogammarus villosus</i>	Ponto-Caspian	1997	Present study	+++	40
		<i>Echinogammarus berilloni</i>	Iberian Peninsula	1925	Wouters, 2002	+	2
		<i>Gammarus fossarum</i>	Indigenous			+	
		<i>Gammarus pulex</i>	Indigenous			++++	34
		<i>Gammarus roeseli</i>	Southern Europe	1937	Collection R.B.I.N.S.	++	4
		<i>Gammarus tigrinus</i>	North America	1991	Present study	++++	49
	Talitridae	<i>Orchestia cavimana</i>	Ponto-Caspian	1927	Wouters, 2002	+++	2
Isopoda	Asellidae	<i>Asellus aquaticus</i>	Indigenous			++++	55
		<i>Proasellus coxalis</i>	Southern Europe	1998	Boets et al., unpublished data	++	
		<i>Proasellus meridianus</i>	Southern Europe	1945	Collection R.B.I.N.S.	+++	21
	Janiridae	<i>Jaera istri</i>	Ponto-Caspian	2000	Present study	++	10
Mysida	Mysidae	<i>Hemimysis anomala</i>	Ponto-Caspian	1999	Verslycke et al., 2000	+	
		<i>Limnomysis benedeni</i>	Ponto-Caspian	2005	Lock et al., 2007	+	
Decapoda	Astacidae	<i>Astacus astacus</i>	Indigenous			Extinct	
		<i>Astacus leptodactylus</i>	Eastern Europe	1986	Gerard, 1986	+	
		<i>Pacifastacus leniusculus</i>	North America	1986	Gerard, 1986	+	
	Atyidae	<i>Athyaephyra desmaresti</i>	Southern Europe	1895	Wouters, 2002	++	17
	Cambaridae	<i>Procambarus clarkii</i>	North America	2008	Boets et al., 2009	+	
		<i>Orconectes limosus</i>	North America	1977	Wouters, 2002	+++	40
	Varunidae	<i>Eriocheir sinensis</i>	Southeast Asia	1933	Wouters, 2002	+++	1

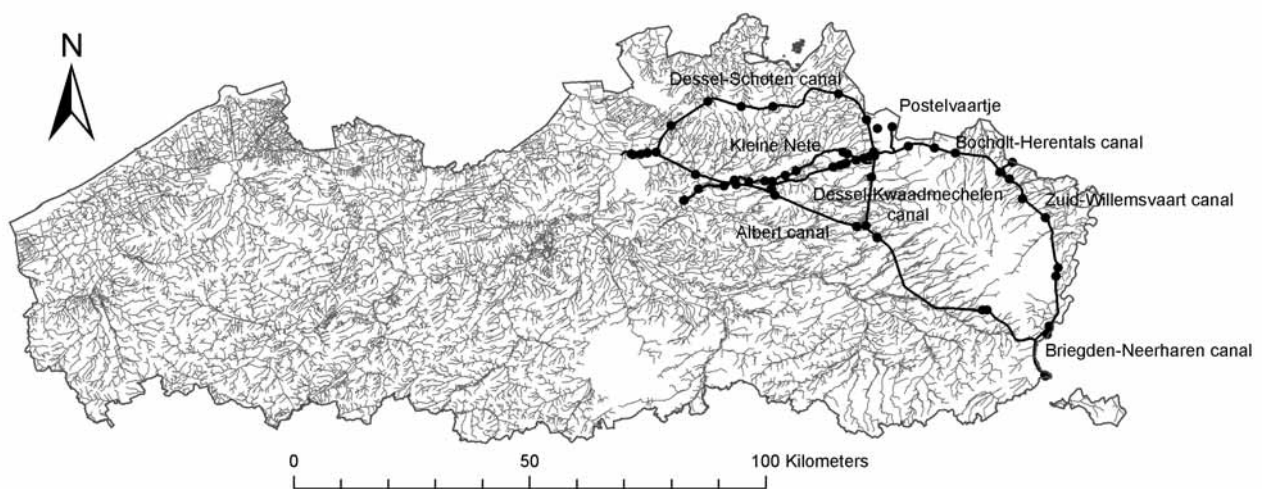


Fig. 1. – Studied watercourses in Flanders with indication of the sampling locations.

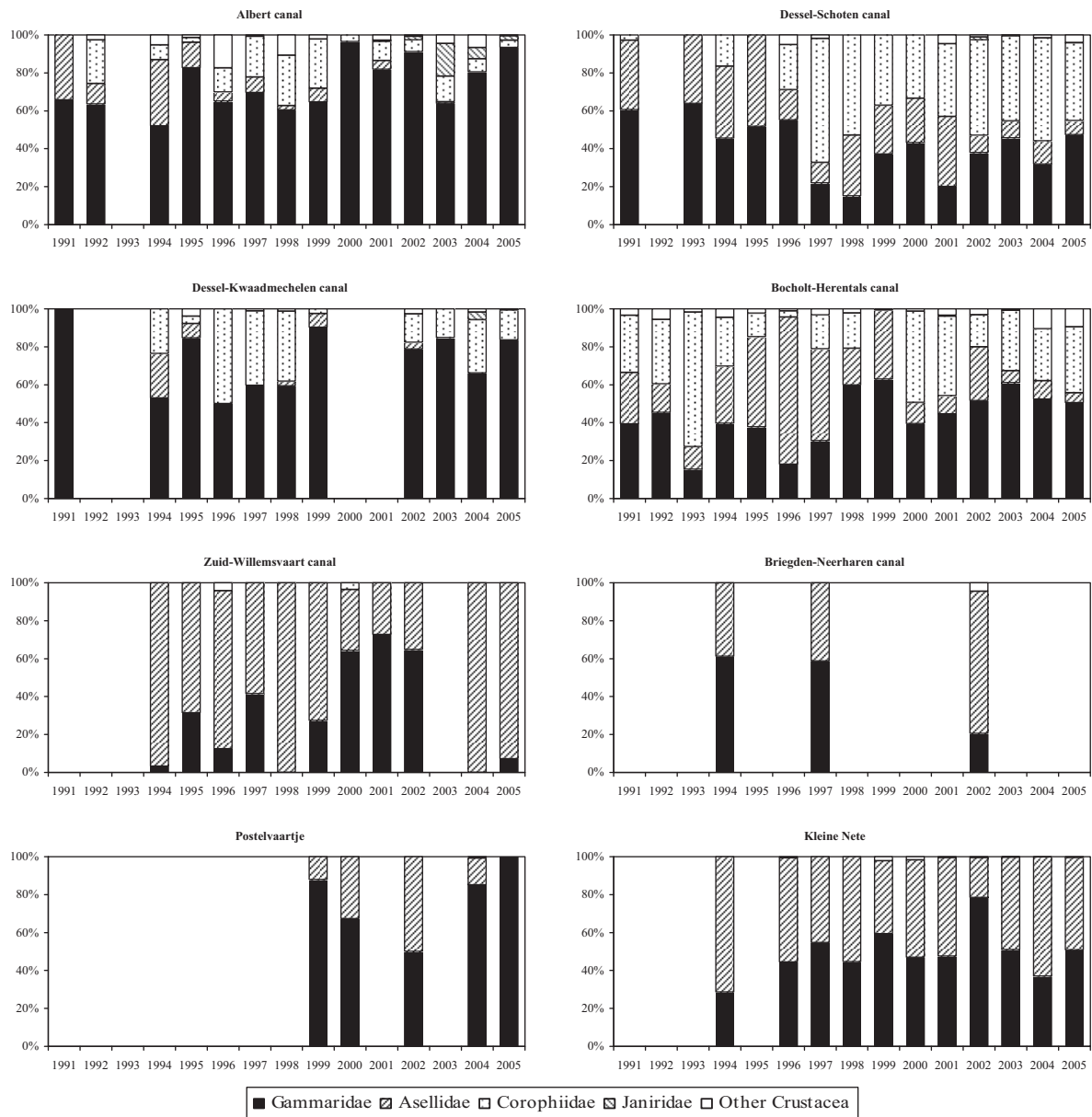


Fig. 2. – Average relative abundance of macrocrustacean families, including alien as well as indigenous species, in the studied watercourses.

## RESULTS

An overview of the freshwater macrocrustaceans that have been detected in the study area and in the rest of Flanders is given in Table 2. More detailed results for each family are given below.

### Gammaridae

Various gammarid species were found in the analysed samples. Although the earliest report of the exotic *D. villosus* in Flanders dated from 2000 (VERCAUTEREN et al., 2006), analysis of the VMM samples showed that this species was already present in 1997 in the East of Flanders, at least in the Albert canal and the Dessel-Kwaad-

mechelen canal. More recent samples revealed its expansion into other canals and watercourses in the region. In all the 14 sampling sites containing *G. pulex*, which were invaded by *D. villosus*, *G. pulex* was no longer present during the last sampling campaign. In only four samples, where at most three individuals of *D. villosus* were encountered, both species were found together. In several canals, the appearance of *D. villosus* led to the complete disappearance of *G. pulex* (Fig. 3). In several sites, including those in the Zuid-Willemsvaart canal (Fig. 3), *D. villosus* also replaced *G. tigrinus*, however, in most sites both species still seemed to be able to co-exist. *D. villosus* was recently also found in several other locations in Flanders: in the Charleroi-Brussels canal in Sint-Pieters-Leeuw near Brussels (VERCAUTEREN et al., 2005), in the

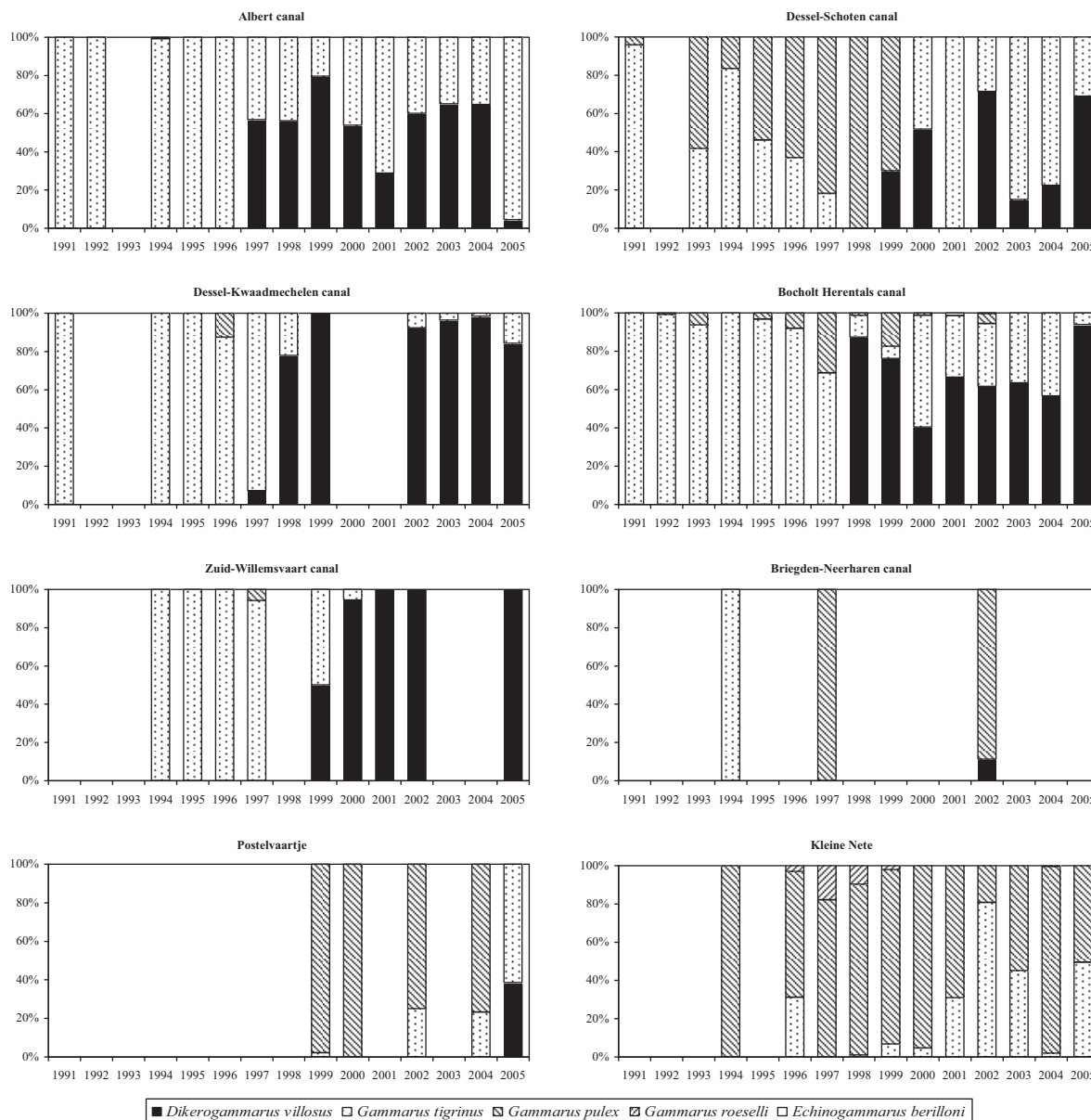


Fig. 3. – Average relative abundance of Gammaridae in the studied watercourses.

Leie-Roeselare canal in Izegem, in a ditch in ‘Prinsenpark’ near Retie, which is connected with canal Bocholt-Herentals (VERCAUTEREN et al., 2006), in a lake in Harelbeke (GHYSELBRECHT, VMM-Ostend), in Kessenich and Heerenlaak, two gravel pits that are connected to the Border Meuse (LOCK et al., 2007) and in the canal Ghent-Terneuzen, the canal Ghent-Ostend and the canal Kortrijk-Bossuyt (BOETS et al., unpublished data).

*G. tigrinus* was reported for the first time for Flanders from 1996 (VERCAUTEREN & WOUTERS, 1999), but it appeared that *G. tigrinus* was already present in the analysed samples of 1991 (Fig. 3). The species has now invaded watercourses all over Flanders (Boets et al., unpublished data).

Both *Echinogammarus berilloni* (Catta, 1878) and *Gammarus roeseli* Gervais, 1835 can be considered as

naturalized, as they have already been present in Flanders for a few decades, but were only found in the Dessel-Schoten canal in 1994 and in the Kleine Nete between 1996 and 1999, respectively (Fig. 3).

### Corophiidae

The Ponto-Caspian invader *Chelicorophium curvispinum* (Sars, 1895) has been observed in the canals in the eastern part of Flanders since 1990 (VERCAUTEREN et al., 2005). The present study revealed that it also occurred in the Kleine Nete in 2005. This species was also found in the ‘Prinsenpark’ in Retie (VERCAUTEREN et al., 2006) and the gravel pits Kessenich and Heerenlaak along the Border Meuse (LOCK et al., 2007).



### Crangonyctidae

*Crangonyx pseudogracilis* Bousfield, 1958 was first reported for Flanders from 1998 in a ditch near Puurs, probably introduced with *Ludwigia grandiflora*. However, analysis of historical samples in a nearby ditch indicated its presence since 1992 (VERCAUTEREN & WOUTERS, 1999). A few specimens were found in the Dessel-Schoten canal in 1997 and 2005 and in the Bocholt-Herentals canal in 1996 and 1998. This species was also found in the Old Meuse in Stokkem, in two brooks in Genk and Herk-de-Stad (VERCAUTEREN, personal communications) and in a lake in Harelbeke (GHYSELBRECHT, personal communications). Since 2002, a growing number of localities in the western part of Flanders have been invaded by this species (GHYSELBRECHT, personal communication).

### Talitridae

*O. cavimana* was observed for the first time in Flanders in 1927 and was found in the Zuid-Willemsvaart canal and Bocholt-Herentals canal in 1980, in the Dessel-Schoten canal in 1981, in other rivers such as the Leie, Scheldt and Dender (WOUTERS, 2002). More recently, the species was also encountered in the river Yser, in the Stenensluisvaart canal in Woumen and the Zuidervaartje canal in Bruges (GHYSELBRECHT, personal communications). However, in this study, this species was found only twice: in the Dessel-Kwaadmechelen canal in 2001 and 2004.

### Asellidae

In the studied area, the family Asellidae was represented by two species: the exotic *Proasellus meridianus* (Racovitza, 1919) and the indigenous *Asellus aquaticus* (Linnaeus, 1758). Although JOSENS et al. (2005) consider *P. meridianus* as an exotic species originating from southern Europe, its exotic status can be questioned. According to different authors, this species originates from Western Europe namely Germany and the British isles (GRÜNER, 1965; MOON & HARDING, 1981). Although *A. aquaticus* is by far the most common species in Flanders, *P. meridianus* is widely distributed as well.

### Janiridae

*J. istri*, an isopod originating from the Ponto-Caspian basin, has been present in the studied watercourses since 2000 (Fig. 2). This species has occurred in the Albert canal since 2001 (VERCAUTEREN et al., 2005). It was observed in the Bocholt-Herentals canal in 2001 and in the Dessel-Kwaadmechelen canal in 2004. Analysis of the samples of the VMM showed that one individual was found in 2004 in Mechelen in a tributary of the Dijle.

### Cambaridae

*Orconectes limosus* has been present in Flanders since 1977 (WOUTERS, 2002) and was regularly found in the studied watercourses. Other locations, according to the dataset of the VMM, are the lake Gavers in Harelbeke, the canal Brussels-Charleroi near Sint-Pietersleuw, the Old Meuse in Stokkem, the river Dender and the Old Scheldt in Oudenaarde. This species was also observed in the 'Prinsenpark' in Retie (VERCAUTEREN et al., 2006), the

gravel pits Kessenich and Heerenlaak along the Border Meuse (LOCK et al., 2007).

### Atyidae

The arrival time of this species in Flanders is estimated around 1895 (WOUTERS, 2002). The species was regularly found in the studied canals and rivers. *Athyaephyra desmaresti* was found in 100 samples (0.7%) in the dataset of the VMM, including the river Scheldt, the analysed canals and rivers, the Old Meuse, the river Wamp and the Roeselare-Leie canal.

### Varunidae

*Eriocheir sinensis* was observed for the first time in 1933 and is now widely distributed throughout Flanders (WOUTERS, 2002). Based upon the review made by WOUTERS (2002), this species has a wide distribution and occurs both in natural habitats as well as in canalised rivers. However, this species was not frequently found in the dataset of the VMM, probably due to the sampling method.

## DISCUSSION

Apart from occasional observations, a large study regarding the occurrence of alien macro-invertebrates in Flanders has not yet been carried out. A detailed study of macrocrustaceans found in six canals, one small artificial watercourse and one natural river in the eastern part of Flanders, which were monitored by the Flemish Environmental Agency, revealed that 14 species of macrocrustaceans were present of which only *G. pulex* and *Asellus aquaticus* can be considered as native (Table 2). However, the exotic status of *Proasellus meridianus* is arguable.

Besides the species that were observed during the present study, several other freshwater macrocrustaceans have been found in Flanders. *Gammarus fossarum* Koch, in Panzer, 1835, is indigenous in small streams in the loamy region. *Proasellus coxalis* (Dollfus, 1892) was first reported from 2005 by VERCAUTEREN & WOUTERS (2008b) in Heist-op-den-Berg. *Hemimysis anomala* (Sars, 1907) was observed for the first time in Flanders in 1999 in a brackish pond 'Galgenweel' (VERSLYCKE et al., 2000). This species mainly occurs in lentic environments and can withstand a wide range of salinities up to 19‰ (BIJ DE VAATE et al., 2002). *H. anomala* was recently also observed in the 'Prinsenpark' in Retie (VERCAUTEREN & WOUTERS, 2008a). *Limnomysis benedeni* Czerniavsky, 1882 was observed in the gravel pits Kessenich (2005-2007) and Heerenlaak (2007) along the Border Meuse (LOCK et al., 2007), in the 'Prinsenpark' in Retie (VERCAUTEREN & WOUTERS, 2008a), in the lake Schulensmeer (LOUETTE et al., 2008) and in the lake Donkmeer (MERTENS, personal communication). *Astacus astacus* (Linnaeus, 1758), an indigenous crayfish species, has not been observed recently. This is due to habitat destruction, pollution and the regulation of watercourses. Furthermore, the introduction of *O. limosus* led to the crayfish plague and this disease almost resulted in the disappearance of *Astacus astacus* in the Netherlands (VAN DER

VELDE et al., 2000) and presumably affected the populations in Flanders as well. Apart from *O. limosus*, two other exotic crayfish species have also been present in Flanders for several decades: *Astacus leptodactylus* Eschscholtz, 1823 and *Pacifastacus leniusculus* (Dana, 1852) (GERARD, 1986). In 2008, a fourth exotic crayfish species was observed in Flanders: *Procambarus clarkii* (Girard, 1852) (BOETS et al., 2009).

The sampling method can have an impact on the assessment of the distribution of certain invaders such as *E. sinensis*, *H. anomala*, *L. benedeni* and *Orchestia cavimana*. Based upon the review by WOUTERS (2002), *E. sinensis* is widely distributed in Flanders. This species can have a large impact on their environment due to burrowing activities, predation and competition (HERBORG et al., 2007; OJAVEER et al., 2007). The low occurrence of this species in the dataset of the VMM is probably a consequence of the sampling method. The species is difficult to catch with artificial substrates since the organisms are highly mobile both on land and in the water. Therefore, other sampling techniques, e.g. with nets would give a more accurate picture of its actual distribution. Invaders such as *H. anomala*, *L. benedeni* and *O. cavimana* are not often observed, which is probably also due to the sampling method (artificial substrates and handnet), since *Hemimysis anomala* and *L. benedeni* are fast swimmers, while *O. cavimana* lives along the river banks in fresh and brackish waters (JOSENS et al., 2005). Additional sampling of the river banks and stone sampling might reveal that the latter species is more abundant than the sampling techniques used suggest, while a hyperbenthic sledge would be a good sampling device to sample mysid shrimp.

Although *G. tigrinus* was reported for the first time in Flanders from 1996 in a pond near Antwerp (VERCAUTEREN & WOUTERS, 1999), it was already present in the analysed canals in 1991, which indicates that it arrived earlier in Flanders, probably via the Scheldt-Rhine connection. Via this route, *G. tigrinus* probably invaded the Albert canal and the other canals. Although *G. tigrinus* invaded the part of the Kleine Nete and Postelvaartje closest to the canal, this species was not able to invade these watercourses completely, despite having had the time and the possibility (connections). This is also the case for *Chelicorophium curvispinum*, *Orconectes limosus* and *Athyaeophya desmaresti*, which are mostly found in large river systems, but less in smaller river systems. Probably, smaller rivers and streams do not provide a suitable habitat for these species.

*D. villosus* was noticed for the first time in the Albert canal in 1997. Its spread was fast and it migrated into the other canals as well. Where it arrived, it always replaced the native *G. pulex* and in some cases the exotic *G. tigrinus* as well. The effect of *D. villosus* on other amphipods could also be observed in neighbouring countries including the Netherlands (VAN DER VELDE et al., 2000; VAN RIEL et al., 2006; 2007) and France (DEVIN et al., 2005a). Therefore, an additional impact assessment of this species on other crustaceans, but also on other macroinvertebrates and fish eggs needs to be performed. Boets et al. (in press) already indicated that in Flanders, this species can especially be found in watercourses with an artificial bank structure with a reasonably good chemical water

quality, which is reflected in a conductivity lower than 500 µS/cm and an oxygen content higher than 90%.

The Ponto-caspian invader *Jaera istri* has been found in the Netherlands since 1997, in the Rhine, the IJssel and the Waal (KELLEHER et al., 2000). Soon after its arrival in 2001 in the Albert canal, it became locally abundant (SCHÖLL, 2000). *J. istri* thrives well on places with stony substrates and in dammed canals (KELLEHER et al., 2000; VERCAUTEREN et al., 2006; VAN RIEL et al., 2007).

It can be concluded that the canals in the East of Flanders are extremely prone to colonisation by invaders because of their high degree of connectivity and structural characteristics. Invaders can expand their area and migrate to other canals and rivers depending on the structural characteristics and hydrological regimes of the recipient habitats. The increasing pace of arrival of new alien species in Flanders since 1980 is remarkable (WOUTERS, 2002). This may be not only due to the higher degree of connection between important waterways, but possibly also supports the invasion meltdown hypothesis (SIMBERLOFF & VON HOLLE, 1999), which states that similar species may facilitate the invasion process of other species originating from the same region, which may cause homogenization of freshwater systems (RAHEL, 2002). In this respect, additional analysis of changes in bio- and functional diversity needs to be done (DEVIN et al., 2005a). Another reason exotic species are so successful is the improved water quality of formerly degraded systems, which has resulted in new habitats with a lot of vacant niches.

Crustacean invaders such as *Dikerogammarus haemobaphes* (Eichwald, 1841), which has already invaded the Meuse (JOSENS et al., 2005), and *Echinogammarus ischnus* (Stebbing, 1899) (WOUTERS, 2002), *Echinogammarus trichiatus* (Martynov, 1932), *Orconectes immunitis* (Hagen, 1870) and *Chelicorophium robustum* (Sars, 1895), which have already invaded the Rhine (BERNAUER & JANSEN, 2006), can be expected to arrive soon in Flanders. Also the Northern crayfish *Orconectes virilis* (Hagen, 1870), the Marbled crayfish *Procambarus* species and the White river crayfish *Procambarus acutus* (Girard, 1852) / *zonangulus* Hobs and Hobs, 1990, which have already been reported in the Netherlands (KOESE, 2008), can be expected. However, their spread will largely depend on their interference with the species that are already present (KLEY & MAIER, 2003; 2006).

Nowadays, different quality assessment methods are based on the presence and diversity of macroinvertebrates in watercourses. Replacement of native taxa by invaders can influence the results of those indices (NGUYEN & DE PAUW, 2002; KELLY et al., 2006). GABRIELS et al. (2005) proposed to use a semi-fixed list so that new taxa can be added. However, additional analysis of the influence of invaders on biological indices should be performed (KELLY et al., 2006). Furthermore, a continuous monitoring of new and formerly established invaders is necessary, in particular regarding their habitat preferences and their effect on native species (DEDECKER et al., 2005), to obtain a better understanding of their impact due to competition and predation. Moreover, migration models (DEDECKER et al., 2006) can be relevant to make better predictions regarding the migration speed of alien macroinvertebrates in rivers.

Alien species are not mentioned specifically in the European water framework directive (WFD; European Community, 2000). However, the precautionary principle in the broad sense could be applicable for the negative effects of exotic species because aquatic ecosystems have to be guarded from decline. Alien species can modify the native biological structure and ecological functioning of aquatic systems and the assessment of alien species as biological pressure should therefore be considered as a part of a catchment management policy together with other pressures. However, effective control measures to eradicate alien aquatic macroinvertebrates are not feasible and at best slow down their dispersal rate. Control measures usually cause more damage (i.e. application of pesticides) or other risks (introduction of natural predator of alien species). The Belgium Forum of Invasive Species proposes a system of lists, which are based on the ability of a species to disperse and its environmental impact. It is suggested that this list can be used to ban import, trade and introduction in the natural environment and for control measures or eradication. In such a system, it is necessary to demonstrate significant damage before a species is listed (now the only macrocrustaceans on the list are *Eriocheir sinensis* and *Pacifastacus leniusculus*, which have already been established in Flanders for several decades). However, by the time damage can be demonstrated, the considered species has usually already invaded the area and nothing can be done to stop its further spread. In our opinion, preventive measures, such as the obligatory treatment of ballast water, are the only possible solution to reduce the influx of alien aquatic macroinvertebrates.

## CONCLUSION

This study about freshwater macrocrustaceans revealed that at least 18 non-native species are currently present in Flemish watercourses. The dispersal routes via the river Meuse and the river Scheldt, the high number of canals, the abundant boat traffic and the increased water quality of formerly degraded watercourses make Flanders highly susceptible for invasions. For this reason, more invaders from neighbouring countries are expected. A continuous monitoring of 'new' and previously introduced species is necessary in order to understand the potential consequences on native and established alien species and their dispersal pathways. As it is hardly possible to eradicate exotic species once they are established, more attention should be given to preventive international measures, which reduce the influx of exotic species.

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## Morphological differentiation of *Limnozetes* Hull, 1916 (Acari: Oribatida: Limnozetidae) in the light of ontogenetic studies

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**ABSTRACT.** Morphological variability of *Limnozetes* Hull, 1916 was investigated in light of the ontogeny of *L. lustrum* Behan-Pelletier, 1989 and *L. rugosus* (Sellnick, 1923). The adults of this genus are similar, but the nymphs are differentiated, either boat-shaped, with thick and stiff posterior gastronotal setae and rather smooth cuticle, as in *L. lustrum*, or stocky, with thin gastronotal setae and plicate cuticle, as in *L. rugosus*. The adult of *L. lustrum* has ten pairs of notogastral setae, while that of *L. rugosus* has 10 or 11 pairs, which is noted here for the first time in *Limnozetes* and has phylogenetic importance. The morphology of juvenile stages of presented species has not been investigated so far. Moreover *L. lustrum* is recorded for the first time from Europe. Morphological groups within *Limnozetes* are proposed: 'ciliatus group', with *L. lustrum* and 'rugosus group', with *L. rugosus*.

**KEY WORDS:** semi-aquatic Oribatida, *Limnozetes lustrum* Behan-Pelletier, 1989, *L. rugosus* (Sellnick, 1923), setation, juvenile stages, ontogeny

### INTRODUCTION

Oribatid mites of the genus *Limnozetes* Hull, 1916 are typically found in transitional, semi-aquatic habitats (BEHAN-PELLETIER, 1989), mainly in wet bogs and at edges of lakes with *Sphagnum*; but also in other mosses and wet meadows. These mites are rather small (270-390µm), but usually abundant, with adults dominating in extracted samples. For example, *L. lustrum* Behan-Pelletier, 1989 reached a density 312 indiv. per 1000cm<sup>3</sup> at the edge of forest lake Bagno Chlebowo (Wielkopolska National Park, Poland, August 19<sup>th</sup> 2007), with a small fraction (9.4%) being juveniles, while *L. rugosus* (Sellnick, 1923) reached a similar density (367 indiv. per 1000cm<sup>3</sup>) at the edge of a small lake in Finse (Norway, September 8<sup>th</sup> 2006), but its juveniles comprised 36% of the individuals.

*Limnozetes* has modest species diversity. Of the 14 named species, SUBÍAS (2004) listed only four of them and one subspecies from Europe: *L. amnicus* Behan-Pelletier, 1989, *L. ciliatus* (Schrank, 1803), *L. onondaga* Behan-Pelletier, 1989, *L. rugosus*, and *L. ciliatus foveolatus* Willmann, 1939. WEIGMANN & DEICHSEL (2006) included only three species (*L. amnicus*, *L. ciliatus* and *L. rugosus*) in their key to German fauna. OLSZANOWSKI et al. (1996) recorded two species and one subspecies (*L. ciliatus*, *L. rugosus* and *L. ciliatus foveolatus*) from Poland, but *L. palmerae* Behan-Pelletier, 1989 was subsequently added by SENICZAK et al. (2007), and *L. lustrum* is added here to the Polish and European fauna.

The diagnoses of most *Limnozetes* species are based exclusively on the morphology of adults, which are sometimes strikingly similar to each other and therefore are not easily differentiated. The diagnosis of the type species, *L. ciliatus* (Schrank, 1803) is old, brief and confusing, while the diagnoses of other species have been successively more detailed (three species were described between 1923-1939 and ten species between 1961-1989). For

example, BEHAN-PELLETIER (1989) described in detail eight new species from north-eastern North America and diagnoses of six species included the juveniles as well. She described and illustrated the tritonymph of *L. latilamellatus* Behan-Pelletier, 1989, *L. guyi* Behan-Pelletier, 1989, *L. borealis* Behan-Pelletier, 1989, *L. onondaga*, *L. palmerae* and *L. amnicus*, and gave measurements of the other juvenile stages of most species, and ontogeny of leg setation of *L. guyi* and *L. onondaga*. Based on the morphology of adults and juveniles, BEHAN-PELLETIER (1989) gave also a new diagnosis of *Limnozetes*, which indicated that the juveniles differ distinctly from the adults. That is why two morphological groups of nymphs can be distinguished: (1) with a wide gastronotum and stocky body, strongly plicate cuticle and thin gastronotal setae, and (2) with more slender gastronotum and boat-shaped body, weakly plicate cuticle and blade-like setae on the posterior part of the gastronotum. The former group is represented by *L. latilamellatus*, *L. guyi* and *L. borealis*, and the latter group by *L. onondaga*, *L. palmerae* and *L. amnicus*.

In a group of the other species of *Limnozetes*, the morphology of a nymph of *L. ciliatus* was investigated and illustrated by MICHAEL (1880); it is boat-shaped, with rather thick and stiff peripheral setae on the gastronotum, and rather smooth cuticle. HAARLØV (1957) described a purported tritonymph of *L. rugosus*, but the mite was wrongly determined; it belongs to *Zetomimus furcatus* (Pearce and Warb., 1906), which has non-plicate cuticle, three pairs of rather long setae in posterior part of gastronotum, and a unique combination of furrows in this region transverse and longitudinal ones in the shape of the letter T - as shown by SHALDYBINA (1969, 1975) and WEIGMANN & DEICHSEL (2006).

This paper aims to investigate the morphological differentiation of *Limnozetes* by comparing the morphology of adult and all juvenile stages. Moreover ontogeny of *L. lustrum* and *L. rugosus*, species from two different morphological groups of *Limnozetes* (BEHAN-PELLETIER,

1989) is compared. The juvenile stages of these species are described and illustrated for the first time, and new diagnoses of species, with morphological characters of juveniles, are given. This study may, therefore, be helpful in the recognition of species in ecological studies, providing more precise diagnoses, and in further studies on phylogeny of oribatid mites.

## MATERIALS AND METHODS

The juvenile stages and adults of *L. lustrum* were collected on the 19<sup>th</sup> of August 2007 from *Sphagnum* at the edge of forest lake Bagno Chlebowo (53°44'17"N, 16°45'26"E, 65m a. s. l.) in the Wielkopolska National Park, Poland, where this species was numerically more abundant than *L. palmerae*. The juvenile stages and adults of *L. rugosus* were collected from *Sphagnum* at the edge of a small lake in Finse (60°35'17"N, 7°30'18"E, 1245m a. s. l.), Norway, where this species was the only member of *Limnozetes*. The description of morphology and drawings of *L. lustrum* include the dorsal aspect of the larva and tritonymph, anal region of the larva and anogenital region of all nymphal stages, where new segments and setae appear during ontogeny; marginal part of gastronotum of *L. ciliatus* is also illustrated. Whereas fundamental aspects of ontogeny are similar in both species, the description of morphology and drawings of *L. rugosus* are limited to the dorsal aspect of the larva and tritonymph, the anal region of the larva and the anogenital region of the tritonymph. The adults of both species are also investigated to document the full development of setation during ontogeny. Illustrations were prepared from individuals mounted on slides. Terminology used follows that of F. GRANDJEAN, developed in papers referenced by TRAVÉ & VACHON (1975).

## RESULTS

### *Limnozetes lustrum* Behan-Pelletier, 1989

#### Diagnosis

Adult (Fig. 1) is well diagnosed by BEHAN-PELLETIER (1989). Setae *ro* and *le* long and rather thick, seta *in* shorter and thin, seta *ex* small, usually covered by large, oval pedotectum 1 (*pd1*). Bothridium well-developed, sensillus (*ss*) short, clavate, tutorium (*tu*) present. Notogaster with ten small setae, including pair *c*<sub>2</sub>. Pteromorph longer than wide (Fig. 2A). Cupule *ia* between setae *la* and *c*<sub>2</sub>, cupule *im* posterior to seta *c*<sub>2</sub>, cupule *ip* between setae *h*<sub>1</sub> and *p*<sub>1</sub>, cupule *ih* anterior and cupule *ips* ventrolateral to seta *p*<sub>3</sub>; opisthonotal gland opening (*gla*) between seta *h*<sub>3</sub> and cupule *ih*. Formulae of legs setae (trochanter to tarsus) and solenidia are: I - 1-4-(3+1)-(4+2)-(15+2); II - 1-4-(3+1)-(4+1)-(14+2); III - 2-3-(1+1)-(3+1)-14; VI - 1-2-2-(3+1)-11; all legs tridactylous. In mites mounted on slides solenidia  $\omega_1$  and  $\omega_2$  on tarsus I usually separated in distal parts (Fig. 3A).

Juveniles oval, body flesh-colored, cuticle of prodorsum and gastronotum rather smooth, with few plicae. Prodorsum triangular, setae *le* and *in* barbed and pointed, seta *ro* thinner and shorter than setae *le* and *in*, seta *ex* short. Sensillus long, setiform. Larva with 12 pairs of gastronotal setae, similar in shape to seta *in*, while nymphs with 15 pairs of these setae; posterior marginal setae thicker, stiffer and slightly longer than other gastronotal setae.

#### Morphology of juvenile stages

Prodorsum of larva (Fig. 4) triangular, cuticle rather smooth, with few folds in lateral and posterior part. Seta *ro* rather small and smooth, but setae *le* and *in* longer, stiff and barbed, seta *ex* minute. Bothridium weakly developed, sensillus long, setiform.

Gastronotum with few folds in anterior part, and 12 pairs of setae, including setae *h*<sub>2</sub> and *h*<sub>3</sub> positioned near paraproctal valves (Fig. 5A). All setae barbed and rather stiff, except for seta *h*<sub>3</sub>, which is small and smooth; length increases from seta *c*<sub>1</sub> to *h*<sub>1</sub>; seta *h*<sub>2</sub> shorter than *h*<sub>1</sub>. Paraproctal valves (segment PS) glabrous. Cupule *ia* posterior to seta *c*<sub>3</sub>, cupule *im* slightly laterally between setae *la* and *lm*, cupule *ip* between setae *h*<sub>1</sub> and *h*<sub>2</sub>, gland opening *gla* between setae *lp* and *h*<sub>3</sub>. Gastronotum slightly wrinkled.

Nymphs slimmer than larva, boat-shaped and flesh-colored. Number of prodorsal setae as in larva, but gastronotum with 15 pairs: pseudanal setae (*p*<sub>1</sub>-*p*<sub>3</sub>) appear in protonymph (Fig. 5B) and remain through ontogeny; seta *p*<sub>1</sub> thick, stiff, pointed, like seta *h*<sub>1</sub>, other setae thin; all setae smooth or with few barbs. Genital valves of protonymph with one pair of small setae, two pairs are added in deutonymph (Fig. 6A) and two more pairs in tritonymph (Fig. 6B). In deutonymph one pair of small aggenital setae (*ag*) appears posterolateral to genital valves, and three pairs of small adanal setae (*ad*<sub>1</sub>-*ad*<sub>3</sub>) appear on segment AD; all setae small and smooth. Paraproctal atrichosy to proto- and deutonymph, but tritonymph with two pairs of small setae. In nymphs cupules *ia* and *im* as in larva, cupule *ip* slightly laterally positioned between setae *h*<sub>1</sub> and *p*<sub>2</sub>, while cupules *ih*, *ips* and *iad* lateral to anterior part of paraproctal valves of proto-, deuto- and tritonymph, respectively, each successively pushed laterally during ontogeny. Gland opening *gla* posterolateral to cupule *ih*. Anogenital region of nymphs slightly wrinkled.

Dorsal aspect of all nymphs is shown in Fig. 7 (tritonymph). Prodorsum with few folds in lateral and posterior part, gastronotum with rare folds, mainly between setae *c*<sub>1</sub> and *dp*. Bothridium and sensillus generally as in larva, central gastronotal setae (*c*<sub>1</sub> and *d*-series) thin but other setae thick and stiff; all setae smooth; setae *c*<sub>3</sub>, *l*- and *h*-series and *p*<sub>1</sub> assume peripheral position on gastronotum (Fig. 8A). Prodorsal and gastronotal setae of nymphs of *L. lustrum* relatively shorter than in larva. Setae *l* on tibia I and seta *ft* on tarsus I thick and rather short; in mites mounted on slides solenidia  $\omega_1$  and  $\omega_2$  usually separated in distal part (Fig. 9A).

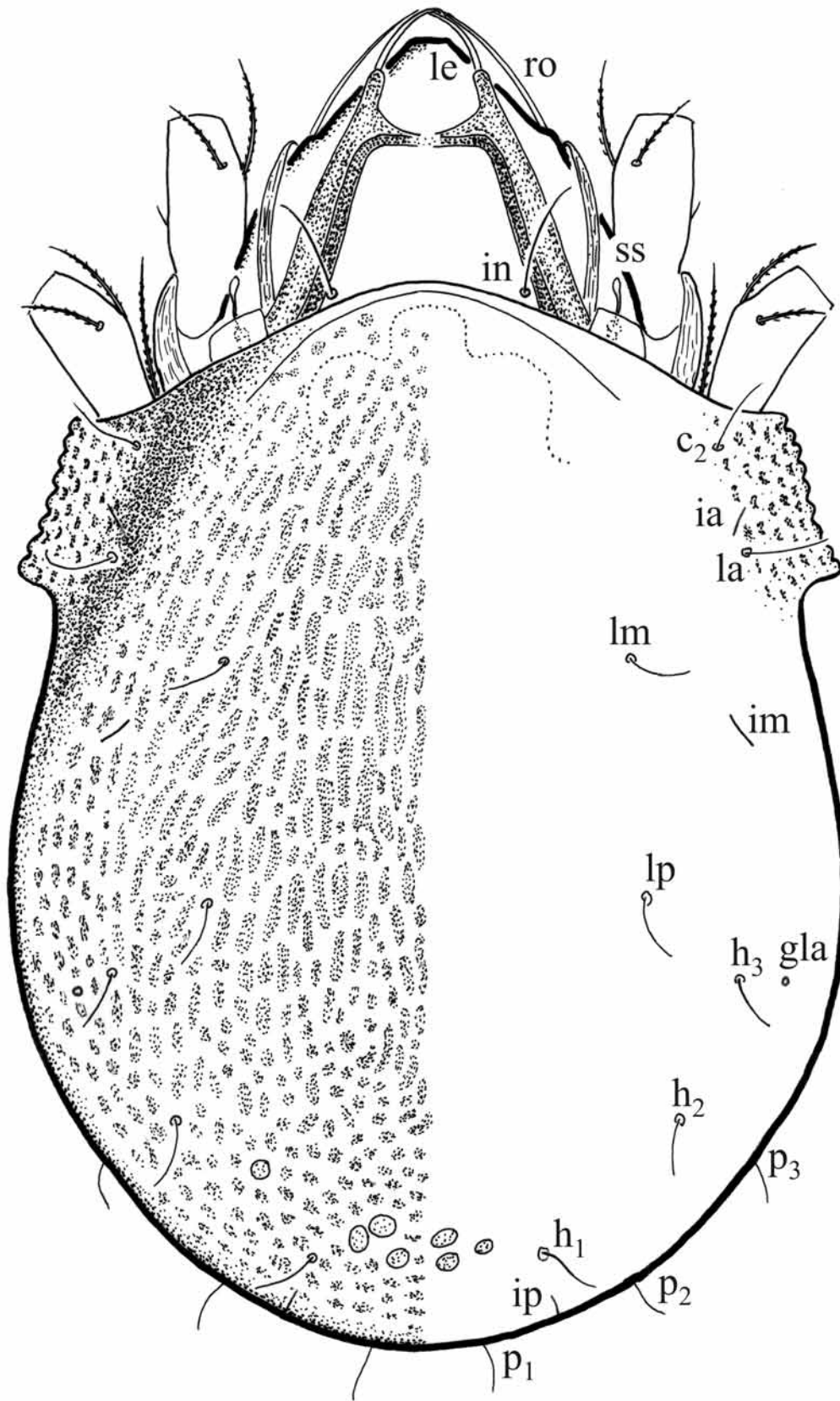


Fig. 1. *Limnozetes lustrum*, adult, dorsal aspect (legs partially drawn).



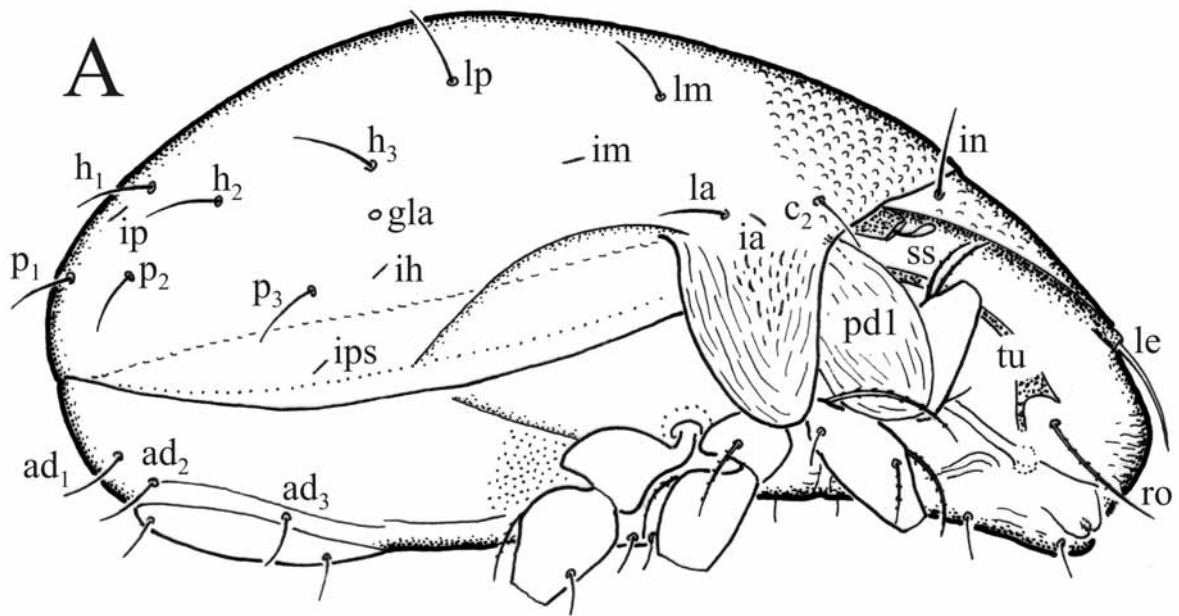


Fig. 2. – Lateral aspect of adult (legs partially drawn): A - *Limnozetes lustrum*,

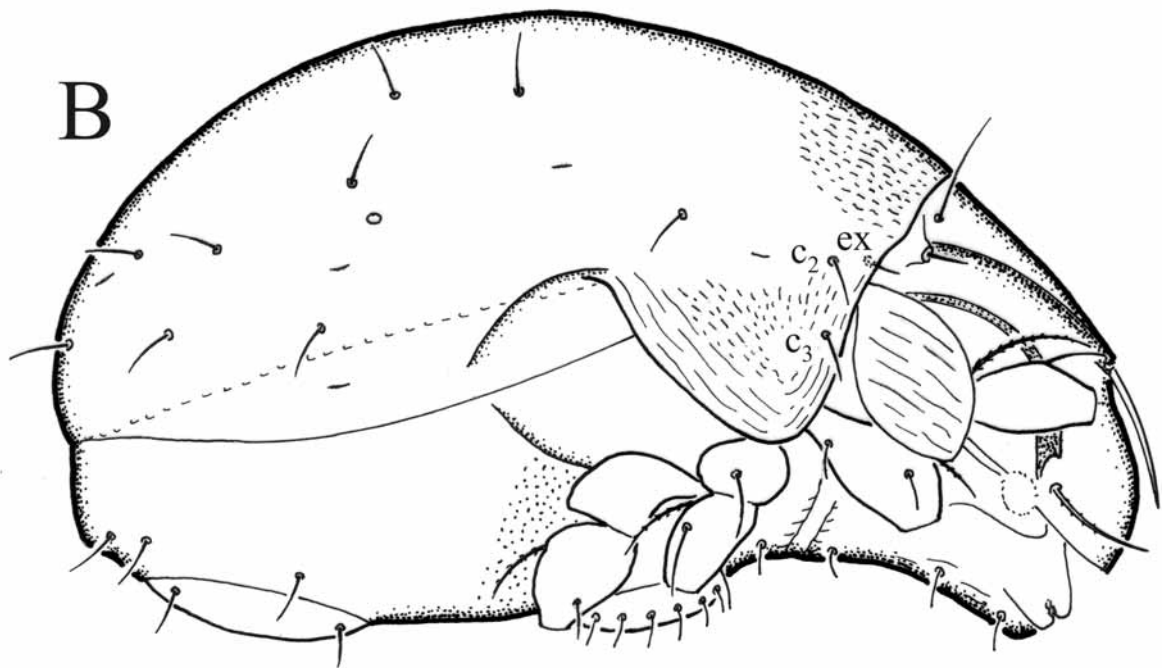


Fig. 2. – Lateral aspect of adult (legs partially drawn): B - *L. rugosus*.

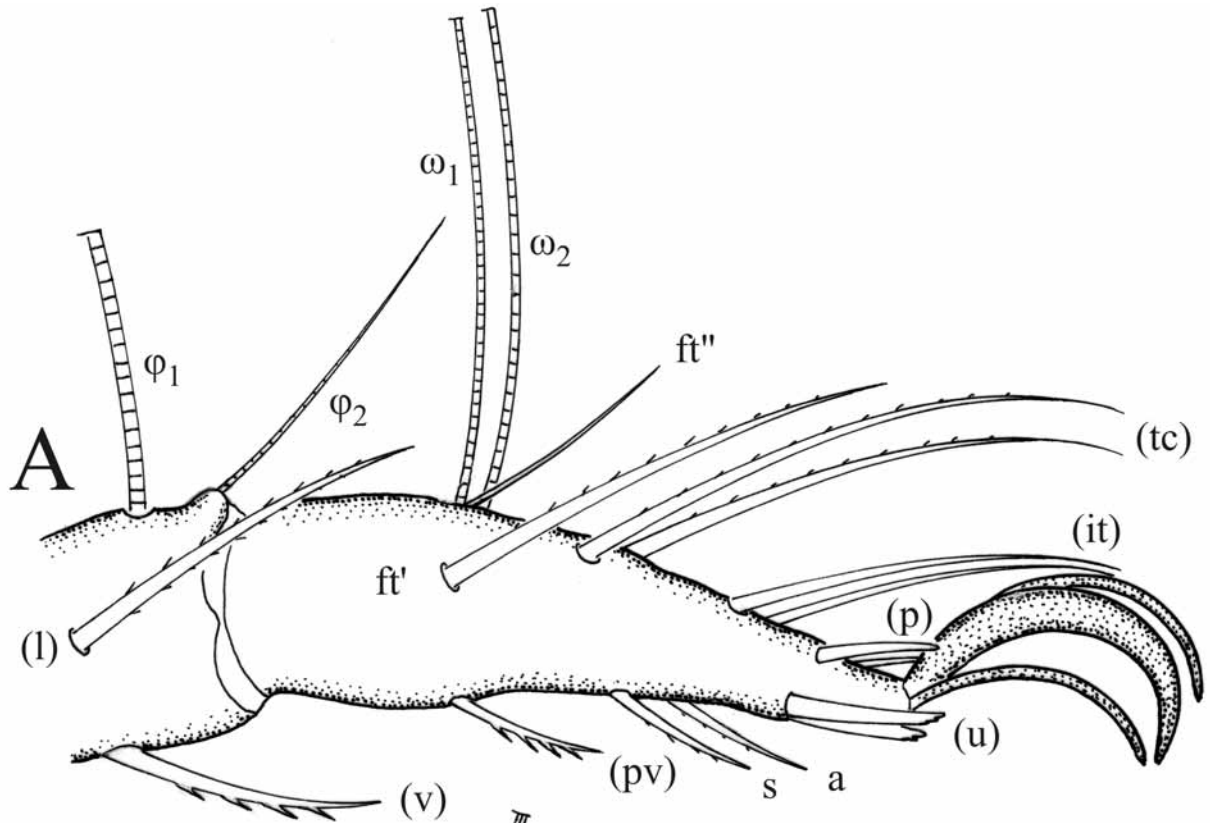


Fig. 3. – Tarsus I of adult, antiaxial aspect: A - *Limnozetes lustrum*.

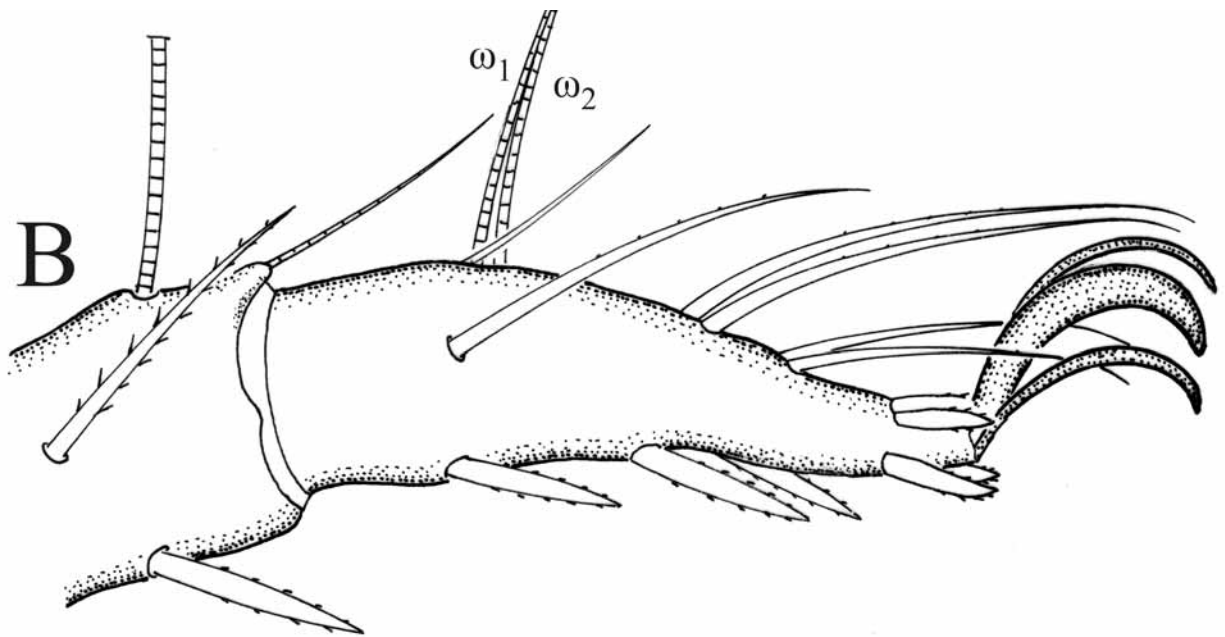


Fig. 3. – Tarsus I of adult, antiaxial aspect: B - *L. rugosus*; pairs of setae in parentheses, some setae are not illustrated.

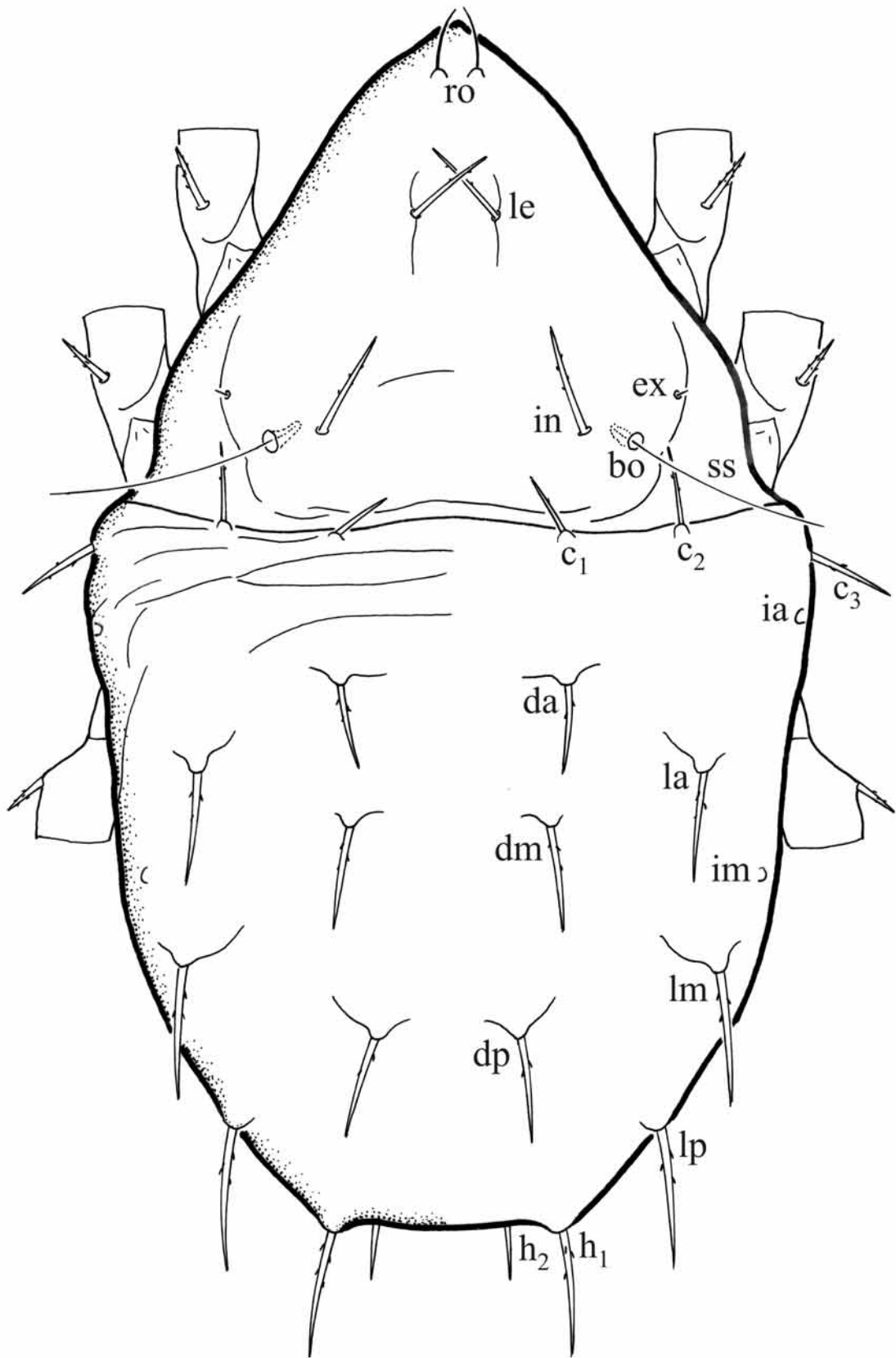


Fig. 4. – *Limnozetes lustrum*, larva, dorsal aspect (legs partially drawn).

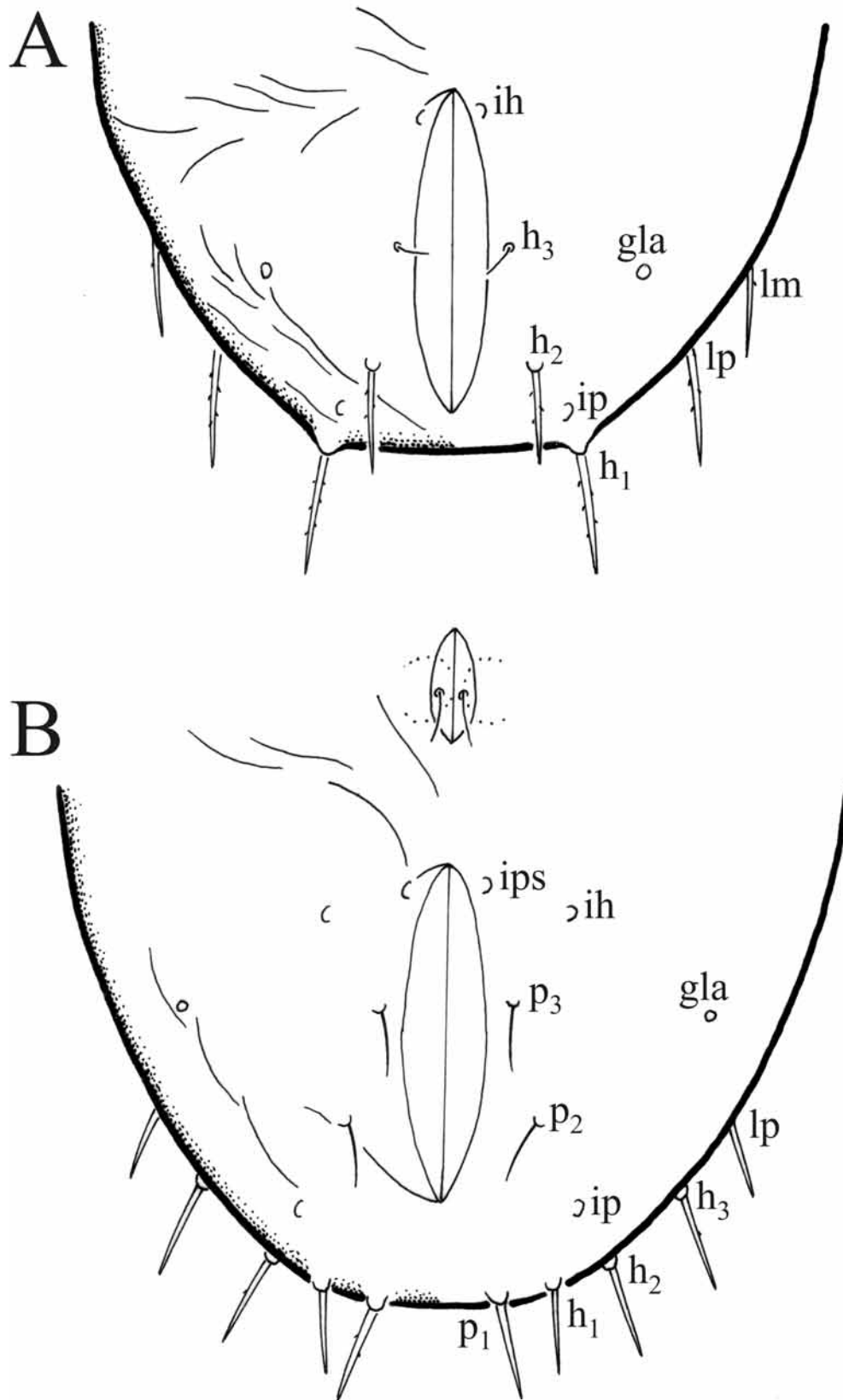


Fig. 5. – *Limnozetes lustrum*: A - anal region of larva, B - anogenital region of protonymph.

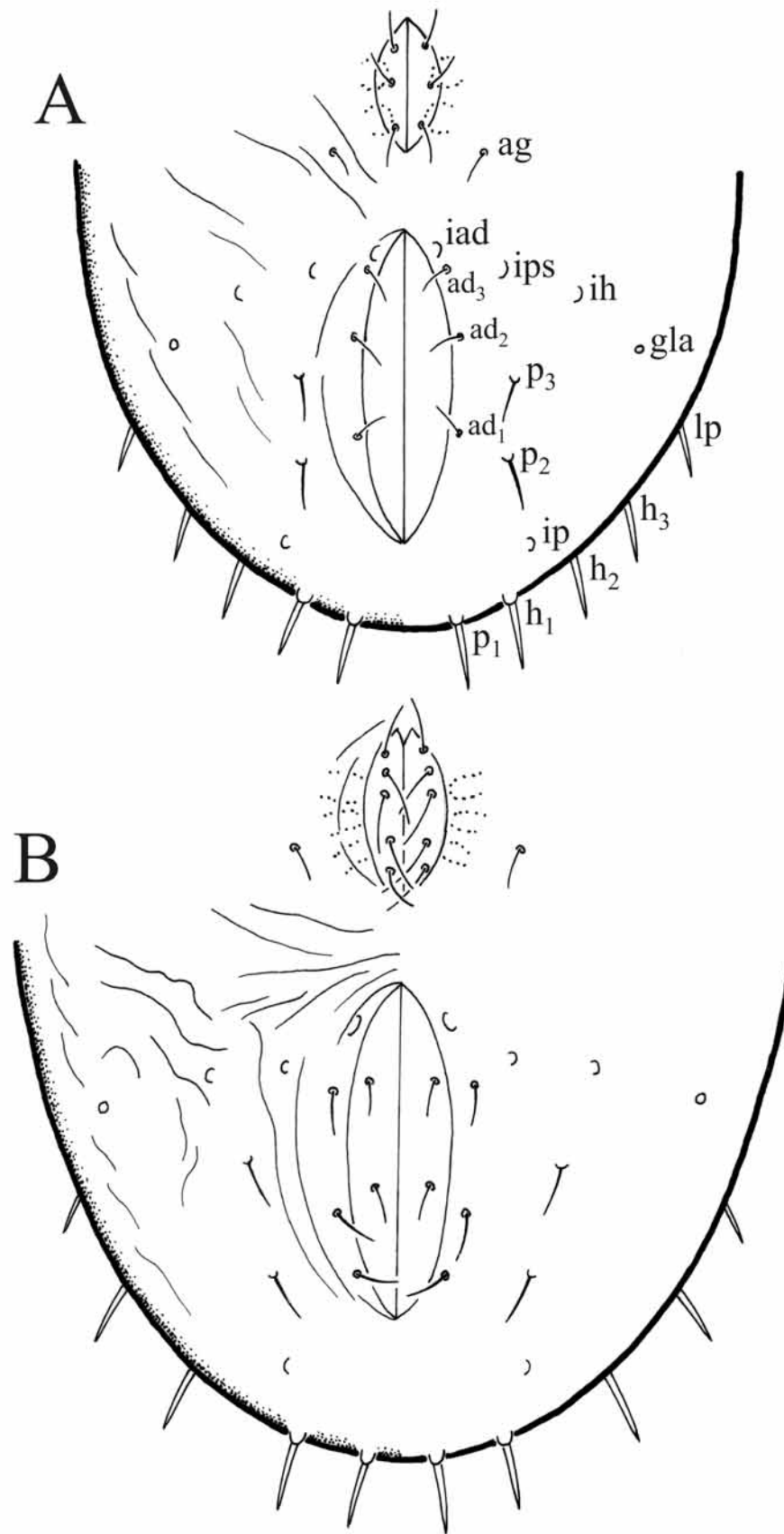


Fig. 6. – *Limnozetes lustrum*, anogenital region: A - deutonymph, B - tritonymph.

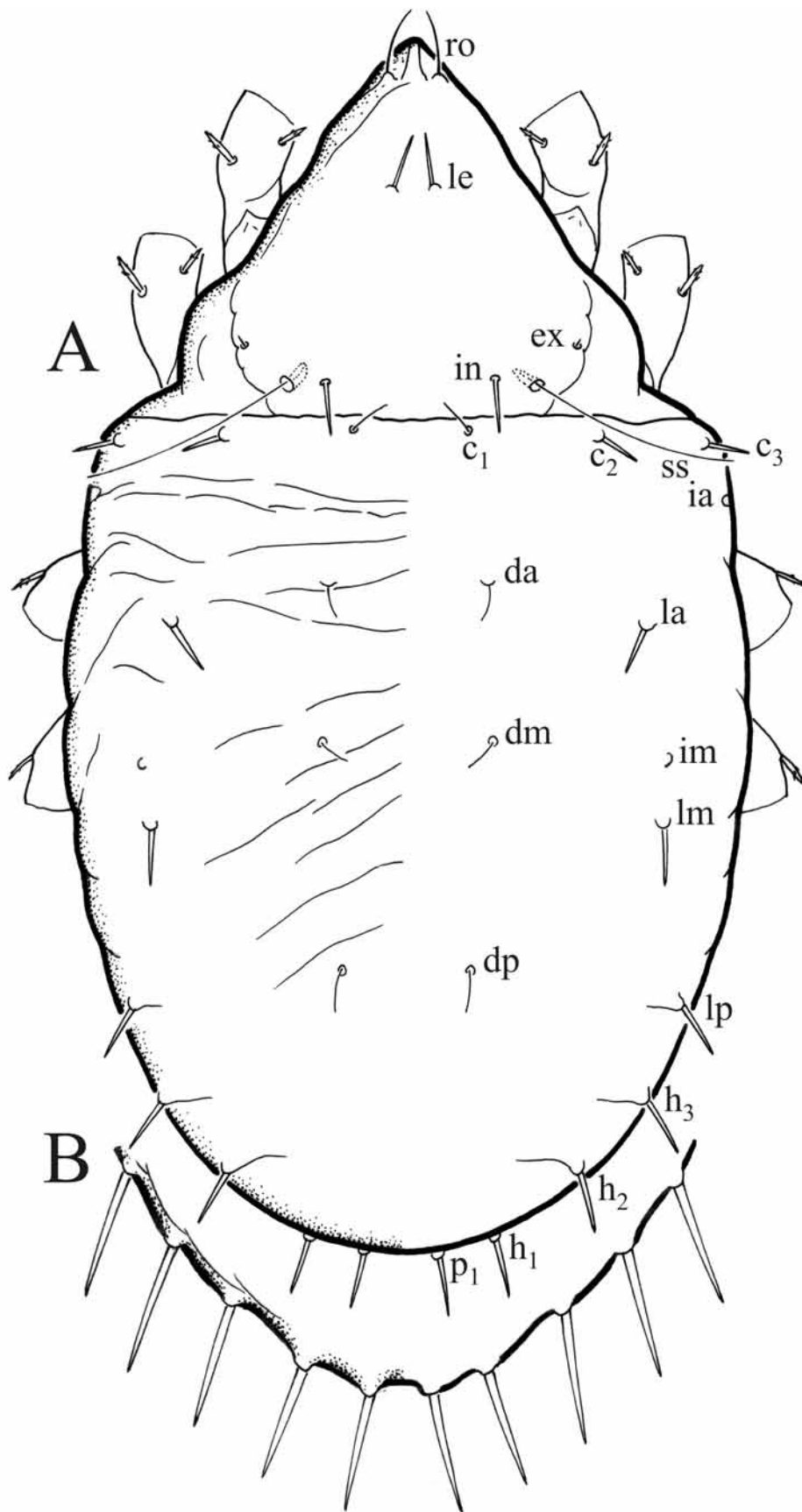


Fig. 7. – Tritonymph, dorsal aspect: A - *Limnozetes lustrum*, B - *L. ciliatus*, marginal part of gastronetum.

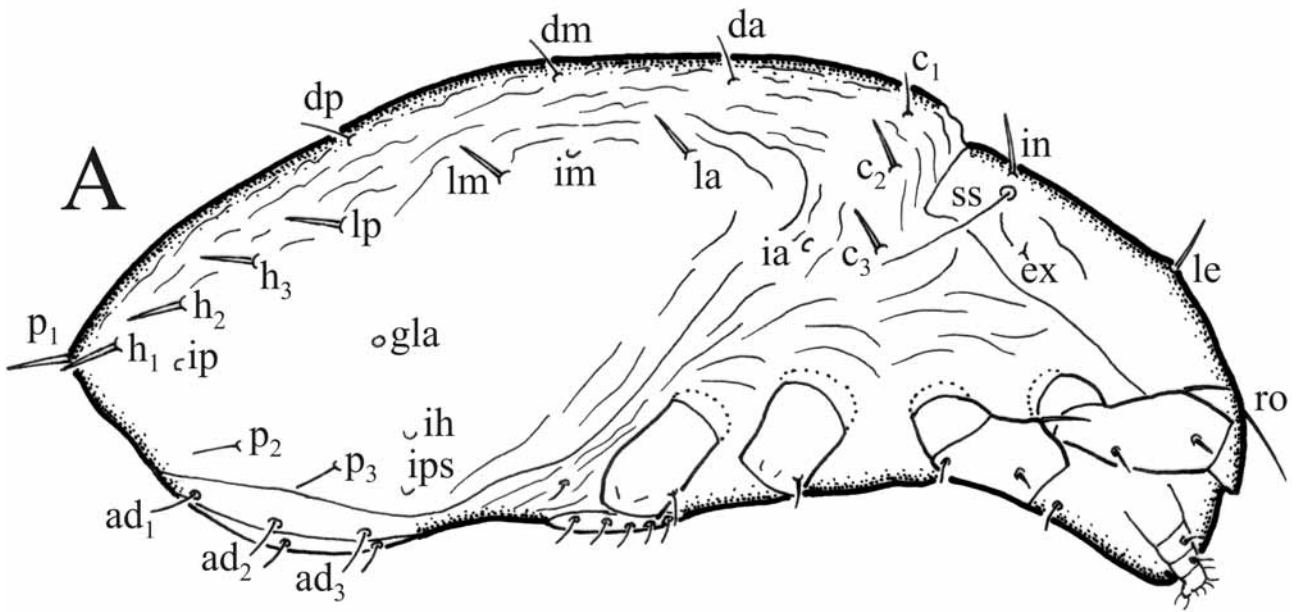


Fig. 8. – Lateral aspect of tritonymph (legs partially drawn): A - *Limnozetes lustrum*.

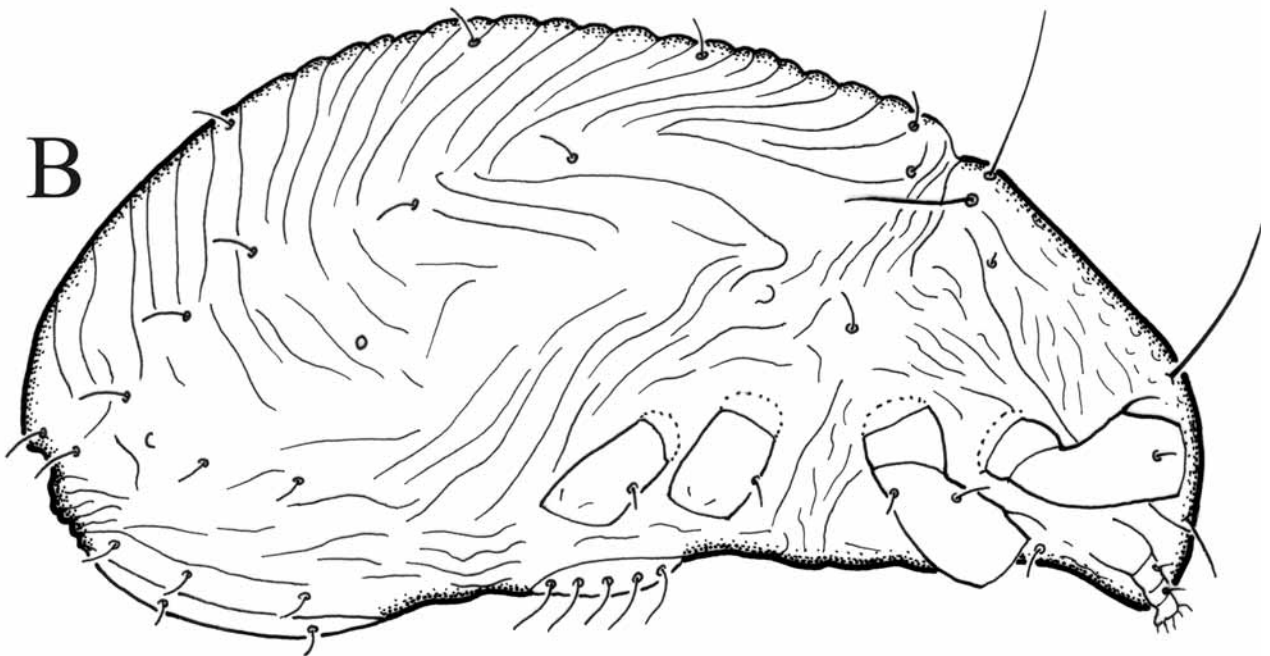


Fig. 8. – Lateral aspect of tritonymph (legs partially drawn): B - *L. rugosus*.

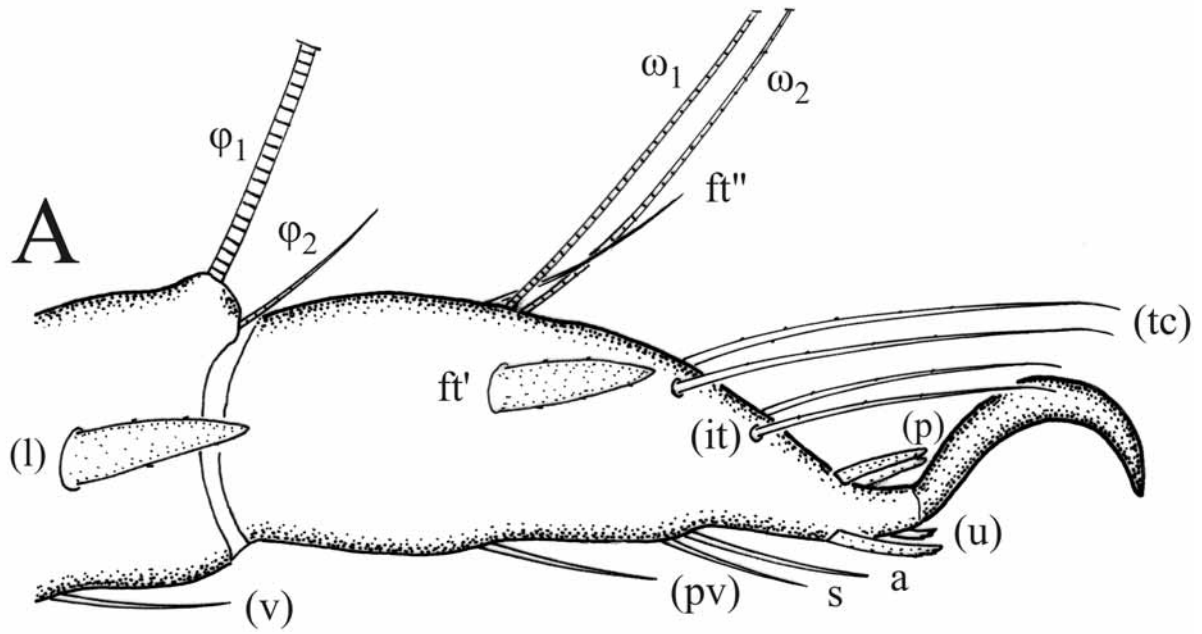


Fig. 9. – Tarsus I of tritonymph: A - *Limnozetes lustrum*, antiaxial aspect.

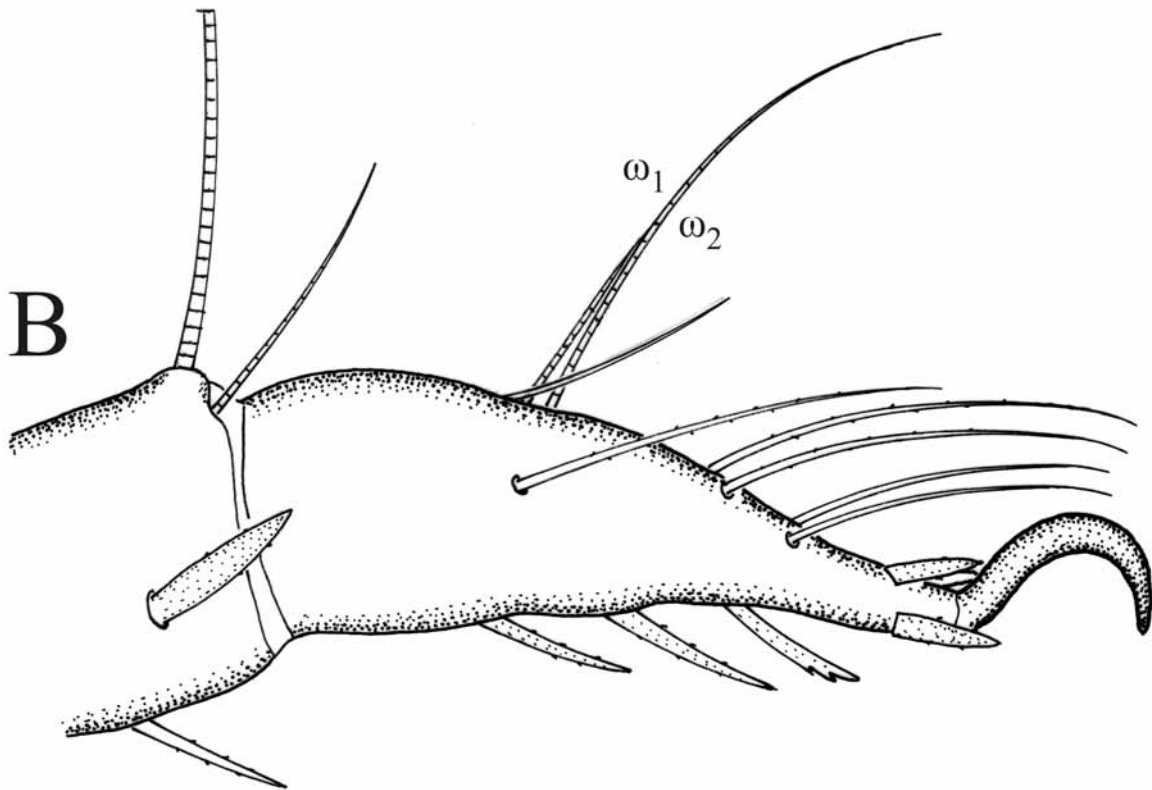


Fig. 9. – Tarsus I of tritonymph: B - *L. lustrum*, region of solenidia, C - *L. rugosus*, antiaxial aspect; pairs of setae in parentheses, some setae are not illustrated.



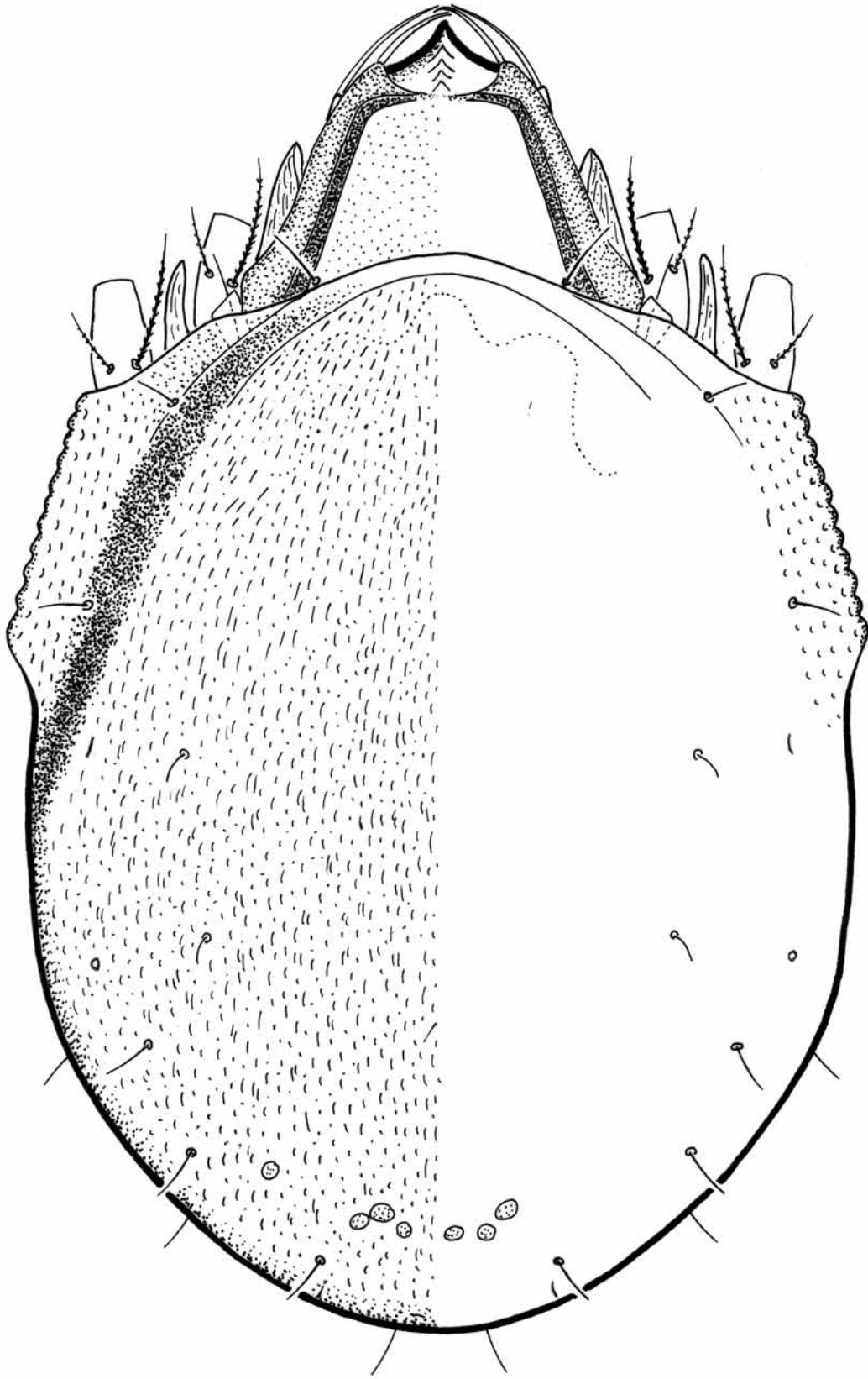


Fig. 10. – *Limnozetes rugosus*, adult, dorsal aspect (legs partially drawn).

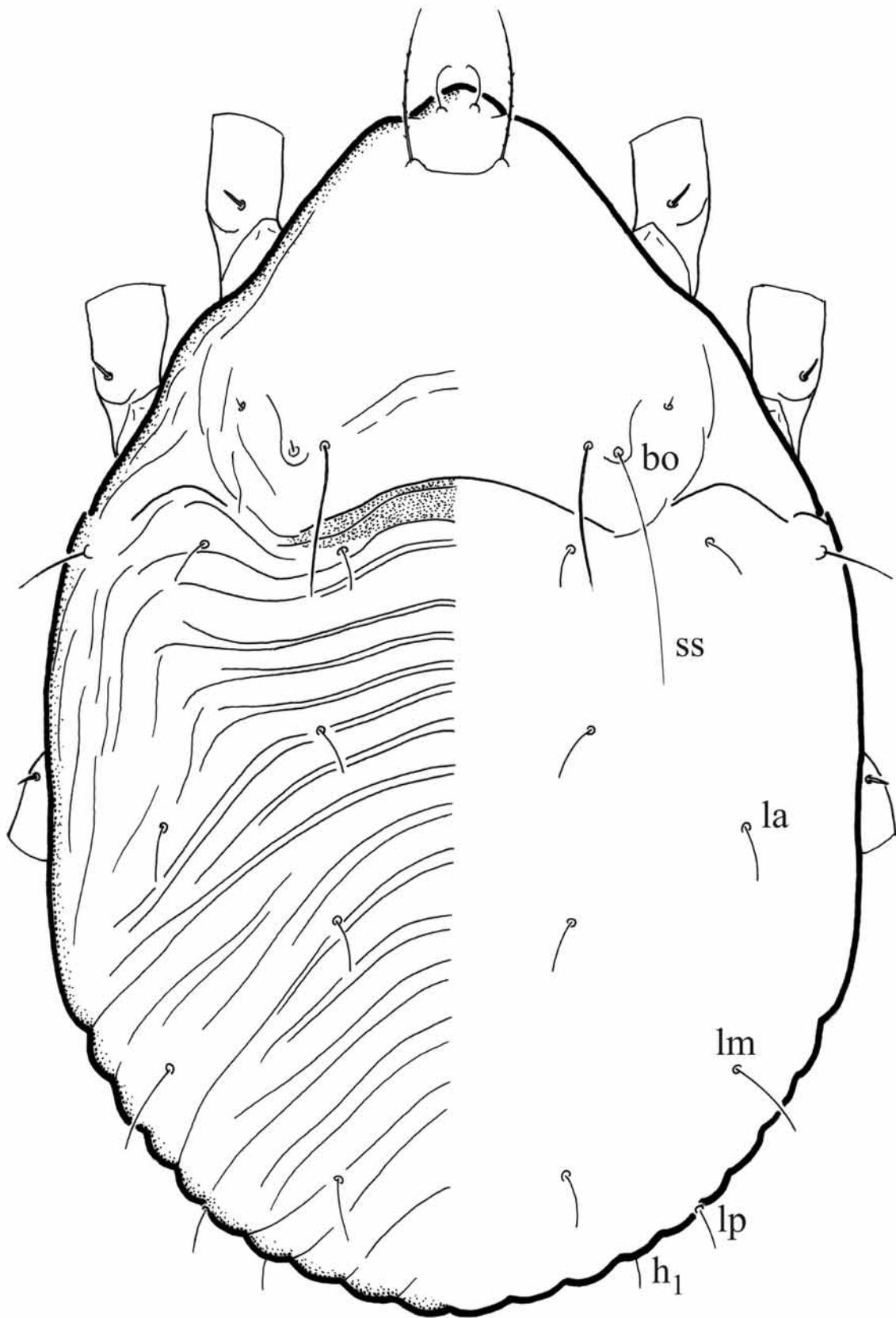


Fig. 11. – *Limnozetes rugosus*, larva, dorsal aspect (legs partially drawn).

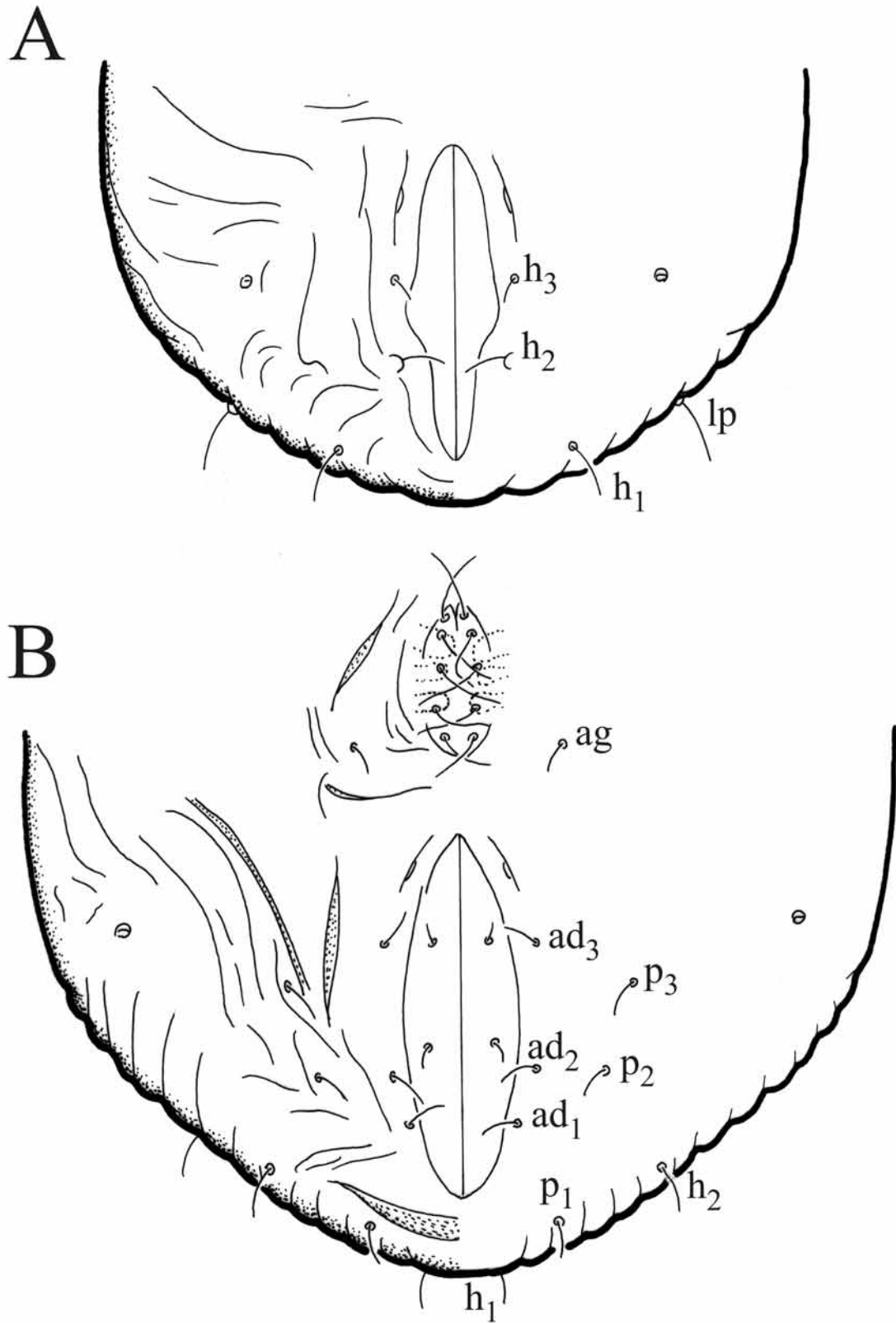


Fig. 12. – *Limnozetes rugosus*: A - anal region of larva, B - anogenital region of tritonymph.

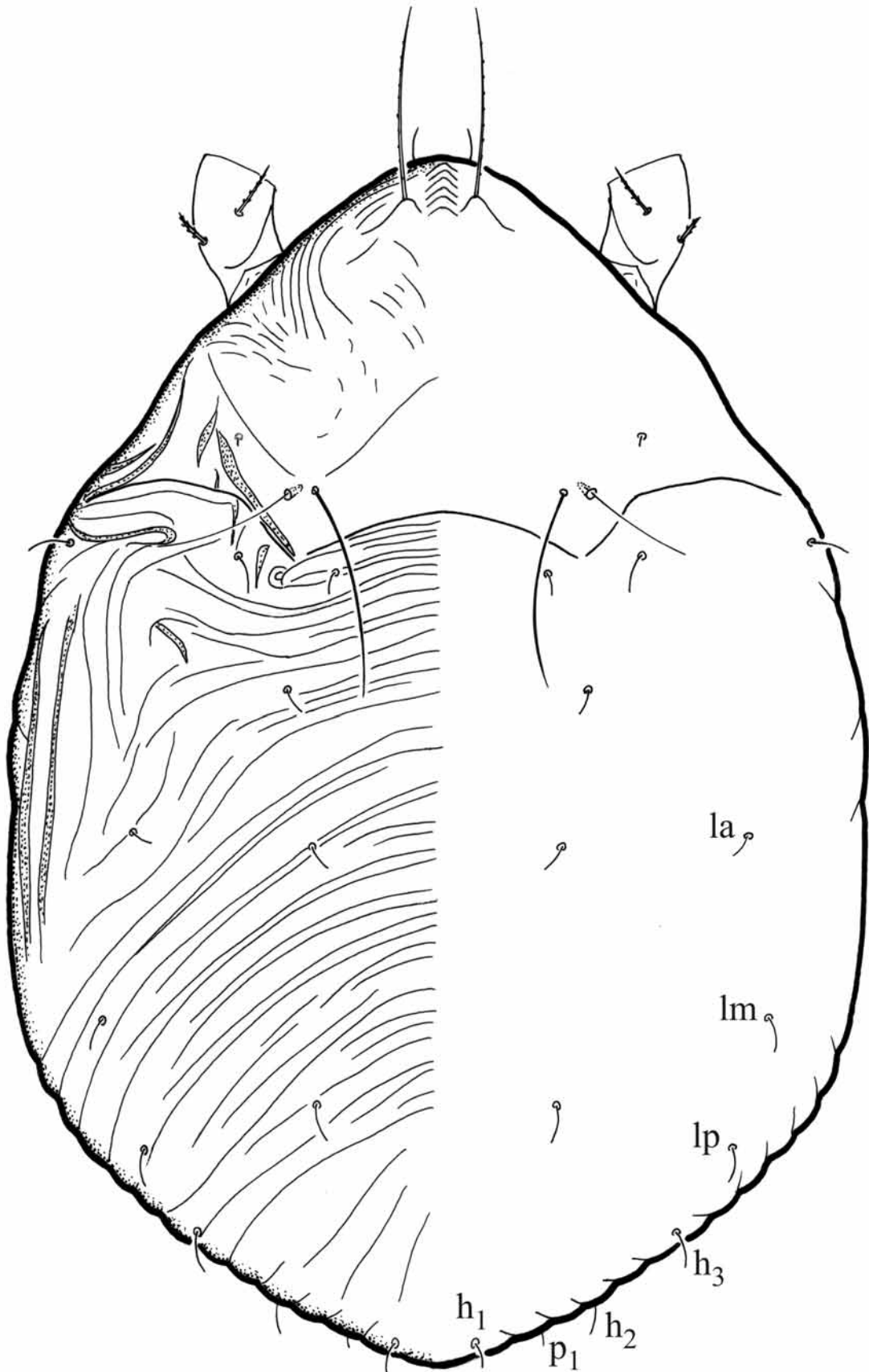


Fig. 13. – *Limnozetes rugosus*, tritonymph, dorsal aspect (legs partially drawn).

### Summary of ontogenetic transformations

The numbers of setae on the prodorsum remain similar during ontogeny, but in the adult setae *ro*, *le* and *in* are distinctly longer than in the juveniles. In the juveniles the bothridium is weakly developed, with long, setiform sensillus, while it is well developed in the adult, with a short, clavate sensillus. During ontogeny the number of gastronotal setae increases from 12 pairs in the larva to 15 pairs in the nymphs, but the adult loses the *d*-series setae and two pairs of *c*-series setae, compared to the tritonymph, leaving only seta  $c_2$ , so the total number of notogastral setae is 10 pairs.

The gastronotal formula of *L. lustrum* (larva to adult) is 12-15-15-15-10, and is consistent with GRANDJEAN (1949), the formula of genital setae is 1-3-5-6 (protonymph to adult), and the formula of aggenital setae is 0-0-1-1-1 (larva to adult). The setal formula of segments PS-AN is 03333-03333-022, respectively, and is consistent with GRANDJEAN (1949), while that of paraproctal setae is 0-0-0-2-2 (larva to adult). The formula of coxisternal setae is 3-1-2 (larva), 3-1-2-1 (protonymph), and 3-1-2-2 (deutonymph, tritonymph and adult). In the larva, seta *lc* is scaliform and covers Claparéde's organ (GRANDJEAN, 1963).

### Distribution and ecology

*Limnozetes lustrum* has been considered a northern Nearctic species (SUBÍAS, 2004), but instead it seems to be a Holarctic species. It lives in wet *Sphagnum* bogs (BEHAN-PELLETIER, 1989), but in Poland inhabits edges of lakes with *Sphagnum* and seems to be common in Pomerania. It was recorded from Wielkopolski National Park (lake Bagno Chlebowo), Tuchola Forest [lake Dury 1 (53°38'20"N, 18°21'14"E, 102m a. s. l.), lake Kozie 1 (53°41'20"N, 17°53'33"E, 119m a. s. l.), lake Kozie 3 (53°41'10"N, 17°52'58"E, 116m a. s. l.) and lake Łyse (53°40'53"N, 18°26'11"E, 87m a. s. l.)], and Brodnica Landscape Park [lake Okonek (53°23'08"N, 19°24'29"E, 84m a. s. l.)]

### *Limnozetes rugosus* (Sellnick, 1923)

#### Diagnosis

Adult (Fig. 10) similar to other European species, but larger (Table 1), with more convex notogaster and pteromorphs wider than longer. Notogaster usually with 10 pairs of setae, including seta  $c_2$ , but some adults also retain pair or one seta  $c_3$  on pteromorph (Fig. 2B). Location of cupules *ia*, *im*, *ip* and *ih*, and gland opening *gla* as in *L. lustrum*. Pedotectum 1 (*pd1*) large, oval. Formula of legs setae (trochanter to tarsus) and solenidia is: I - 1-4-(3+1)-(4+2)-(15+2); II - 1-4-(3+1)-(4+1)-(14+2); III - 2-3-(1+1)-(3+1)-14; VI - 1-2-0-(3+1)-10. In mites mounted on slides solenidia  $\omega_1$  and  $\omega_2$  on tarsus I usually joined in medial and distal parts (Fig. 3B). Legs tridactylous.

Juveniles stocky, yellow-brown, with plicate cuticle. Prodorsal setae *le* and *in* rather long, seta *ro* distinctly

shorter, seta *ex* minute. Bothridium weakly developed, sensillus long, setiform, commonly broken. Setae on gastronotum and anogenital region short and smooth.

### Morphology of larva and tritonymph

Prodorsum of larva (Fig. 11) triangular, lateral and posterior parts plicate. Seta *ro* short, smooth and curved, setae *le* and *in* distinctly longer; seta *le* with small barbs, seta *in* smooth; seta *le* on apophysis; seta *ex* minute. Bothridium weakly developed, sensillus setiform, often broken.

Gastronotal region with 12 pairs of setae, including setae  $h_2$  and  $h_3$  positioned near paraproctal valves (Fig. 12A); length decreases from seta  $h_1$  to  $h_3$ . Other gastronotal setae short and smooth. Gastronotum plicate.

Shape of tritonymph body (Figs 12B; 13) and prodorsal setae, bothridium and sensillus as in larva. Setae *in* and *le* rather long, *in* curved posteriorly (Fig. 8B). Gastronotum with 15 pairs of setae; all setae small, thin and smooth, locations of setae as in *L. lustrum*. Genital valves of tritonymph (Fig. 12B) with 5 pairs of small setae, seta *ag* present. Two pairs of small setae on paraproctal valves, adanal ( $ad_1$ - $ad_3$ ) and pseudanal ( $p_1$ - $p_3$ ) setae slightly longer; all setae smooth. Lateral and posterior parts of prodorsum, gastronotum and anogenital region plicate. Cupule *ia* posterior to seta  $c_3$ , cupules *im* and *ip* difficult to observe in plicate cuticle; cupules *ih*, *ips* and *iad* lateral to anterior part of paraproctal valves of proto-, deuto- and tritonymph, respectively. Setae *l* on tibia I thick and rather short, but seta *ft* on tarsus I thin and long; in mites mounted on slides solenidia  $\omega_1$  and  $\omega_2$  usually joined in medial and distal parts (Fig. 9A).

### Ontogenetic transformations

The ontogeny of prodorsal and gastronotal setae, bothridium and sensillus is similar to that of *L. lustrum*. Compared to the tritonymph, the adult loses the *d*-series setae and usually two pairs of *c*-series setae, leaving seta  $c_2$ , but some specimens retain a pair of  $c_3$  or unilaterally one seta on the pteromorph. The formulae of genital setae, aggenital setae, segments PS-AN, paraproctal setae and coxisternal setae are similar to those of *L. lustrum* (Table 2), while the formula of gastronotal setae is 12-15-15-15-(10-11).

### Distribution and ecology

*Limnozetes rugosus* has been considered a northern European species (SUBÍAS, 2004; WEIGMANN, 2006). It lives in wet *Sphagnum* bogs (WILLMANN, 1931; SELLNICK, 1960; SITNIKOVA, 1975; SUBÍAS, 2004; WEIGMANN, 2006), and at the edges of forest lakes with *Sphagnum*. In Poland it was recorded from Tuchola Forest [lake Dury 1, lake Dury 2 (53°38'20"N, 18°21'22"E, 104m a. s. l.), lake Dury 4 (53°37'56"N, 18°21'42"E, 102m a. s. l.), lake Kozie 1, lake Kozie 3, lake Łyse, lake Małe Gacno (53°47'11"N, 17°33'10"E, 143m a. s. l.) and lake Wielkie Gacno (53°47'32"N, 17°33'27"E, 133m a. s. l.)].

TABLE 1

Measurements of some morphological characters of juvenile stages of *Limnozetes lustrum* and *L. rugosus* (mean measurements of 10 specimens in  $\mu\text{m}$ ): larva (L), protonymph (PN), deutonymph (DN), tritonymph (TN) and adult (AD), nd – not developed, <sup>a/</sup> – if present.

Species	Morphological characters	Developmental stages				
		L	PN	DN	TN	AD
<i>L. lustrum</i>	Body length	198	247	284	335	350
	Body width	108	160	163	169	225
	Length of : seta <i>le</i>	13	9	13	12	45
	seta <i>in</i>	15	10	12	18	28
	seta <i>c</i> <sub>3</sub>	15	10	11	17	lost
	seta <i>da</i>	12	9	8	12	lost
	seta <i>dp</i>	18	10	8	13	lost
	seta <i>lp</i>	21	18	17	20	17
	seta <i>h</i> <sub>1</sub>	20	15	16	19	18
	genital opening	nd	17	27	35	40
	anal opening	46	59	70	86	68
<i>L. rugosus</i>	Body length	215	257	304	350	390
	Body width	147	175	211	257	251
	Length of : seta <i>le</i>	31	31	44	48	27
	seta <i>in</i>	28	31	47	54	38
	seta <i>c</i> <sub>3</sub>	9	9	9	9	14 <sup>a</sup>
	seta <i>da</i>	8	8	8	8	lost
	seta <i>dp</i>	7	9	8	8	lost
	seta <i>lp</i>	12	13	13	13	8
	seta <i>h</i> <sub>1</sub>	10	9	9	9	9
	genital opening	nd	20	29	38	50
	anal opening	57	67	77	92	79

TABLE 2

Chosen morphological characters of *Limnozetes lustrum* and *L. rugosus*.

Morphological characters	<i>L. lustrum</i>	<i>L. rugosus</i>
Adult :		
presence of seta <i>c</i> <sub>3</sub>	absent	absent or present
shape of pteromorph	longer than wider	wider than longer
solenidia $\omega_1$ and $\omega_2$ on tarsus I	usually separated	usually joined
shape of setae <i>pv, s, a</i> on tarsus I	thin	rather thick
Juveniles :		
pattern of cuticle	rare folds	plicate
colour of body	flesh-colored	yellow-brown
Larva :		
shape of setae <i>lm, lp, h</i> <sub>1</sub>	thick, stiff	thin
Nymphs :		
shape of : body	boat shaped	stocky
setae of <i>h</i> -series, <i>p</i> <sub>1</sub>	thick, stiff	thin
solenidia $\omega_1$ and $\omega_2$ on tarsus I	usually separated	usually joined
shape of setae <i>pv, s, a</i> on tarsus I	thin	rather thick
setae <i>l'</i> on tibia I	thick	thin
Formulae of gastronotal setae	12-15-15-15-10	12-15-15-15-(10-11)

## DISCUSSION

Adults of *L. lustrum* and *L. rugosus* are, at first glance, similar to each other, while their juvenile stages are morphologically differentiated. The juveniles of the former species are flesh-colored, with more slender body and cuticle with few folds, while those of the latter species are yellow-brown, stocky, with plicate cuticle. Additionally, the nymphs of *L. lustrum* are boat-shaped, with rather thick and stiff peripheral setae on the gastronotum, like those of *L. onondaga*, *L. palmerae* and *L. amnicus*, which were described by BEHAN-PELLETIER (1989). To this group belongs also *L. ciliatus*, the nymph of which is generally similar to that of *L. lustrum*, but has longer peripheral setae on the gastronotum (Fig. 7B) and more genital setae (GRANDJEAN, 1951b; SENICZAK & SENICZAK, 2009). The nymphs of *L. amnicus*, *L. ciliatus*, *L. lustrum*, *L. onondaga* and *L. palmerae* seem to be similar to typical aquatic species of *Hydrozetes* (SENICZAK et al., 2007; 2009; SENICZAK & SENICZAK, 2007), whose nymphs are also boat-shaped, with thick and stiff setae on the posterior part of gastronotum.

In contrast, the juveniles of *L. rugosus* are stocky and have thin gastronotal setae, as are those of *L. latilamellatus*, *L. guyi* and *L. borealis*, which were described by BEHAN-PELLETIER (1989). Stocky body, short gastronotal setae and plicate cuticle are also found among the juveniles of Achipteridae (SENICZAK & SENICZAK, 2007). The ontogeny of setation in *L. lustrum* and *L. rugosus* is generally similar, except for retention of a pair, or singleton seta  $c_3$  in a number of adult specimens in the latter species. Among 750 investigated adults of this species collected from *Sphagnum* at the edge of a small lake in Finse (Norway), 4.8% of population had a pair of setae  $c_3$ , 18.4% of population had one seta  $c_3$ , and the other individuals lost these setae.

Presence of seta  $c_3$  in some adults of *L. rugosus* has been noted here for the first time in *Limnozetes* and may be considered ancestral, and also could be argued to be derived, in the concepts of GRANDJEAN (1949; 1951a; 1968). In the higher Oribatida (Brachypylina or Circumdehiscentia) often two pairs of  $c$ -setae ( $c_1$ ,  $c_3$ ) are subject to loss in the adult during phylogeny, relative to the tritonymph, such that only seta  $c_2$  remains, as in *L. lustrum*. However, there are two possible sequences of loss of these setae during phylogeny. According to GRANDJEAN (1951a, 1968) first pair  $c_3$  is lost and then pair  $c_1$ ; such a pattern occurs in the adults of the subfamily Sphaerozetinae (SENICZAK et al., 1990) sensu SHALDYBINA (1975). In contrast, SHALDYBINA (1972) considered pair  $c_1$  to be lost first, and then pair  $c_3$ ; this pattern of loss of notogastral setae is observed in *Hydrozetes* Berlese, 1902 (SENICZAK et al., 2007; SENICZAK & SENICZAK, 2008a, b). Therefore, we can question which pattern of loss of notogastral setae occurs in the adult of *L. rugosus*. The answer may lie in the fact that *Limnozetes* and *Hydrozetes* – while different in many ways – may be closely related.

GRANDJEAN (1951b) compared the morphology of *Limnozetes* and *Hydrozetes* with that of some other aquatic genera of oribatid mites and considered the former genus to be more closely related to the latter than to the other brachypylina genera. In most oribatid species the formula

of genital setae is 1-3-5-6, while some species of *Limnozetes* and the 'lacustris group' of *Hydrozetes* express genital neotrichy. For example, the formula of *Limnozetes ciliatus* is 2-4-6-7 (GRANDJEAN, 1951b; SENICZAK & SENICZAK, 2009), and that of *Hydrozetes parisiensis* Grandjean, 1948 is 2-(4-5)-(6-7)-(7-8) (SENICZAK et al., 2009). If we compare the nymphs of European species of *Limnozetes* (except *L. rugosus*) and *Hydrozetes*, they are boat-shaped and have rather strong peripheral setae at least on the posterior part of the gastronotum, as noted for *L. lustrum*. The sensillus has undergone some level of regression in *Limnozetes* and *Hydrozetes*, both in the juvenile stages and in the adult. Moreover these genera share a tendency to parthenogenesis, which occurs in all hitherto known species of *Limnozetes*, in the 'lacustris group' of *Hydrozetes* (SENICZAK et al., 2009) and the other taxa with plicate nymphs show parthenogenesis, e.g., *Tectocephus* sp.

BEHAN-PELLETIER (1989) compared the morphology of adult and juvenile stages of *Limnozetes* and *Hydrozetes*, and also considered these genera closely related. She envisioned character transformations in *Limnozetes* that led to the absence of an alary carina and the presence of pteromorphs in the adults, a slimmer body shape and thinner setae in the juveniles, and reduction of some leg setae.

Based on the observations of GRANDJEAN (1951b) and BEHAN-PELLETIER (1989), *Limnozetes* is closely related to *Hydrozetes*, and presents a similar pattern of loss of notogastral setae in the adult, e.g. the first of the  $c$ -series to regress is pair  $c_1$ , and then pair  $c_3$ . However, these genera differ markedly in the regression of the dorsocentral series of setae. In *Hydrozetes* all setae of the  $d$ -series are retained by adults, regardless of the presence or absence of  $c_1$  and  $c_3$ , while in adults of *Limnozetes* these setae are always absent, despite the variable presence of  $c_3$  in *L. rugosus*. In some other families, the presence or absence of dorsocentral setae is quite variable as well. For example, in the ceratozetid subfamily Sphaerozetinae sensu SHALDYBINA (1975) first  $c_1$  was regressed, then pair  $c_3$  and then the dorsocentral setae (SENICZAK et al., 1990). The loss of setae  $da$  and  $dm$  in this group has small systematic value, and in *Fuscozetes setosus* (C. L. Koch, 1941) it is the source of variability in the number of notogastral setae (SENICZAK et al., 1990), while seta  $dp$  is lost in a closely related species - *F. taticus* Seniczak, 1993, and only alveolus of this seta remains (SENICZAK, 1993).

*Limnozetes lustrum* and *L. rugosus* differ also in the number and shape of some setae on legs, which is consistent with the findings of BEHAN-PELLETIER (1989), who also observed different patterns of leg setation in the investigated species. In the adult of *L. lustrum* mounted on slides solenidia  $\omega_1$  and  $\omega_2$  on tarsus I are usually separated, like in the adult of *L. onondaga* (BEHAN-PELLETIER, 1989), while in *L. rugosus* they are usually joined in medial and distal parts, similar to the adult of *L. guyi* (BEHAN-PELLETIER, 1989). However, this morphological character needs more study on the other species of *Limnozetes*.

Based on the number of notogastral setae of adults, which has systematic value, and morphology of nymphs, we can divide the species of *Limnozetes* into two groups: 'ciliatus group' (*L. amnicus*, *L. ciliatus*, *L. lustrum*, *L.*

*onondaga* and *L. palmerae*) and 'rugosus group' (*L. borealis*, *L. latilamellatus*, *L. guyi* and *L. rugosus*). The 'ciliatus group' includes the adults with ten pairs of notogastral setae, and boat-shaped nymphs, with rather thick and stiff peripheral setae on the gastronomum and rather smooth cuticle. The 'rugosus group' comprises the adults with 10–11 pairs of notogastral setae, and stocky nymphs, with thin gastronomal setae and plicate cuticle.

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**The ultrastructural immunolocalization of loricrin  
in the hairy epidermis of the platypus  
(*Ornithorhynchus anatinus*, Monotremata)  
indicates it contributes to the formation of the cell corneous envelope**

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**ABSTRACT.** The fine localization of loricrin, a major protein of the cell corneous envelope, is known for the epidermis of few species of placental mammals. The localization of this protein has been studied by immunocytochemistry in the hairy epidermis (orthokeratotic) of the platypus, a representative of monotremes, and compared with the localization of the protein in the epidermis of placental mammals. In the hairy epidermis small keratohyaline granules of 0.1-0.3 $\mu$ m are present in transitional cells of the stratum granulosum. An anti-loricrin antibody labels the pale component of keratohyaline granules, the corneous mass of transitional corneocytes, and mainly the corneous cell envelope of corneocytes in the stratum corneum. The last pattern resembles that of the hairy epidermis of placental mammals and differs from the diffuse distribution of loricrin previously described in the parakeratotic epidermis of the platypus. The study confirms that the final pattern of distribution of loricrin in the hairy epidermis of the platypus is as previously observed in corneocytes of placental mammals.

**KEY WORDS:** monotremes; hairy epidermis; loricrin; immunocytochemistry; immunogold

## INTRODUCTION

The evolution of the mammalian integument from that of its reptilian ancestors required numerous modifications in both the dermis and epidermis (CHUDINOV, 1968; FINDLAY, 1970; MADERSON, 1972). The epidermis of basic amniotes, either scaled or un-scaled, probably possessed only a form of alpha-keratinization (soft keratin). In comparison to the dry and resistant epidermis of extant reptiles, the general epidermis of mammals is soft, elastic and moisturized, properties that have been associated with the fine action of mammalian musculature, while the pelage has mostly taken over the role of mechanical protection together with thermal insulation (SPEARMAN, 1964; 1966; FINDLAY, 1970; MADERSON, 1972; SOKOLOV, 1982; ALIBARDI, 2006a). The softness of the mammalian epidermis may be related to the evolution of a granular layer that produced a soft stratum corneum (orthokeratotic) from the parakeratotic epidermis of reptilian progenitors (SPEARMAN, 1964). The latter was characterized by the presence of nuclei in cells of the stratum corneum (corneocytes) and lacked keratohyaline granules.

Keratohyaline-like granules characterize the granular layer of the epidermis of mammals, and have been categorized into F-granules, L-granules and composite granules (HOLBROOK, 1989; STEVEN et al., 1990; RESING & DALE, 1991; HARDMAN et al., 1998; ISHIDA-YAMAMOTO & IIZUKA, 1998).

Most morphological, physiological and molecular information is available for the epidermis of only a few mammalian species such as mouse, human, rat, and cow (MATOLTSY, 1986; HOLBROOK, 1989; FUCHS, 1990; MEHREL et al., 1990; RESING & DALE, 1991; DALE et al.,

1994; ISHIDA-YAMAMOTO et al., 2000; KALININ et al., 2002). For the remaining mammals, only general histological information is available (SOKOLOV, 1982).

Those studies have shown that some proteins (filaggrin and trichohyalin) function as an interkeratin matrix for the formation of corneous material while other proteins (involucrin and loricrin) are mainly deposited along the plasmalemma to produce the resistant cell corneous envelope of mature corneocytes. Information on the expression and localization of keratins and associated proteins involved in the process of cornification is not available for most mammalian species, including marsupials and monotremes (ALIBARDI, 2004; ALIBARDI & TONI, 2005).

A previous study on the epidermis of the interdigital membrane of the Duckbill platypus (*Ornithorhynchus anatinus*) showed that the epidermis in this area is parakeratotic (nucleated) (ALIBARDI & MADERSON, 2003; ALIBARDI, 2006a). Loricrin was intracellularly diffuse in corneocytes instead of concentrated along the cell corneous membrane, as in corneocytes of the other mammals. A similar type of localization has been found in reptilian and avian corneocytes and may represent the primitive pattern of redistribution for this protein in the mature corneocytes of the epidermis.

In the present study, the previous observations on the localization of loricrin in the web epidermis have been compared with the localization of this protein in the epidermis of a hairy area of the platypus limb, the digit close to the interdigital membrane (web). This comparison allows us to establish whether or not the final pattern of distribution of the protein in monotreme epidermis is similar to that of other mammals.

## MATERIALS AND METHODS

The present study was conducted on samples of the skin from three adult individuals of platypus, *Ornithorhynchus anatinus*, (Shaw 1799), collected in the field around Sydney, Australia (see details in ALIBARDI & MADERSON, 2003; ALIBARDI, 2004).

Briefly, small pieces of digit skin containing some hairs (1x2mm) were collected from captured individuals of platypus that were re-marked and released in the field, and the tissues were immediately fixed for 8 hours in 2.5% glutaraldehyde in phosphate buffer 0.1M at pH 7.4, postfixed with 2% osmium tetroxide in water, dehydrated with ethanol, and embedded in Spurr's resin. Other samples were instead fixed in 3% paraformaldehyde in phosphate buffer for 4-5 hours, rinsed with phosphate buffer, dehydrated with ethanol, and embedded in the resin Lowicryl KM4 under ultraviolet light at 0-4°C for three days.

Using an ultramicrotome, sections of 1-4µm were obtained and stained with 0.5% toluidine blue. Forty- to 90nm-thick sections were collected on copper grids, and stained with uranyl acetate and lead citrate according to routine methods for electron microscopic study of the skin. Other sections were collected on gelatin-coated slides from tissues embedded in Lowicryl KM4 for the following light microscopic immunocytochemical study.

Tissues were pre-incubated for 30 minutes in 5% normal goat serum with 2% BSA in 0.05M Tris/HCl buffer at pH 7.6, incubated overnight at 4°C in the buffer containing the primary antibody (rabbit anti-mouse loricrin 15-amino acid specific epitope, Babco, California, USA, diluted 1:300), which was omitted in the controls. After several rinses in the buffer, the sections were incubated for 1 hour at room temperature in the same medium, containing 1:50 anti-rabbit-IgG FITC-conjugated secondary antibodies. After rinsing, sections were mounted in Fluoromount (EM Sciences, USA) and observed under a Zeiss epifluorescence microscope equipped with a fluorescein filter.

For immuno-electronmicroscopy, 40-90nm-thick sections embedded in Lowicryl resin were collected on nickel grids, incubated for 10 minutes in 1% cold-water fish gelatin in Tris buffer as above, and immunostained with the anti-loricrin antibody (1:100 dilution). The primary antibody was omitted in controls. Anti-rabbit IgG conjugated to 10nm-large gold particles (Sigma, USA) was used as the secondary antibody at the concentration of 1:70 in buffer. The incubation of the tissue with the secondary antibody was carried out for one hour at room temperature. After a light staining with uranyl acetate (7 minutes) the sections were observed under a CM-100 Philips electron microscope.

## RESULTS

The multi-layered epidermis of the hairy digits of the platypus possessed a linear or undulating outline with some epidermal papillae, often containing numerous

melanocytes (Fig. 1A). Generally a granular layer was not visible under the light microscope while a thin stratum corneum was present (Fig. 1B). The immunostaining for loricrin showed that only the transitional and corneous layers were positive while the other epidermal layers and the dermis were negative (Fig. 1C).

The ultrastructural analysis showed that many keratinocytes of the basal layer and of the spinosus layer contained numerous melanosomes which appeared even more numerous within the flattening cells of the upper stratum spinosus (Fig. 2A). The keratinocytes of the stratum spinosus contained mainly tonofilaments of low to medium electron-density, some of which converged to desmosomes. Numerous vesicular and some lamellar bodies were present in the cytoplasm of the upper keratinocytes of the upper spinosus layer, and were localized among the tonofilaments (data not shown, see ALIBARDI & MADERSON, 2003).

The keratinocytes of the upper layers of the stratum spinosus contained numerous dense tonofilaments and also sparse and dense keratohyaline granules, 0.1-0.2µm in diameter. These granules were more numerous, with variable but irregular size (0.1-0.3µm) in the transitional layer (pre-corneous) where they appeared merged with a pale corneous material (Fig. 2B). The latter corneous material did not contain tonofilaments but was mainly amorphous. Therefore these granules were identified as composite keratohyaline granules (darker F-granules surrounded by paler amorphous material). A 10-20nm thick cell corneous membrane was seen in corneocytes of the transitional layer (pre-corneous).

In the stratum corneum, cornified cells (corneocytes) showed an irregular or dentate surface that interlocked these cells to form a compact tissue. The corneocytes of the stratum corneum were surrounded with a 10-20nm thick and dense cell corneous membrane. The corneous material within corneocytes was not homogenous but contained irregular dense areas among paler material, probably derived from cell or nuclear degeneration.

The ultrastructural immunogold analysis showed that no specific labeling for loricrin was present in keratinocytes of the basal and spinosus layers. In the upper layers of the spinosus layer, and in the corneocytes of the transitional (pre-corneous) layer, the pale areas and the irregular granules present between the dense keratohyaline or between the condensing keratin bundles, were decorated with gold particles (Fig. 2C-D). Also in the composite granules, the labeling appeared mainly associated with the pale material present amongst the coarse network of dense material forming the F-granules (Fig. 2E).

In the transitional corneocytes the immunolabeling was diffuse (Fig. 3A) but in mature corneocytes of the stratum corneum, the labeling tended to localize along the plasma membranes or on the cell surface of mature corneocytes (Fig. 3B). Gold particles were also seen along parts of the desmosome remnants connecting the corneocytes of the stratum corneum, while the labeling for loricrin almost disappeared in the central corneous mass of mature corneocytes.

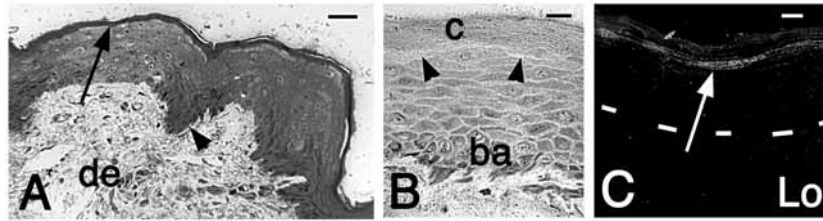


Fig. 1. – Light microscopy of the skin (hairy epidermis) of platypus (semithin sections). **A**, histological view of epidermis with thin stratum corneum (arrow) and small papilla (arrowhead) into the dermis (de). Toluidine blue stain. Bar=10 $\mu$ m. **B**, detail of thick epidermis with irregular basal layer (ba), spinosus and transitional agranulated layer (arrowheads), and the corneous layer (c). Toluidine blue stain. Bar=10 $\mu$ m. **C**, Immunofluorescent staining for loricrin (Lo) of the corneous layer (arrow). Dashes underline the basal layer of the epidermis. Bar=10 $\mu$ m.

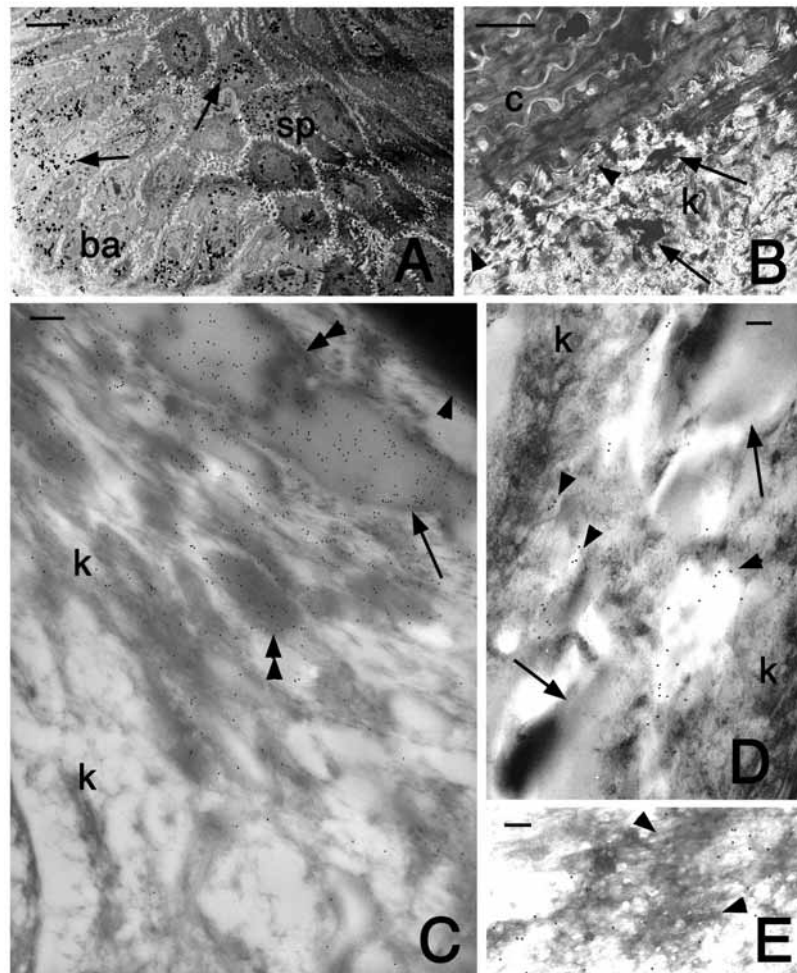


Fig. 2. – Ultrastructure (**A-B**) and Immunogold labeling (**C-E**) of the hairy epidermis of platypus. **A**, basal (ba) and spinosus (sp) layers of the thick epidermis. Arrows indicate the numerous melanosomes incorporated in these cells. Bar=5 $\mu$ m. **B**, detail of the transitional layer beneath the stratum corneum (c). Transitional cells contain pale granules (arrowheads) and composite keratohyaline granules (arrows) among bundles of keratin (k). Bar=1 $\mu$ m. **C**, diffuse loricrin immunolabeling in transitional cell (the arrowhead indicates the corneous layer). Most labeling is seen over pale material (arrows) associated with denser (double arrowheads) material forming the irregular keratohyaline granules (k). Bar=200nm. **D**, detail of cytoplasm of spinosus cell with diffuse labeling (arrowheads) among keratin bundles (k) and pale areas (arrows). Bar=100nm. **E**, detail of composite keratohyaline granule (arrowheads on coarse filaments forming the network) with labeling in the pale components. Bar=100nm.

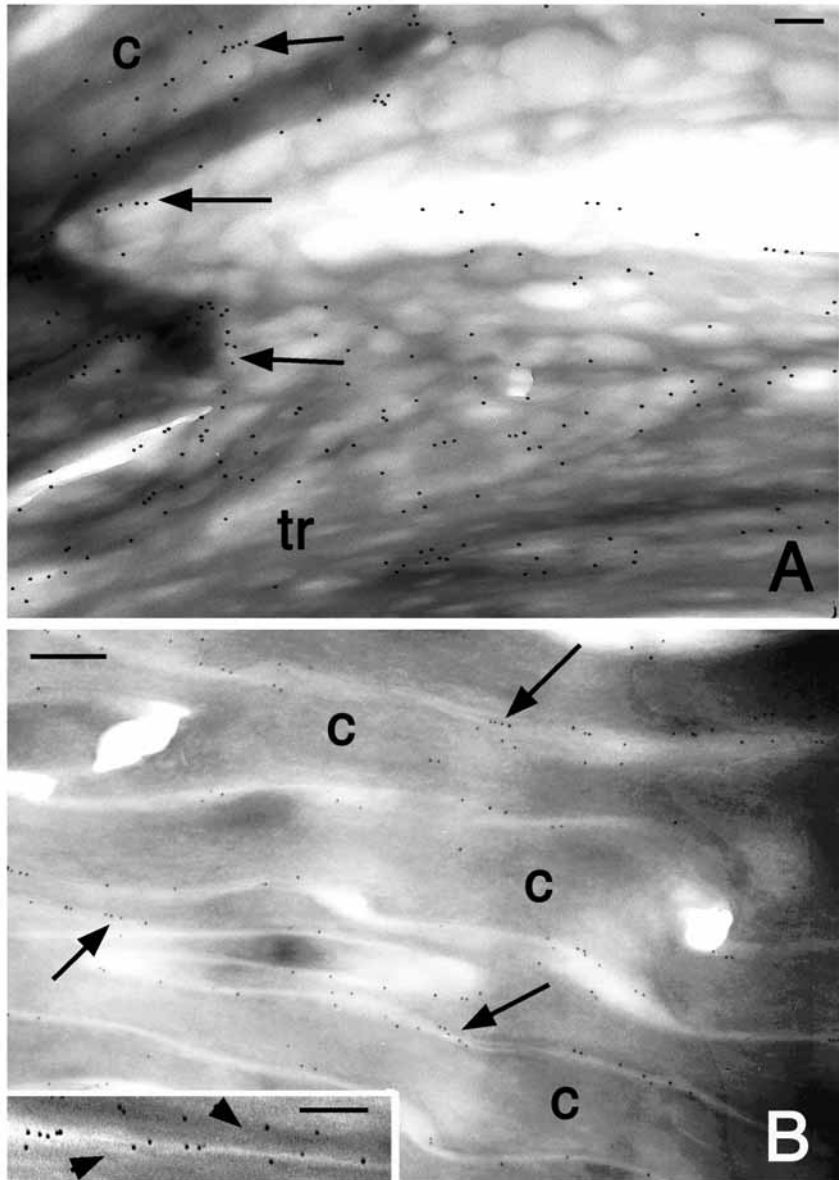


Fig. 3. – Immunogold labeling of external epidermal layers of the hairy epidermis of platypus. **A**, transitional layer (tr) with diffuse distribution of gold particles that tend to align along the plasma membrane of the corneocytes of the stratum corneum (arrows). **B**, loricrin-immunolabeling in corneocytes (c) of the stratum corneum where most gold particles are localized along the plasma membrane (arrows). Bar=100nm. The inset details the linear distribution of gold particles, mainly along the dense border (arrowheads) of corneocytes (Bar=100nm).

## DISCUSSION

The present study shows that keratohyaline granules in the hairy epidermis of the platypus are of small dimension (around 0.2µm), and therefore not easily detected under the light microscope. An extensive survey on the skin of many domesticated and wild mammals has shown some histological variations in the epidermis, in particular in relation to the presence, reduction or even disappearance of the stratum granulosum (SOKOLOV, 1982). In fact, the stratum granulosum is

not clearly seen (in marsupials, pholidotans, dermopters, chiropterans, cetaceans, sirenians, arctiodactylans, proboscideans), discontinuous (in insectivores, carnivores, xenarthrans), or present with different degrees of size and location (in lagomorphs, primates, rodents, pinnipeda). It is possible, however, that, as in marsupials, so also in the epidermis of the placentals where the stratum granulosum is not visible under the light microscope, keratohyaline granules of submicroscopical dimensions may be present (smaller than 0.4µm), as in the case of bat epidermis (ALIBARDI, 2006b).

The scattered distribution of loricrin-immunolabeling previously detected in the parakeratotic interdigital (web) epidermis of the platypus resembles the immunolabeling of reptilian and avian corneocytes (ALIBARDI & MADERSON, 2003). In corneocytes of the hairy epidermis of the platypus, however, loricrin or loricrin-like immunolabeling is present over the electron-pale and amorphous material that is localized among keratin bundles and denser keratohyaline granules.

Parakeratosis in mammalian epidermis is often associated with pathological conditions (psoriasis, eczema, ichthyosis, and sometimes hyperkeratosis), and has been considered as a reversion to a more primitive form of cornification, possibly present in the first cotylosaurian reptiles from which therapsids and later mammals derived (SPEARMAN, 1964; 1966; MADERSON, 1972; ALIBARDI, 2003).

The process of terminal differentiation in both ortho- and para-keratotic corneocytes eventually determines the formation of the cell corneous envelope, indicating that at least some proteins synthesized in these terminally-differentiated cells become cross-linked to the plasma membrane (KALININ et al., 2002). A loricrin-like immunoreactivity has been not only found in mammalian epidermis, but also in the epidermis of reptiles and birds, suggesting that a loricrin-like protein or a similar sulfur-rich protein could be an ancient structural protein necessary for the formation of the resistant cell corneous membrane of amniote epidermis (ALIBARDI, 2003; ALIBARDI & TONI, 2004). The immunological evidence on the presence of loricrin-like proteins in the epidermis of lower vertebrates, further suggests that its protective role was established very early during the evolution of the stratum corneum in tetrapods (ALIBARDI, 2006a).

It is, however, uncertain whether the diffuse distribution of loricrin is really primitive in mammals, since human and rat keratinocytes also show a diffuse labeling for loricrin in some body areas, and specific organelles (L-granules) are also not seen in some regions of the epidermis (STEVEN et al., 1990; HARDMAN et al., 1998; ISHIDA-YAMAMOTO et al., 1996; 2000). A random distribution of loricrin is also present in human corneocytes forming the stratum corneum in some pathological conditions, suggesting that the mechanism of distribution of loricrin is defective in these specific pathological conditions (ISHIDA-YAMAMOTO et al., 1996, 2000).

In conclusion, in the monotremes, here represented by the platypus, loricrin contributes to the formation of the cornified cell corneous membrane of the hairy epidermis of digits, as it does in the epidermis of the other mammals.

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## Early Formation of the Coelomo-Cardiovascular Complex in the Chick Blastoderm

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**ABSTRACT.** Although it is known that small areas in the unincubated avian blastoderm undergo regulation under influence of the surrounding large Rauber's (Koller's) sickle-dependent anlage fields, this seems not to be true for these anlage fields themselves. Indeed, after removal of whole anlage fields or regions in the unincubated avian blastoderms, no restoration of a complete embryo occurs, suggesting predisposition (mosaicism). In the unincubated chicken blastoderm, in the absence of Rauber's (Koller's) sickle horn regions, the isolated median region (with included middle part of Rauber – Koller's sickle) is not able, after culture, to give rise to primary heart tubes. Our present study indicates that the earliest anlage field of the coelomo-cardiovascular system in the unincubated chicken blastoderm (giving rise to both the area vasculosae laterales and the area vasculosa caudalis) is localized in the upper layer between the definitive endoderm anlage field (in the concavity of Rauber – Koller's sickle) and the more rostral and lateral neighbouring sickle-shaped lateral plate anlage.

**KEY WORDS:** chick embryo, mosaic development, Rauber - Koller's sickle, sickle horns, coelomo-cardiovascular system, coelomates.

### INTRODUCTION

Since the experimental studies *in ovo* of LUTZ (1949) LUTZ et al. (1963) and the *in vitro* studies of SPRATT & HAAS (1960), it has generally been accepted that the avian blastoderm always presents a highly regulative development, i.e., that any isolated major part of it could develop into a normal symmetrical embryo. However, we have shown that mosaicism also can be provoked, under certain circumstances, in unincubated avian blastoderm parts depending on the spatial distribution of RAUBER - Koller's sickle (1876) material and its relationship with the upper layer (CALLEBAUT et al., 2007). We use here the term mosaic development as originally defined by CONKLIN (1905) in ascidian species: each region of the whole fertilized egg would be able to form more or less independently on its own. The development of the entire embryo was regarded as being the sum of the development of the interacting individual parts. In the present work, we studied the mosaic or regulation phenomena occurring before and during the early formation of the coelomo-cardiovascular system. We define the coelomo-cardiovascular system as the intimate association of blood islands (which will give rise to the cardiovascular system) with the more superficial coelomic vesicles (giving rise to the coelomic cavity) (CALLEBAUT et al., 2004). Both mosaic and regulation phenomena and the development of the coelomo-cardiovascular system are closely related since they are successively influenced by the localization of Rauber - Koller's sickle material (junctional endoblast). Until now, the earliest known localization of the cells of the prospective cardiovascular system in avian embryos has been determined in pregastrular blastoderms (HATADA & STERN, 1994) and in the intermediate primitive streak stages (GARCIA-MARTINEZ & SCHOENWOLF, 1993; LOPEZ-SANCHEZ et al., 2001). The latter authors localized heart

and lateral plate precursor cells just lateral to, and parallel with, the cranial part of the primitive streak. In the caudal blastoderm region they found precursors of lateral plate and extraembryonic mesoderm. Explants from the caudal region of pregastrula chicken blastoderms give rise to blood tissue (haemoglobin) (GORDON-THOMSON & FABIAN, 1994). Caudal deep layer cells seem to play a role in cardiac myogenesis in pregastrular upper layer (YATSKIEVYCH et al., 1997). In these studies, however, no precise relationship with Rauber - Koller's sickle material was described, since the fundamental inductive effect on gastrulation (CALLEBAUT & VAN NUETEN, 1994) and of the Rauber - Koller's sickle-derived junctional endoblast on the formation of the coelomo-cardiovascular system was only shown more recently (CALLEBAUT et al., 2002a; 2004). By our present ablation experiments, we were able to localize retrospectively the anlage field of the coelomo-cardiovascular system in the upper layer at the unincubated chicken blastoderm stage.

### MATERIALS AND METHODS

We used unincubated chicken (*Gallus domesticus*) eggs. We studied, in culture, the effect on general development or more particularly the development of the coelom and associated cardiovascular system, after ablation experiments in unincubated chicken blastoderm or parts of it. The effect of removal of one or both lateral parts (containing the sickle horns) of the blastoderm was also investigated. Finally the evolution, in culture, of isolated caudal parts (containing the medium part of Rauber - Koller's sickle) was followed. Each experimental procedure is represented in a scheme accompanying the photomicrographs. The blastoderm parts were cultured according to the technique of SPRATT (1947). The semi-

solid culture media allow microsurgery and further culture on the same substrate. Stereomicroscopic photographs were taken in the same direction at the beginning, during and at the end of the culture period. After fixation, the blastoderms were stained with Unna *in toto* to visualize the localization of blood-containing structures in surface views (VAKAET, 1962). Embedding in paraffin was performed as mentioned in earlier studies (CALLEBAUT et al., 2004). The blastoderms were sectioned perpendicularly to the visible or presumed axis. The deparaffinized sections were stained with Harris's or Heidenhain's haematoxylin and eosin.

## RESULTS

### 1/ Unilateral excision of a sickle horn region and accompanying lateral tissue, according to the procedure schematically represented on the map of the anlage fields (CALLEBAUT et al., 2000) in Fig. 1A (n=6)

After culture, fixation and *in toto* staining with Unna, an apparently normal embryo is seen (Fig. 1B). However, the coelomo-cardiovascular system is only normally developed in the unoperated side (containing the remaining sickle horn region) and in the caudal region (median part of Rauber - Koller's sickle), respectively visible as an area vasculosa lateralis and an area vasculosa caudalis. In the operated side the area vasculosa lateralis is absent. Sections confirmed these observations and also revealed that in the operated side no coelomo-cardiovascular anlage was seen and the intraembryonic cavity was closed laterally (Fig. 1C).

### 2/ Bilateral removal of the sickle horns and neighbouring lateral parts as represented schematically on the map of the anlage fields of the unincubated chicken blastoderm (CALLEBAUT et al., 2000) (Fig. 2A) (n=7)

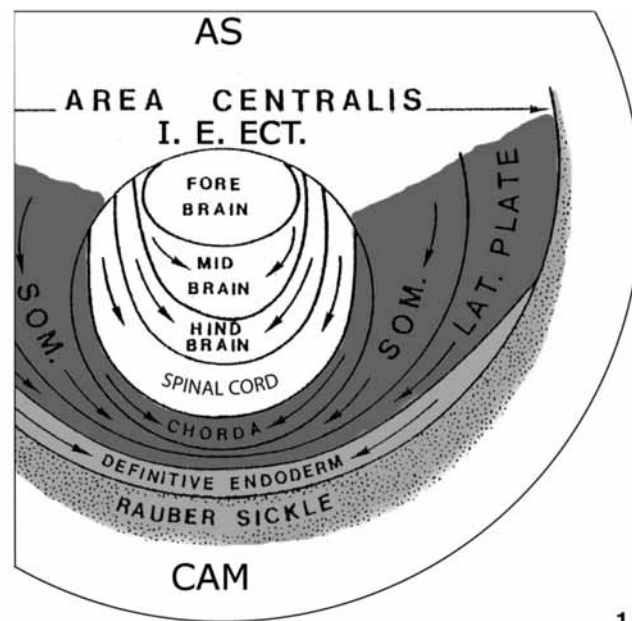
In Fig. 2B, such an operated chicken blastoderm is seen at the start of the culture period. After thirty hours of culture (Fig. 2C) no area vasculosae laterales were visible. Only in the caudal region was a reduced area vasculosa caudalis discernable. In sections, it is seen that a neural plate has developed in the cranial half of the blastoderm. In and below the middle part of this neural plate, the so-called midline structures (floor plate, prechordal plate, notochord, pharyngeal endoderm) are seen (Fig. 2D). They are formed from Hensen's node material (LE DOUARIN et al., 2000) and are originally derived from median upper layer cells localized in the concavity of Rauber - Koller's sickle and induced by the median part of the latter (CALLEBAUT et al., 1996; CALLEBAUT et al., 2006). In the caudal half of the blastoderm a short primitive streak and two paraxial mesoblast mantles are obvious (Fig. 2E). These are derived from the presomite material that is amply present in the truncated fate map of the unincubated chicken blastoderm (Fig. 2A). Laterally, these mesoblast mantles end abruptly near the cut edge of the blastoderm (Fig. 2E). Since neither junctional endoblast nor lateral plate anlage is present and no regulation occurs, no blood islands or coelomic vesicles develop

more laterally, which explains the absence of area vasculosae laterales (Fig. 2C). By contrast, more caudally, blood islands and associated coelomic vesicles are present, forming the caudal area vasculosa. In some bilaterally-truncated blastoderms, after prolonged culture, the cranial part (with neurulation phenomena) and the caudal part (with reduced gastrulation and coelomo-cardiovascular phenomena) are only joined by a narrow tissue bridge along which individual somites can be seen bilaterally (Fig. 2F). The morphogenetic movements that occur in bilaterally truncated blastoderms after culture are represented in Fig. 2G. The peripheral anlage fields (definitive endoderm, cardiovascular tissue, lateral plate and finally somites) in the early incubated chicken blastoderm are successively induced point by point by the Rauber - Koller's sickle material in their immediate neighbourhood by sliding along its concavity (CALLEBAUT et al., 1996; CALLEBAUT et al., 2003a) and finally they ingress in the primitive streak. This results in the so-called "polonaise" movement of the upper layer cells in the area centralis (GRÄPER, 1929; WETZEL, 1929; PASTEELS, 1937). Since the bulk of the lateral plate anlagen and cardiovascular system are localized in the immediate neighbourhood of the inducing sickle horns (Figs 1A; 3), it is quite obvious that in the absence of sickle horns and associated lateral plate upper layer cells, no coelomo-cardiovascular system (area vasculosae lateralis) can develop. Thus our experiments indicate that the median part of Rauber - Koller's sickle alone can not regulate the formation of a whole embryo. Thus no reorganization takes place in the remaining upper layer after unilateral or bilateral removal of the sickle horn regions.

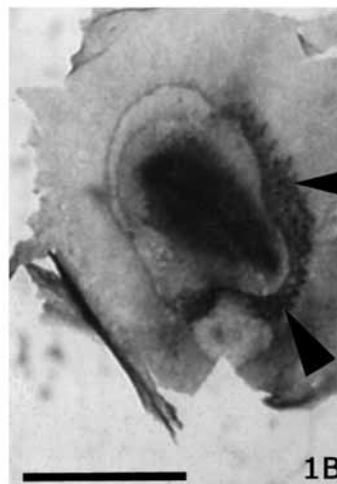
## DISCUSSION

The sickle horn regions have thus an indispensable role in the development of the area vasculosae laterales, which give rise to the latero-cranial part of the coelomo-cardiovascular system including the heart anlage. Indeed, bilateral removal of the sickle horn regions and neighbouring lateral blastoderm parts (experiment 2) results in the absence of a coelomo-cardiovascular system in the embryo. Thus, no heart develops when no sickle horn regions are present. Moreover, our experiments suggest that a local, direct influence (by positional information; CALLEBAUT et al., 2003a) of the sickle horns on the closely upper layer cells (anlage of the lateral plates) already exists at the unincubated stage. The different parts of Rauber - Koller's sickle behave as an early preformed skeleton, and by positional cues, build up the whole blastoderm (CALLEBAUT et al., 2003b). That the median massive part of Rauber - Koller's sickle induces blood and large blood vessels but no heart (comparable with the area vasculosa caudalis) has been observed after transplantation of the median part of Rauber - Koller's sickle (responsible for the formation of the area vasculosa caudalis) on the isolated uncommitted upper layer of the anti-sickle region (CALLEBAUT et al., 2002b). Also, LOPEZ-SANCHEZ et al. (2001) found lateral plate and extraembryonic mesoderm but no heart precursor cells in the most caudal median region of the intermediate primitive streak chicken blastoderm. Indication of the performed surgical

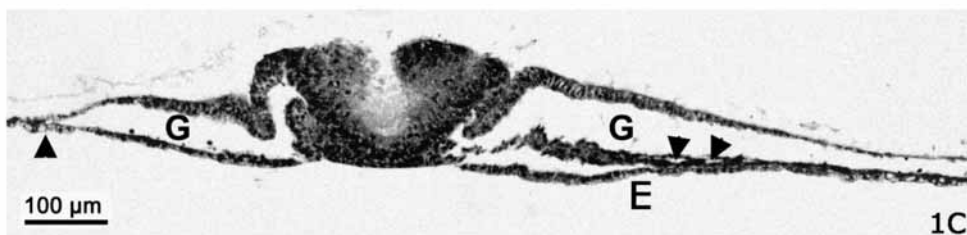




1A



1B



1C

Fig. 1A. – Procedure of the unilateral excision of a sickle horn region and neighbouring lateral tissue, schematically represented on the map of the anlage fields in the upper layer of an unincubated chicken blastoderm (ventral view). The endophyll layer is not represented. AS, anti-sickle region; I.E. ECT, intraembryonic ectoderm; SOM, somites; CAM, caudal marginal zone.

Fig. 1B. – Blastoderm treated as represented in Fig. 1A, after 28h of culture, fixation and *in toto* staining with Unna. A normal area vasculosa caudalis (caudal arrowhead) and area vasculosa lateralis (lateral arrowhead) are seen in the unoperated side. At the operated side no area vasculosa lateralis is seen as the consequence of the removal of the Rauber's sickle horn in this region and thus no heart can be formed in this side. Bar=2mm.

Fig. 1C. – Section through a similar embryo after 28h of culture. G, intraembryonic cavity closed at the operated side (indicated by the arrowhead directed upwards) contains no mesoblast. In the unoperated side the intraembryonic cavity (G) extends far peripherally. It contains a mesoblast mantle, which, laterally, forms blood islands close to the endoderm (E) that are domed by coelomic vesicles (indicated by 2 arrowheads directed downwards). Bar=100μm.

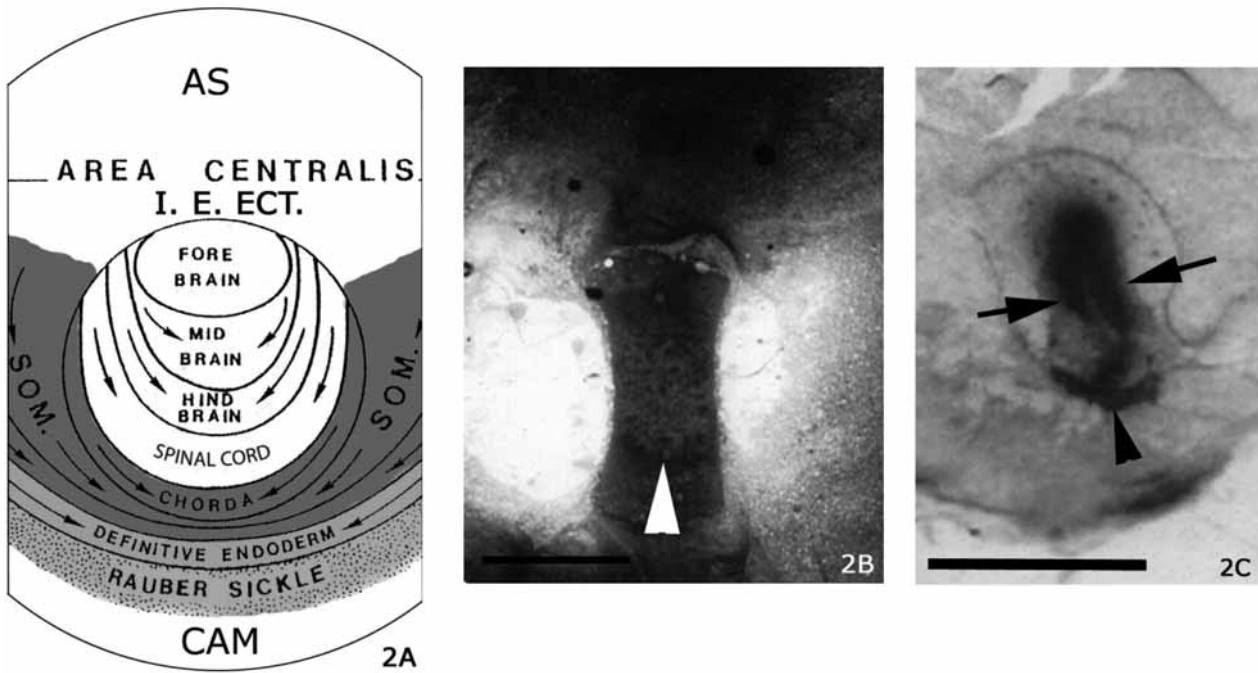


Fig. 2A. – Procedure of the bilateral removal of the sickle horn regions and neighbouring lateral tissue, schematically represented on the map of the anlage fields in the upper layer of the unincubated blastoderm (ventral view) (identical indications as seen in Fig. 1A).

Fig. 2B. – Unincubated blastoderm treated as represented in Fig. 2A., at the start of the culture. White arrowhead indicates median part of Rauber's sickle. Bar=2mm.

Fig. 2C. – The same blastoderm after 30h of culture after fixation and *in toto* staining with Unna. Bilaterally no area vasculosae laterales and thus no heart tubes have formed because both sickle horn regions have been removed and no junctional endoblast has developed. Only the caudal area vasculosa (arrowhead) has developed because the median part of Rauber's sickle remained intact. In the middle region of the embryo proper, two paraxial denser zones of presomitic material (bilaterally indicated by two arrows) are seen; these correspond to the two paraxial tissue condensations seen in sections (Fig. 2E). Bar=2mm.

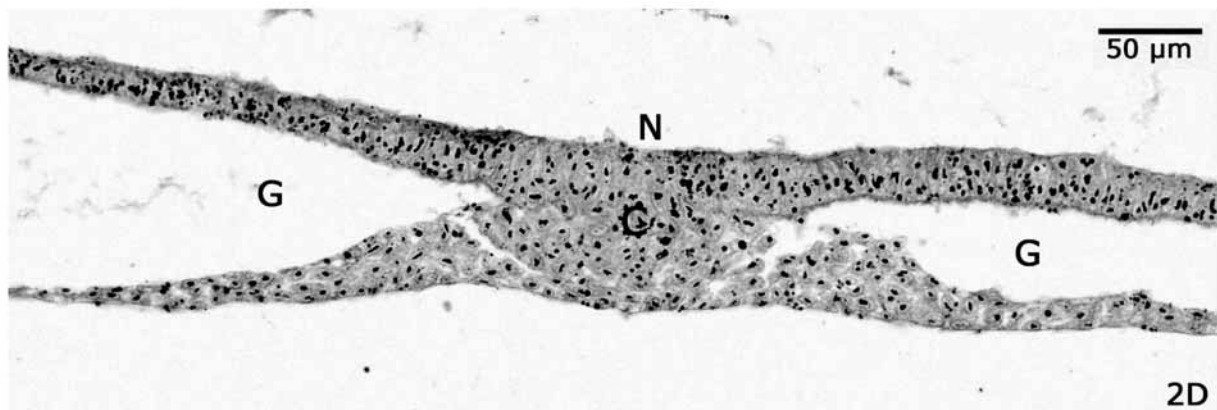


Fig. 2D. – Section through the cranial part of the embryo seen in Fig. 2C; N, neural plate; C, prechordal plate and notochord; G, intraembryonic cavity. Heidenhain hematoxylin. Bar=50μm.

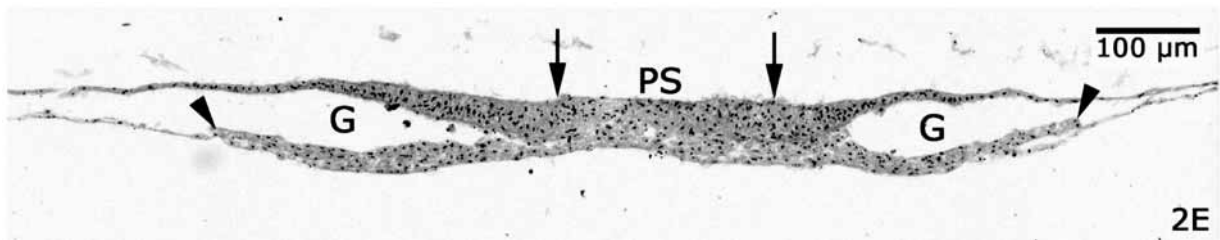


Fig. 2E. – Section through the middle region of the embryo seen in Fig. 2C. The lateral arrows indicate paraxial condensations of tissue (probably presomite material) laterally from an atypical primitive streak (PS). Arrowheads indicate the lateral cut ends of the mesoblast mantle in the intraembryonic cavity (G). More laterally neither blood islands nor coelomic vesicles are present, because the junctional endoblast (normally derived from the Rauber's sickle horns) is absent. Heidenhain hematoxylin. Bar=100 $\mu$ m.

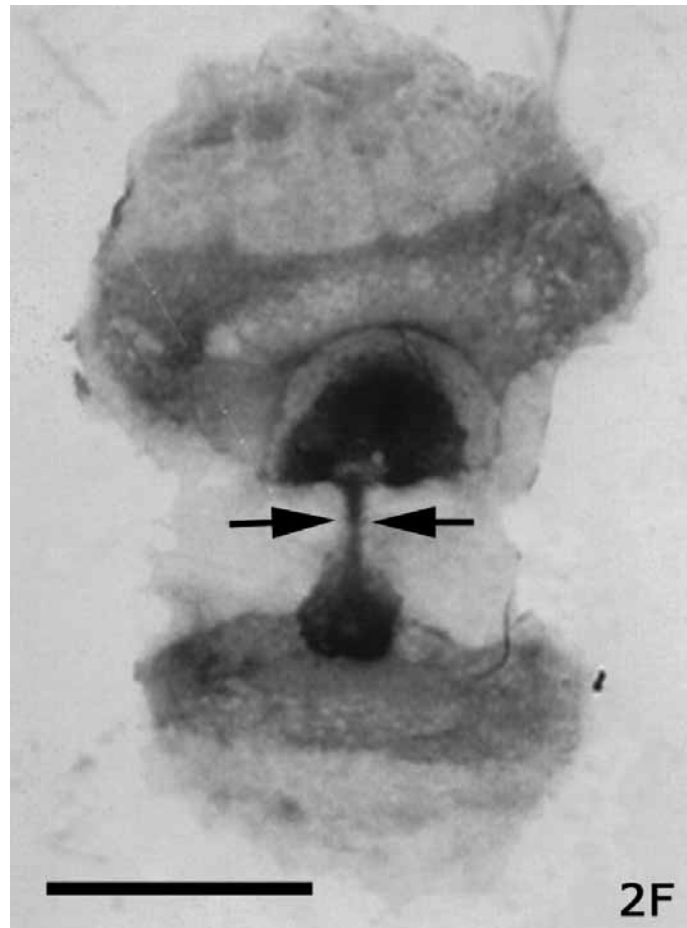


Fig. 2F. – Chicken embryo operated as represented in Fig. 2A after 28h of culture (fixation and Unna staining). A narrow bridge (indicated by arrows) has formed between the cranial and caudal part of the embryo. Bar=2mm.

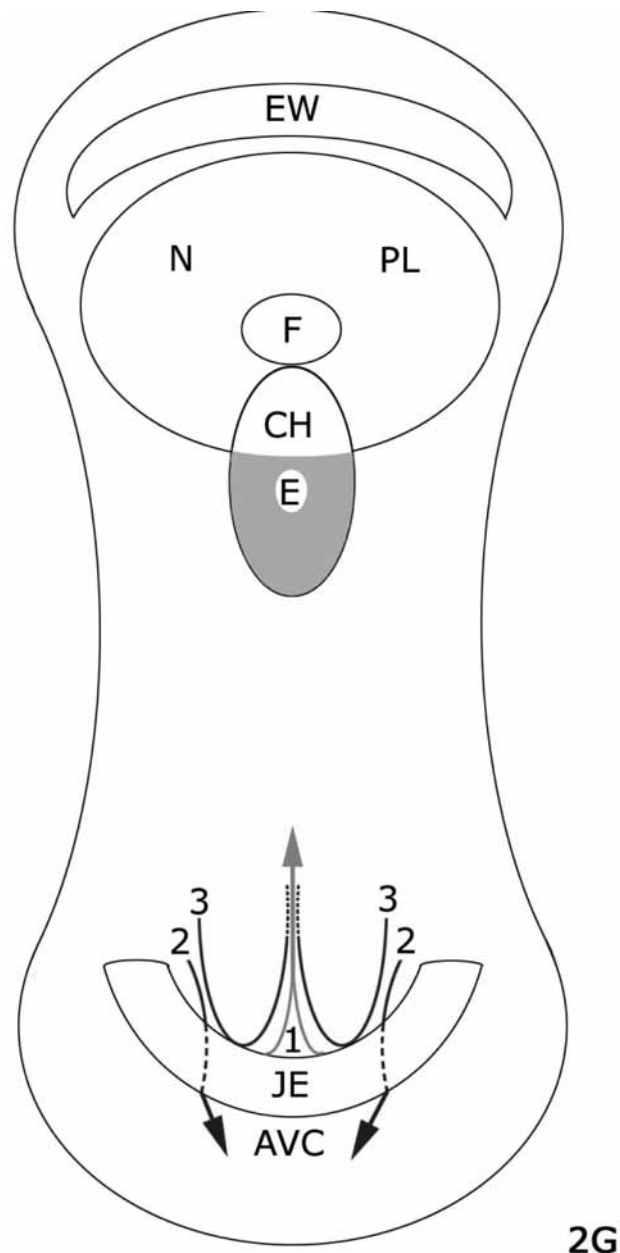


Fig. 2G. – Schematic representation (ventral view) of the successive migration pathways (1-3) of the different cell groups in bilaterally truncated embryos after the excisions represented in Fig. 2A. 1: Bilateral “polonaise” movement (GRÄPER, 1929): the cells of the anlage field of the definitive endoderm ingress into the primitive streak, migrate cranially (median arrow) and become finally localized in E (endoderm). The anlage fields of the so-called midline structures; floorplate (F), notochord (CH), are also displaced cranially. (N PL) neural plate; (EW) endophyll wall. 2: Remainder of the lateral plate mesoderm sliding medially between remainder of the junctional endoblast (JE) and the epiblast, forming the area vasculosa caudalis (AVC). 3: Finally the “polonaise” movement (GRÄPER, 1929) of the anlage field of the paraxial mesoderm, forming somites (interrupted lines).

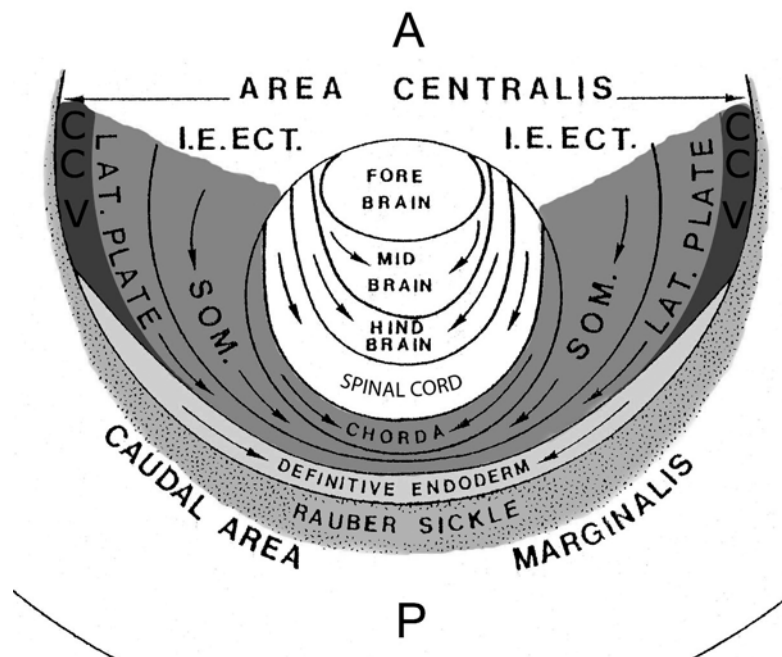


Fig. 3. Adapted schematic representation of the main localization of the predisposed anlage fields in the upper layer of a chicken unincubated blastoderm (ventral view, endophyll not represented). CV, anlage of the coelomo-cardiovascular system localized between definitive endoderm and lateral plate anlagen.

ablations on the map of the anlage fields of the unincubated chicken blastoderm (according to CALLEBAUT et al., 1996; CALLEBAUT et al., 2000), allows the evolution of the remaining blastoderm parts after culture to be better followed, understood and sometimes predicted. In this anlage field map, we could now add the localization of the earliest anlage field of the cardiovascular system (Fig. 3).

In his fate map of birds (unincubated blastoderm stages; -1,0,1), VAKAET (1985) localized the area vasculosa (extraembryonic mesoblast, according to him), outside the area centralis, thus already early *in utero*, exterior to the Rauber - Koller's sickle. According to our experiments, however (CALLEBAUT et al., 2002a; b; 2004), the area vasculosa (coelomo-cardiovascular system in our terms) is only formed much later, after primitive streak formation and by peripheral migration of cells of the mesoblast mantle below the epiblast and over the junctional endoblast, under inductive influence of the latter. That the coelomo-cardiovascular system (typically found in coelomates; DOLLANDER & FENART, 1973), forms one embryological entity has been demonstrated in avian blastoderms (CALLEBAUT et al., 2002a; 2004). Thus, the coelomo-cardiovascular system develops both phylogenetically and ontogenetically later than the general body structures. It is part of three associated elements; endoderm, blood islands and coelomic epithelium. Later, even in adult structures, this epithelial covering of coelomic organs can contribute to the vasculature of the heart (MIKAWA & GOURDIE, 1996; MÄNNER, 1999) or, in the intestinal tract, by developmental processes of epithelial-

mesenchymal transition, migration and differentiation into vascular endothelial cells, vascular smooth muscle cells and pericytes (KAWAGUCHI et al., 2007). We observed the effects of the most cranially- and laterally-extending parts of the Rauber - Koller's sickle (later junctional endoblast) on the formation of the hemi-pericardial cavities and endocardium on both sides. The far-extending lateral localization of the sickle horns is probably the reason for the original double anlage of the heart. At stage 7 (HAMBURGER & HAMILTON, 1951) (23-26h incubation) the first reported markers of terminal myocardial differentiation were detected in the primary heart fields (BISAHA & BADER, 1991; HAN et al., 1992). MARTINSEN (2005) localizes gastrulated precardiac cells at stage 7 (HAMBURGER & HAMILTON, 1951) in an elongated, sickle-shaped region that corresponds to the also-sickle-shaped region at the lateral border of the embryo proper, i.e., at the inner limit of the area vasculosa (CALLEBAUT et al., 2002a; 2004).

#### Heart and pericard development

LUTZ (1949) described experiments in which the median part of unincubated chicken blastoderms had been transplanted on a chorioallantoic membrane and cultured *in ovo* for a prolonged growth period. After histological examination of the graft, he found axial structures; nervous system, notochord, pharynx and hypophysis - but no heart. By contrast, grafts of lateral parts of unincubated blastoderms developed rudimentary hearts. SPRATT & HAAS (1960) excised triangular caudal segments (less than one fourth of the whole surface of unincubated blast-

oderms). The segments were cultured. Histological examination revealed that these explants formed embryonic axial systems, many of which were bilaterally-symmetrical bodies containing brain, spinal cord, notochord, pairs of somites but obviously no heart. We can explain the absence of heart formation in both mentioned cases in view of our present results. Indeed, we demonstrated here that in the absence of sickle horns no area vasculosae laterales developed and thus no coelomo-cardiovascular system appeared, at that level leading to the absence of heart and pericard formation. We made a distinction in localization and function between the area vasculosae laterales (extending cranially, giving rise to the primary heart tubes) and the area vasculosa caudalis. This suggests some similarity with the two spatially-distinct populations of progenitors for blood and endothelial cells described in developing *Xenopus* embryos by WALMSLEY et al. (2002). The first population gives rise to embryonic blood and vitelline veins and to the endocardium of the heart in the anterior ventral blood island. The second population resides in the dorsal lateral plate mesoderm and contains precursor adult blood stem cells and the major vessels.

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## SHORT NOTES

## Non-breeding reed warblers *Acrocephalus scirpaceus* (Hermann, 1804) in June in southern Europe: local or still migrating birds?

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Accurate estimations of population structures for breeding birds are essential for assessing population-specific parameters, such as adult survival rates, breeding site fidelity or productivity (1-3). However, not all adult birds observed in a certain breeding area should be considered as breeders: non-breeding local birds and, in migrant species, individuals that are still migrating could also be present. Taking the proportions of birds in these categories into account is crucial for estimating unbiased population-specific parameters (4; 5).

In migrant bird species with large distribution areas, the overlap between local breeding birds and still-migrating ones is frequent, particularly at low latitudes (6-8). Compared to non-migrants (e.g., breeding birds), migrants normally show higher mean fuel loadings, i.e., higher energy stores that are used during the migratory flights (9). Birds tend to have longer and more pointed wings as well as shorter tails related to body size with increasing migration distances (10; 11) as is confirmed in several species of Holarctic passerines (12-15). Therefore, both a higher mean fuel load and different morphologies more resembling the long-distance-type in non-breeders may indicate that these birds are not local but still migrating birds.

Floaters present the non-territorial fraction of a territorial population (16) and are often older, less-experienced or handicapped birds (17), that would normally not breed. Because of their transient behaviour, they are less likely to be recaptured (4). The reed warbler *Acrocephalus scirpaceus* (Hermann, 1804) is a widespread breeding songbird in western Europe that passes the winter in tropical Africa (7). In spring, reed warblers reach southern Europe by late-March or April (7). Thus, most (if not all) adults should have reached their breeding areas by June, at least in southern Europe (18). The question whether some northern European birds that are still migrating could be found in southern European breeding areas has rarely been investigated. Our aim here was to determine whether non-breeding reed warblers captured in June in southern Europe were local or migrating birds, by considering traits such as fuel load, flight-associated morphology and recaptures.

Reed warblers were captured in Jaizubia (43°21'N, 01°49'W, Fuenterrabía, N Iberia), a 38.1ha small tidal

river area located in the Txingudi marshlands. Birds were captured with mist nets (240 linear m) placed across reed beds and opened for four hours starting at dawn, during 10 consecutive days in June (05.06–14.06), July (10.07–19.07), and August (07.08–16.08) 2006. Once captured, reed warblers were ringed (or the ring was read) and their age was determined as adults (second-year birds or older, EURING code 4) or juveniles (first-year birds, EURING code 3). The following variables were also recorded: wing length (method III,  $\pm 0.5$ mm; 19), tail length ( $\pm 0.5$ mm), length of primary feathers P2 to P9 (from outermost to innermost,  $\pm 0.5$ mm; P1 is vestigial), body mass (TANITA digital balance,  $\pm 0.1$ g) and fat score (scaled from 0 to 8; 20). Finally, we checked whether birds had brood patches (females; the brood patch is used for incubating the eggs, has no feathers and is highly vascularized) or cloacal protuberances (males, scaled from 0 to 9 (21)). We only investigated adult birds because our aim was to analyse the characteristics of non-breeding birds in a breeding population.

Flight-associated morphology (wing length, wing morphology, tail length/wing length) and fuel load (body mass/wing length and fat score; here, wing length is used as a proxy of body size; 22) were compared between breeding and non-breeding adult reed warblers captured in June. A bird was considered as breeder if it showed apparent breeding signs in June, July, or August. Because data on flight-associated morphology and body mass/wing length fitted the normal distribution (K-S test,  $P > 0.05$ ),  $t$ -tests were used for statistical comparisons. In contrast, fat score was not normally distributed (K-S test:  $Z = 2.466$ ,  $P < 0.001$ ), which is why a  $U$  test was applied. In order to avoid pseudo-replication, each individual was considered only once.

Wing morphology was assessed by the  $C_2$  index which expresses wing pointedness (23). Positive values of the index are associated with rounded wings, while negative values are associated with more pointed wings.

Of the 77 adults captured in June, 47 (61.0%) showed signs of breeding (either in June or later, in July or August), and were considered to be breeders. This value is similar to that found in some Nearctic passerines (4) and reinforces the idea that a significant fraction of adult birds in a certain local population consists of non-breeders during the breeding season (24).

Breeding and non-breeding reed warblers captured in June had the same flight-associated morphology but differed in relation to their fuel load (Table 1). This supports



the hypothesis that non-breeders may be either local birds or non-local birds from populations breeding in areas relatively close to Txingudi, because if these were birds from more northern areas, we would expect more pronounced long-distance morphology (13). A higher fuel load in non-breeders may indicate that they are still migrating birds breeding in areas close to Txingudi. Alternatively, these individuals could be local, either birds arriving later in their breeding areas (with some remaining fuel accumulated for the spring migration period, from Africa to Europe) or birds starting to gain the fuel required for the migration beginning in summer (from Europe to Africa).

None of the non-breeding birds captured in June was recaptured afterwards, whereas 40.4% of breeders at Txingudi were recaptured in July or August ( $\chi^2_1=16.101$ ,  $P$ -exact<0.001; Table 2). This confirms that the recapture probability was relatively high throughout the season and suggests that non-breeders did not remain at Txingudi for a long time. This result is consistent with the idea that non-breeders could either still be migration birds or local transient birds (floaters), unable to defend a territory (25; 4).

The presence of non-breeders can be very important at a local level, and in our opinion, this should be considered in studies dealing with the dynamics of breeding bird populations. This is particularly relevant in programs surveying parameters such as annual survival, population size or productivity (1). All of these parameters are fundamental to our understanding of population trends in particular species and, therefore, are also directly linked to the conservation of biodiversity.

TABLE 1

Differences in flight-associated biometrics and fuel load (mean±SE) between breeding and non-breeding adult reed warblers captured in June in the Txingudi marshlands (northern Iberia).

Variable	Breeding	Non-breeding	Statistics	P
Wing length	63.9±0.3 (N=47)	63.9±0.4 (N=30)	$t_{75}=0.011$	0.991
Wing morphology (roundness)	0.75±0.02 (N=43)	0.73±0.02 (N=29)	$t_{70}=0.948$	0.346
Tail length/Wing length	0.84±0.01 (N=47)	0.84±0.01 (N=30)	$t_{75}=0.428$	0.670
Body mass/Wing length	0.16±0.01 (N=47)	0.17±0.01 (N=30)	$t_{75}=2.830$	0.006
Fat scores	0.7±0.1 (N=43)	2.5±0.2 (N=29)	$U=199.0$	<0.001

TABLE 2

Number of breeding and non-breeding adult reed warblers captured at Txingudi in June and recaptured at the same site in July or August.

	Captured only in June	Captured in June and July/August
Breeding	28	19
Non-breeding	30	0

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## Aphids on *Ammophila arenaria* in Belgium: first reports, phenology and host range expansion

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This paper reports the discovery of three species of aphids (Hemiptera: Aphidoidea) previously unknown to occur in Belgium (7), namely the species *Schizaphis rufula* (Walker 1849), *Laingia psammae* (Theobald 1922) and *Metopolophium sabiniae* (Prior 1976). All species were collected on the dune grass *Ammophila arenaria*. (L.) Link, a dominant grass species in sand dunes along all European coasts south of latitude 63°N (6). This plant grows most vigorously in the open habitat of mobile dunes where it regularly gets buried by windblown sand (1). It also tolerates the extreme exposure and wide fluctuations of soil temperature typical of this habitat (6). Based on morphology and distribution, two subspecies can be distinguished. *Ammophila arenaria arenaria* is present along the European North Atlantic coast, whereas *A. arenaria arundinacea* occurs at southern Atlantic latitudes and along the Mediterranean (9).

*S. rufula* has been described across Europe, including Britain, Corsica, Denmark, Finland, Germany, Ireland, Poland, Sicily, Sweden, the Netherlands and Ukraine (8). This species is known to live on leaves of *A. arenaria* and *Elymus arenarius* in sand dune areas, where leaves of these plants can turn yellow as a result of the infestation (3). During a large field survey in 2007 at six spatially-separated locations within the Flemish nature reserve Westhoek, this species was caught in large numbers. We sampled plants by sweep-netting, which yielded up to 485 individuals on a single plant. A field survey was conducted in the same nature reserve in the summer of 2008 with 60 *A. arenaria* plants belonging to the two subspecies. Again, *S. rufula* was the most numerous aphid species encountered. In both years this species of aphid was present from June onwards during the whole summer, in 2007 even remaining present up to October (Figs 1 & 3).

*L. psammae* is known from Britain, Bulgaria, Czech Republic, Denmark, Finland, Germany, Hungary, Poland, Romania, Russia, Sicily, Slovakia, Spain, Sweden, the

Netherlands and Ukraine (8). The aphids are known to live between the flowers or fruits, in the inflorescences of *A. arenaria* and *Calamagrostis epigeios*. In Sweden, the species is also collected on plants of the genus *Elymus*, *Calamagrostis arundinacea* and *Deschampsia caespitosa* (2). Interestingly, we encountered this species quite often on plants with no inflorescences, living on the leaves. This species was a lot less abundant than *S. rufula* during the 2007 sampling (Fig. 2), but in the field survey of 2008 the species was better represented (Fig. 3). It seems to be reaching peak densities earlier in the season than *S. rufula*.

*M. sabiniae* has up till now only been found in Britain and France (8). The observation of *M. sabiniae* feeding and reproducing on *A. arenaria* is the first (5). This aphid species is known from *Festuca rubra* and *Vulpia membranacea* in dune areas (4). Although of this species only 10 individuals in total were discovered in the 2008 survey (Fig. 3), it was recorded at 14/07, 30/07 and 24/09, suggesting persisting populations rather than spillover from other plant species. Moreover, we were able to breed this species on *A. arenaria* for several generations in the laboratory.

Another noteworthy observation during the same field survey was the development of large populations of the aphid species *Rhopalosiphum padi* (Linnaeus 1758) on *A. arenaria* (Fig. 3). This is to our knowledge the first report of *R. padi* successfully reproducing on *A. arenaria* (5). The primary host of this species is *Prunus padus* and occasionally some other *Prunus* species. The aphids hatch from eggs on these hosts in early spring. In late spring, alate viviparous females colonise secondary hosts. These are various grasses, including cereals such as barley (*Hordeum*), wheat (*Triticum vulgare*) and oats (*Avena sativa*). It occasionally occurs on other monocotyledons and on *Capsella bursa-pastoris*. In early autumn, the species migrates back to the primary host. This aphid species is a cosmopolitan pest that can do much harm to cereals and is a potential vector of plant viruses (3). Therefore this finding might be of some economical relevance, since *A. arenaria* is traditionally planted to stabilise sand and to control erosion, for example to prevent flooding (10).

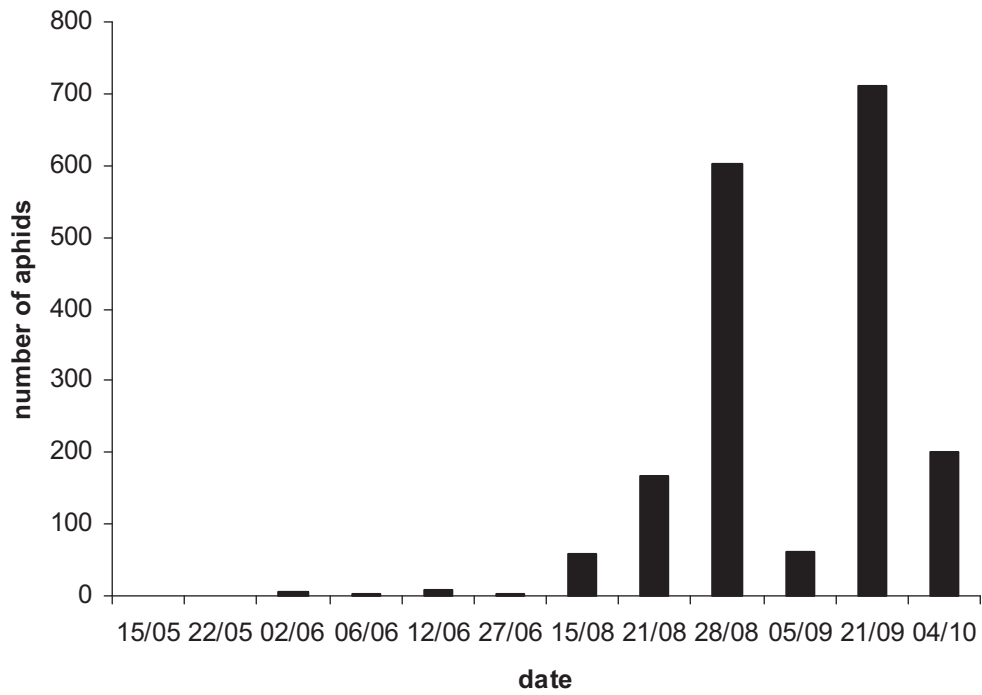


Fig. 1. – Phenology of *S. rufula* in the year 2007 based on sweep netting catches at different field sites.

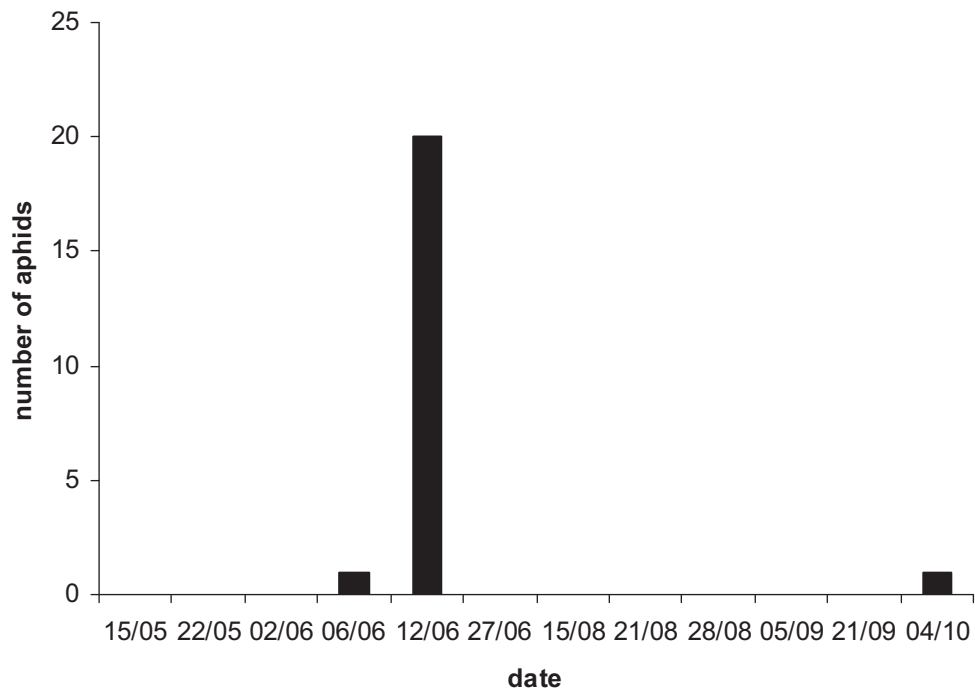


Fig. 2. – Phenology of *L. psammae* in the year 2007 based on sweep netting catches at different field sites.

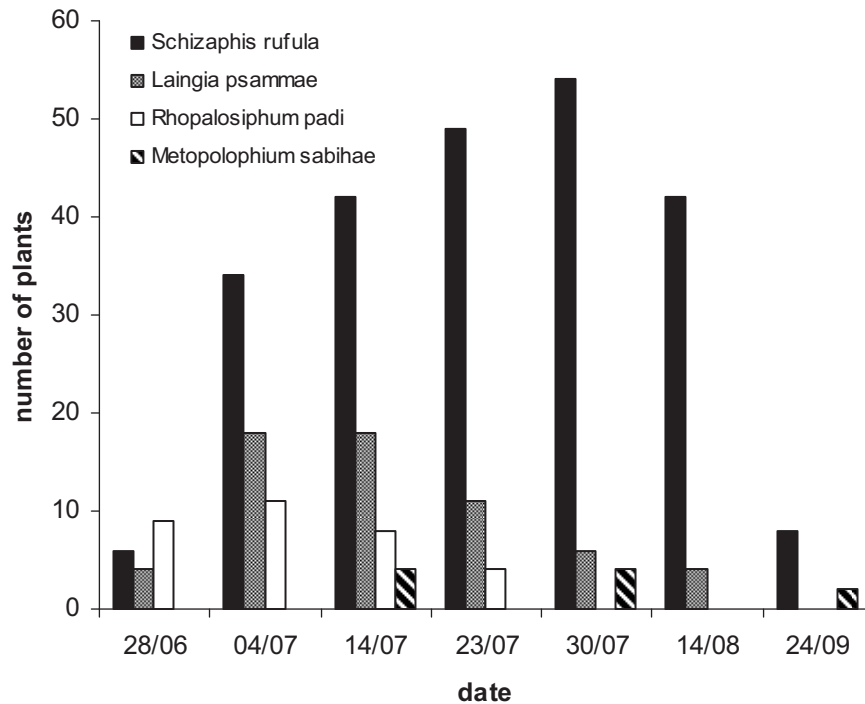


Fig. 3. – Phenology of *S. rufula*, *L. psammae*, *R. padi* and *M. sabiniae* in the year 2008 based on the number of plants out of 60 replicates, on which the aphid species was recorded during a field survey.

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