Hydrobiologia 175: 237–256, 1989

On the morphology and karyology of the genus *Archilopsis* (Meixner) (Platyhelminthes, Proseriata)

Paul M. Martens[1], Marco C. Curini-Galletti[2] & Ileana Puccinelli[3]
[1]*Research group Zoology, Department SBM, Limburgs Universitair Centrum, Diepenbeek, Belgium*; [2]*Dipartimento di Scienze dell'Ambiente e del Territorio, Università di Pisa, Pisa, Italy*; [3]*Istituto di Zoologia ed Anatomia Comparata, Università di Pisa, Pisa, Italy*

Received 7 October 1987; in revised form 20 January 1988; accepted 10 March 1988

Key words: Platyhelminthes, Proseriata, Archilopsis, karyology, phylogeny

Abstract

Two new *Archilopsis* species are described: *Archilopsis marifuga* sp.n. and *Archilopsis arenaria* sp.n.; *Archilopsis unipunctata* (Fabricius, 1826) and *Archilopsis spinosa* (Jensen, 1878) are redescribed. The latter taxon is re-established. The descriptions are based on morphological and karyological data. The four species of the genus can unequivocally be recognized by the morphology of the cirrus and the presence or absence of a stylet.
The genus *Archilopsis* is regarded as a monophyletic group with at least two autapomorphies. The relationship between the four species is discussed and a cladogram for the genus proposed. The existence of two sistertaxa (each with two species) is recognized.

Introduction

Fabricius (1826) described *Planaria unipunctata* on very superficial characteristics (according to our present criteria). It was redescribed and transferred to the genus *Monocelis* by Oersted (1844). Claparède (1861) described specimens (as *M. unipunctata*) which deviate from the descriptions of Fabricius and Oersted, while Jensen (1878) described a new species, *M. spinosa*, on the basis of some features not present in the descriptions of *M. unipunctata*. Nonetheless, this species was synonymized with *M. unipunctata* by von Graff (as *Automolus unipunctata* in 1882, as *M. unipunctata* in 1913), while Maristo (1938) on the other hand regarded only *M. spinosa* Jensen, 1878 as a valid species. Finally, Meixner (1938) introduced the new genus-name *Archilopsis* with the single species *A. unipunctata* (Fabricius, 1826) (see also Ax, 1951). Two more descriptions must be taken into consideration: Luther (1960) and Karling (1974: photograph on p. 95 of which the material was available for our study).

While studying the Turbellaria on the Belgian, Dutch and Northern French coasts we found two species which both fitted the descriptions of *A. unipunctata*. A careful morphological (on light- and electronmicroscopic level) and a karyological analysis confirmed that two different species were involved indeed. In the neighbourhood of the Huntsman Marine Laboratory (St. Andrews, Passamaquody Bay, Canada) we found a third species, different from the former two but still fitting the descriptions of *A. unipunctata*. Finally we received from Dr. B. Sopott and Dr U. Noldt (Göttingen) slides of specimens labeled *A. uni-*

Table 1. Sampling places, period, collectors and collected material of the populations studied by the Authors.

Locality	Period	Collector	Material
Archilopsis unipunctata			
Iceland:			
Eyjafjördur	July 1947	Westblad	1 specimen serially sectioned
Sweden:			
Söderby	June 1940	Bock	2 specimens serially sectioned
Vettersö	July 1940	Bock	2 specimens serially sectioned
Gälnan	July 1940	Bock	1 specimen serially sectioned
Gullmmaren		Karling	1 whole mount
Kristieneberg	June 1969	Schockaert	1 whole mount
Kristieneberg	July 1985	Schockaert	4 whole mounts
Kristieneberg	July 1986	Schockaert	5 whole mounts
			5 specimens for karyology
Finland:			
Hagnö	March 1940	Karling	1 specimen serially sectioned
Germany:			
Sylt	Febr. 1984	Noldt	2 whole mounts
Canada:			
Passamaquoddy bay (St. Qndrews)	August 1984	the authors	1 specimen semi-thin sectioned 1 specimen for karyology 4 whole mounts
Archilopsis arenaria			
Belgium:			
De Panne	Aug. 1981	the authors	1 specimen serially sectioned
De panne	May 1983	the authors	3 specimens for karyology
			1 specimen semi-thin sectioned
Oostende	May 1983	the authors	4 specimens for karyology
Zwin (beach)	May 1983	the authors	10 specimens for karyology
Heist	Oct.-Febr. 1983/84	Jouk	10 whole mounts
Zwin (beach)	Jan., March 1984	Revis	10 whole mounts
Germany:			
Sylt	Sept. 1985	the authors	10 specimens for karyology
France:			15 whole mounts
Roscoff	July 1986		
		the authors	6 specimens for karyology
Archilopsis marifuga			
Belgium:			
Zwin (lagoon)	May 1983	the authors	4 specimens for karyology
Zwin (lagoon)	Sept.-Apr. 1983/84	Revis	3 whole mounts
Netherlands:			
Texel	June 1982	the authors	1 whole mount
France:			
Ambleteuse (estuary of the Slack)	May 1979	E. Martens	2 specimens semi–thin sectioned 10 specimens serially sectioned
	May 1984	the authors	4 whole mounts
			2 specimens for karyology
Canada:			
Passamaquoddy bay (St. Andrews)	August 1984	the authors	2 whole mounts 2 specimens for karyology
Archilopsis spinosa			
England:			
Plymouth	?	Capstick	2 specimens serially sectioned
Germany:			
Sylt	June 1985	Noldt	3 whole mounts
Sylt	Sept. 1985	the authors	2 specimens serially sectioned
			1 specimen semi-thin sectioned
			9 whole mounts
			9 specimens for karyology
France:			
Roscoff	July 1986	the authors	6 specimens for karyology

punctata collected at Sylt (Germany). These specimens appeared to be different from the three other species we had studied so far.

It has thus become clear that *Archilopsis unipunctata* (Fabricius, 1826) actually consists of a complex of at least four species. Those species are here described and for two of them a new taxon is proposed.

Material and methods

Of the four species involved as many specimens as possible from various localities have been studied alive, in whole mount, in sectioned material and from a karyological point of view (Table l). The *Archilopsis* material of the Swedish Museum of Natural History has been reviewed. Of two species also E.M. data are available and elsewhere presented (E. Martens & Schockaert, 1981 and E. Martens, 1986, see discussion).
Animals were extracted from the sand with the MgCl2 decantation technique (see P. Martens 1984a). Specimens studied alive were preserved as whole mounts with lactophenol or polyvinyl-lactophenol. Specimens for paraffin sectioning were fixed with Bouin's fluid, sectioned at 5 μm and stained with iron hematoxyline – eosine. Mallory's, or Masson's triplestain. Some specimens were fixed with 2% glutaraldehyde, post-fixed with 2% OsO4 (in cacodylate or phosphate buffer) and upon embedded, serially sectioned (with Reichert Ultracut) in 1 μm sections (semi-thin) (alternating with ultra-thin sections).

Figures without a scale are freehand drawings, those with scale were made with the camera lucida. Photographs of the extremely squeezed animals for cirrus and stylet comparisons are all reproduced at the same magnification. Relative pore indices are given according to Karling (1966).

Animals used for karyological study remained alive in 0.2% colchicine for 3–4 hours, than transferred in 2% acetic acid (2 min.) stained in lactic-aceto-orcein and squashed under a coverslip. These preparations have also been used for the study of the morphology of the cirrus (Papi, 1951).

The male germinal line was used for karyological purposes. The haploid chromosome number was ascertained from primary and secondary spermatocytes and the diploid number from spermatogonial mitoses.

Relative lengths (r.l. = length of chromosome x 100/total length of haploid genome) and centromeric indices (c.l. = length of the short arm x 100/length of the entire chromosome) were obtained from measurements of ten metaphase plates in each population, but the karyotype morphology was confirmed by a higher number of plates from different specimens. We have not considered it relevant to report the c.l. values of the acrocentric chromosomes in the four species, as they showed some variations in the different plates, due to the different degree of coiling of the long arm. The short arm can be considered of almost equal length in the various pairs of the set. The idiograms of the four species are based on the mean values of all populations examined. The chromosome nomenclature employed is that of Levan *et al.* (1964).

Type and voucher material is deposited in the zoological collection of the department SBM, Limburgs Universitair Centrum, Diepenbeek, Belgium.

Descriptions

A. Morphology (Figs. 1–8)

The *habitus* of the *Archilopsis* species is that of the majority of the Monocelididae: slender and elongated, 3–4 mm in *A. unipunctata* and *A. spinosa*, 2–3 mm in *A. marifuga* and *A. arenaria*. They are without eyes or pigment. The gut may be light-green to yellowish depending on its content. The anterior tip is slightly narrowing and rounded, with 'olly' droplets in front of that statocyst. The posterior end is rounded and with numerous adhesive papillae.

The *epidermis* in all species is of the insunk type and ciliated over the whole body, only the tail region is bared. Cilia are 2–3 μm in *A. unipunctata* and *A. arenaria*, 4–5 μm in *A. marifuga* and *A. spinosa*. No rhabdite-like inclusions were

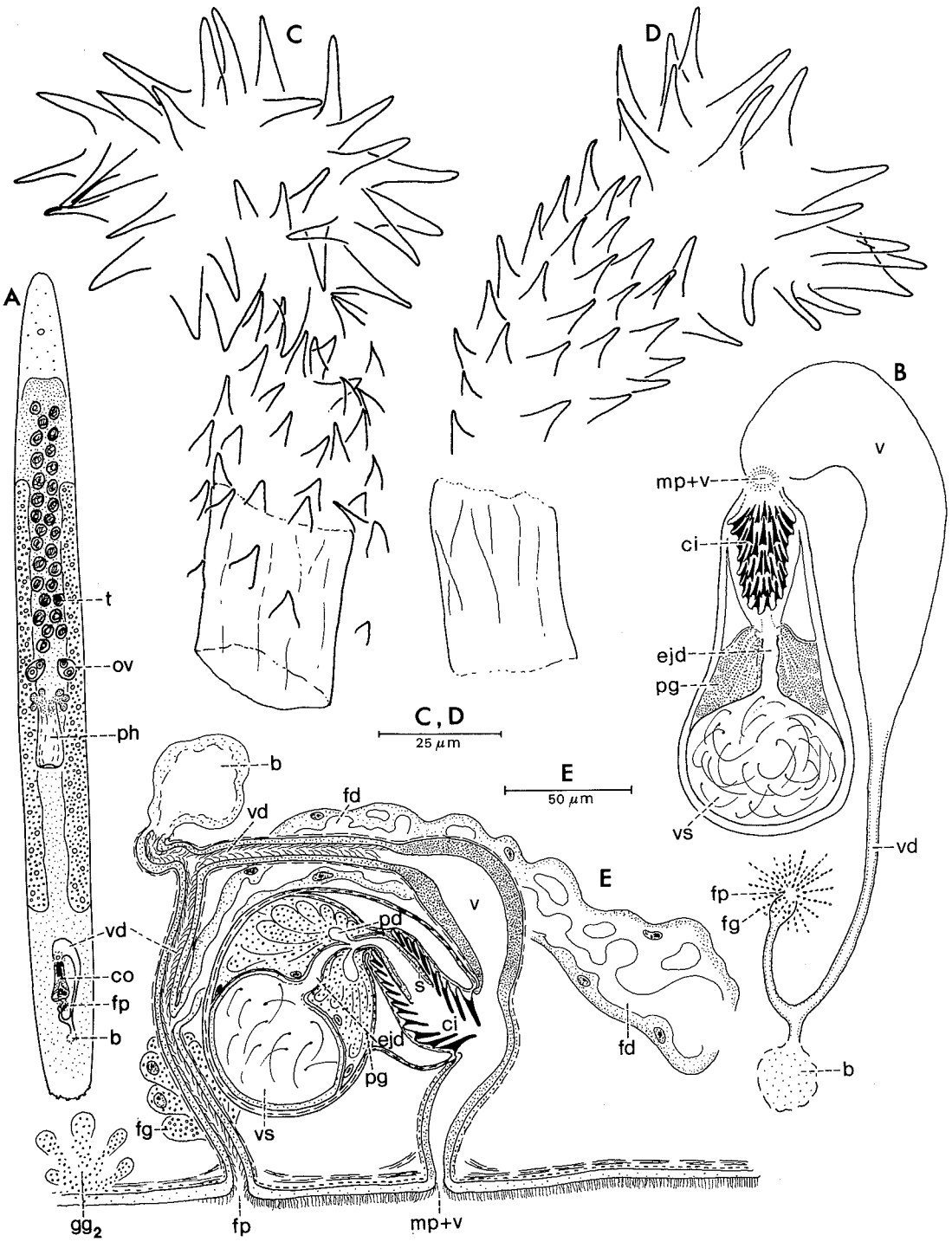

Fig. 1. Archilopsis unipunctata: A. Habitus. B. Copulatory organs (from living animal; stylet not visible). C and D. Cirrus with stylet (from squeezed animals). E. Reconstruction of the genital organs from serial sections (seen from the right).

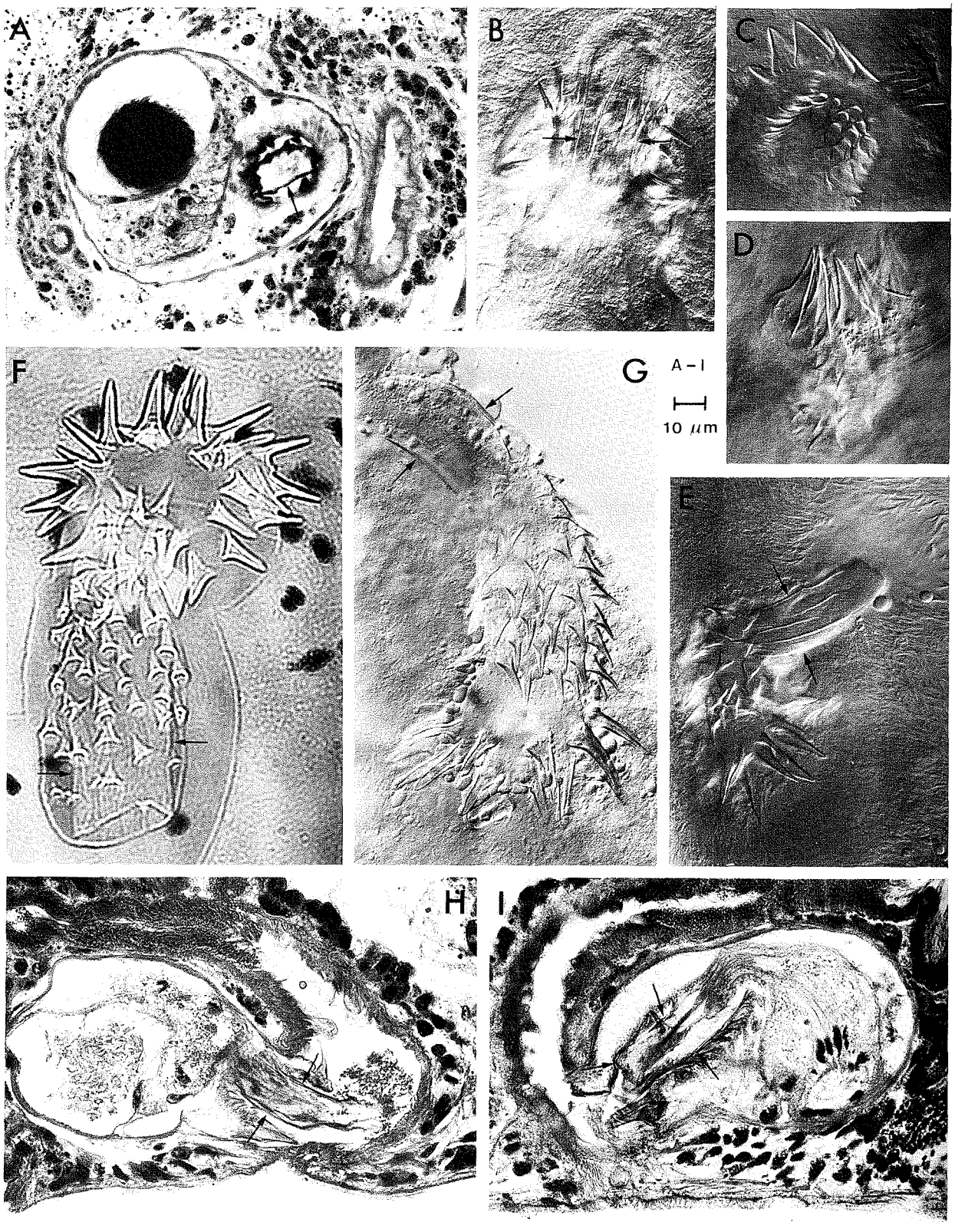

Fig. 2. Archilopsis unipunctata: photographs of the cirrus from different populations. A. Section through the cirrus with stylet (specimen from Eyjafjördur, Iceland). B and E. From Kristinenberg (Sweden). C and D. Sylt (Germany). G. Gullmmaren (Sweden). F. St. Andrews (Canada). H. Vettersö (Sweden). I. Gälnan (Sweden). A,G-I from the collection of the Swedish Museum of Natural History).

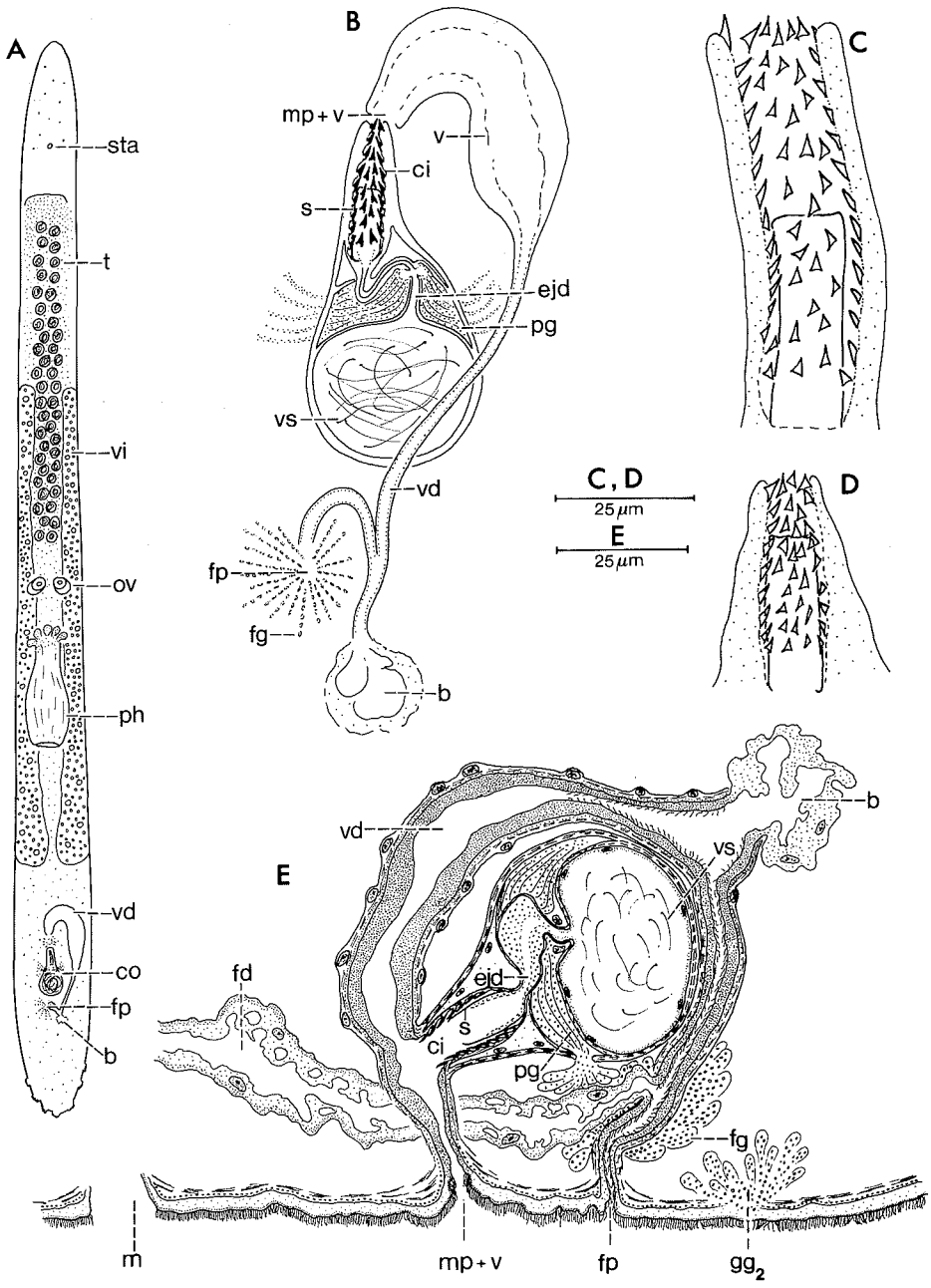

Fig. 3. Archilopsis arenaria: A. Habitus. B. Copulatory organs (from living animal). C and D. Cirrus with stylet (from squeezed animals). E. Reconstruction of the genital organs, from serial sections (seen from the left).

observed within the epidermis. In addition to the adhesive organs three kinds of glands (or glandular organs) associated with the epidermis can be found in sectioned material: (1) numerous small elongated sack-like glands with a fine eosinophilous secretion in the anterior body part (very small in *A. arenaria*), (2) a group of eosinophilous glands (gg_2) behind the female pore ('kittdrusenkomplex' (Ax, 1959a)) and (3) brown to yellowish stained ovoid vesicles with a flat

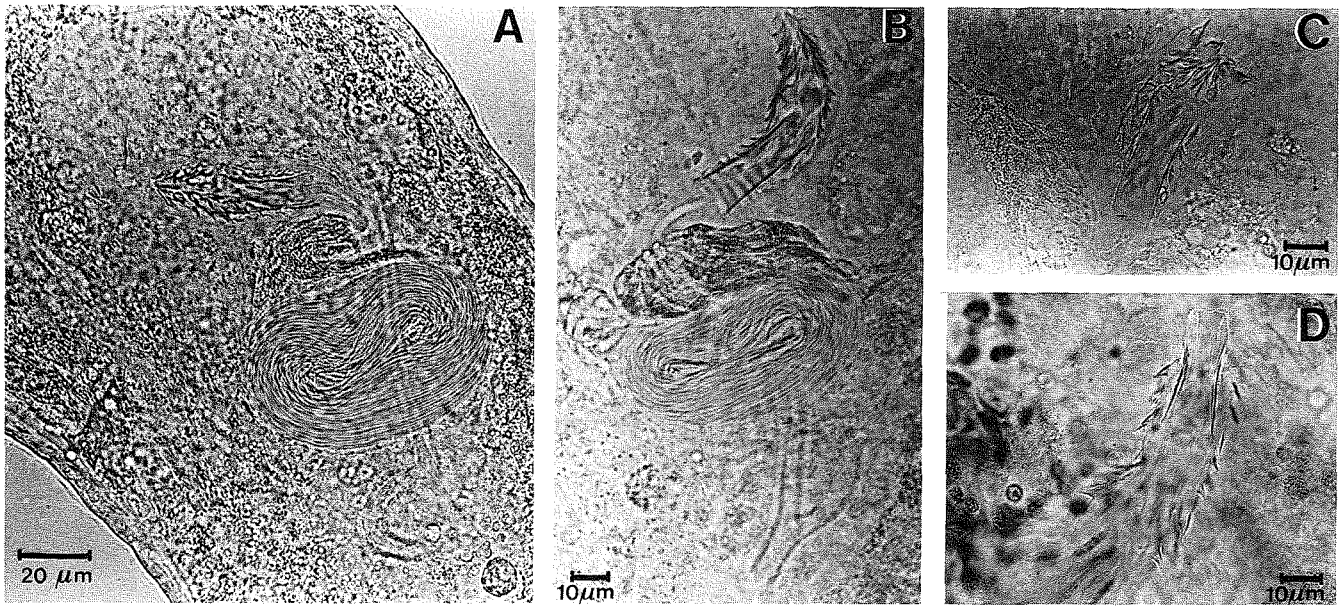

Fig. 4. Archilopsis arenaria: Photographs of the cirrus all from the Belgian coast (A from living animal: B-D from squeezed animals).

nucleated epithelium ('rhammites': Hyman (1951) or 'rhamniten': Maristo (1938) and 'gg_1': P. Martens (1983)). In living animals this latter type of glands can be seen as bundles of long fine rods, very obvious in *A. unipunctata* and *A. spinosa*.

The *pharynx* lies in the second half of the body. As in most Monocelididae it is tubiform, in horizontal position, and orientated backwards, with longitudinal muscles at the epithelial side, circular muscles at the parenchyma side, external and internal epithelium with insunk nuclei and ciliated over the whole length; only the very distal tip of the pharynx is devoid of cilia. A distinct oesophageal part is missing.

Male genital organs. The number of testes varies within the species: 32–36 in *A. spinosa*, 24–36 in *A. arenaria*, 26–30 in *A. unipunctata* and 20–26 in *A. marifuga*.

The *copulatory organ* in the four species is basically of the same construction. A detailed description can be found in E. Martens & Schockaert (1981) incl. E.M. data (this description is actually based on *A. marifuga* and not on *A. unipunctata* (see discussion below). The presence of the two prostate ducts could be confirmed in all four species on the lightmicroscopic level. A central stylet within the cirrus is found in *A. unipunctata* and *A. arenaria*. This stylet is to be considered a part of the ejaculatory duct (papilla), permanently everted into the cirrus. without an external epithelium, but with a thickened basement membrane (see also E. Martens, 1986).

In *A. unipunctata* (Figs. 1B and E) the bulb has a total length of 150 µm while the cirrus is about 75 µm long. The cirrus gradually widens from its proximal to its distal end. Distally it is provided with a girdle of large spines, 18–22 µm long (up to 25 µm, see Luther, 1960), while the proximal spines are 5–7 µm. These proximal spines are more or less triangular in shape with a rounded tip. Most of the large distal spines are broad at the basis, narrow abruptly and end into slender elongated tips, rounded at the edge. Among these distal spines 2 or 3 are slightly larger than the other ones and do not have the abrupt narrowing. In the proximal half of the (inverted) cirrus a large

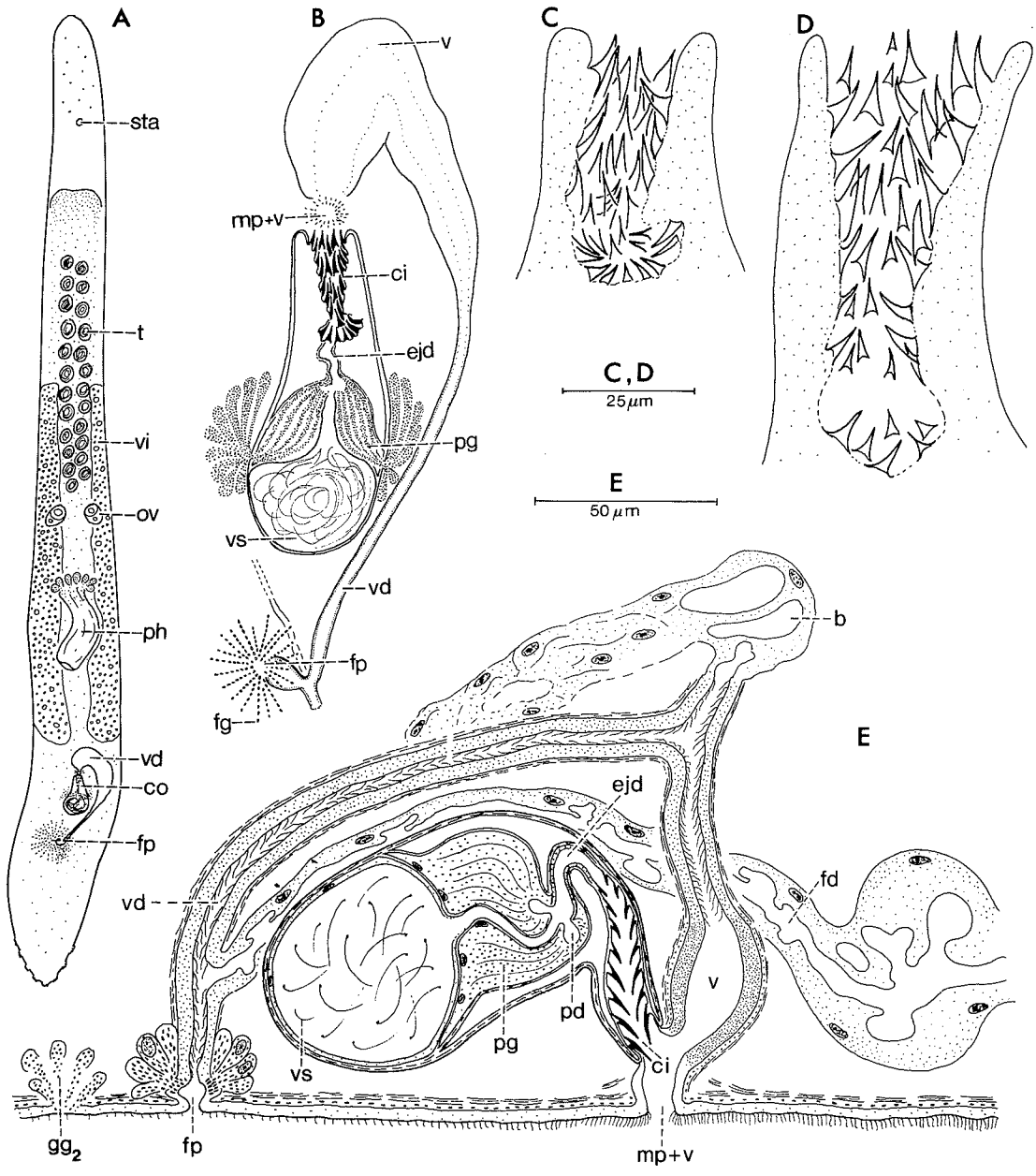

Fig. 5. Archilopsis marifuga: A. Habitus. B. Copulatory organs from living animal. C and D. Cirrus (from squezed animals). E. Reconstruction of the genital organs, from serial sections (seen from the right).

central stylet like structure is present, 40–45 µm long and 27–30 µm broad. It is clearly visible in well squeezed animals, especially when the stylet protrudes from the exerted cirrus (Figs. 1C–D and 2). In sectioned material the stylet becomes apparent as a prolongation of the ejaculatory duct which penetrates the cirrus. The epithelium above the thickened basale lamina has been lost. This structures *not* visible in living or poorly squeezed animals (Figs. 1A–B) can easily be overlooked in

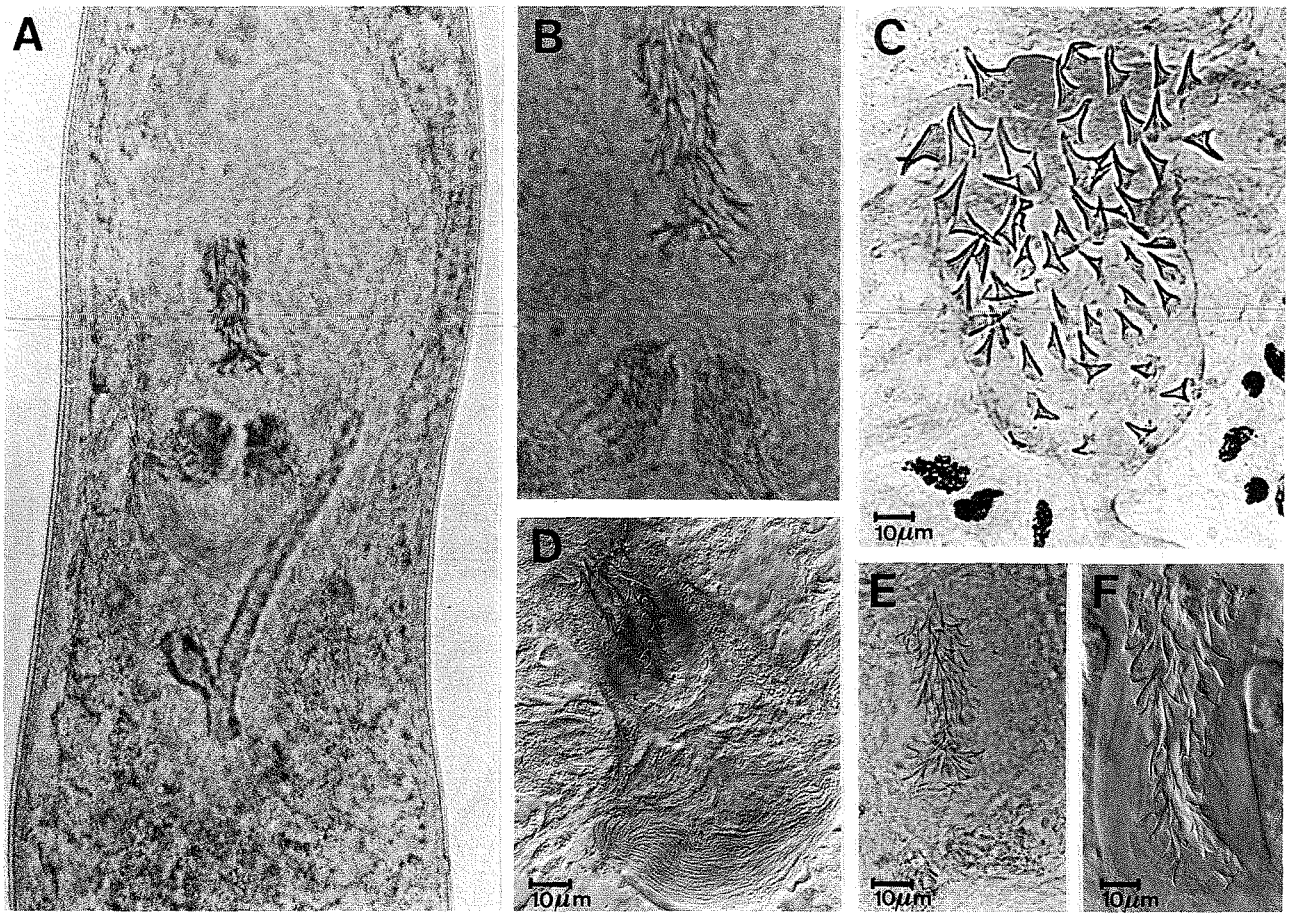

Fig. 6. Archilopsis marifuga: Photographs of the cirrus from different populations. A and B Texel (Netherlands) C. St. Andrews (Canada) D. Ambleteuse (France) E and F. Zwin (Belgium) (A, B from living animals; C-F from squeezed animals).

sectioned material because the stylet wall is laying close to the spines of the cirrus. The part of the ejaculatory duct between the stylet and the prostate ducts is short.

In *A. arenaria* (Figs. 3B and E) the copulatory bulb has a total length of about 100 μm, the cirrus is about 40 μm long with a stylet within its proximal part (27–37 μm long and 10–13 μm broad). The proximal 2/3 of the cirrus is a straight tube (due to the presence of the stylet) and narrows in its distal part (Figs. 3AB, 4A and B). The spines are rather uniform and triangular, the distal spines being slightly larger than the proximal ones (7 μm to 4 μm). Here the ejaculatory duct between the stylet and the prostate ducts is also rather short.

In *A. marifuga* (Figs. 5B and E. 6) the copulatory bulb has a total length of 150 μm and the cirrus is about 50–55 μm long. No inner stylet is present. The proximal part of the cirrus is slightly broadened and is followed by a constriction from where the cirrus again widens distally. The spines of the cirrus are uniform in shape, narrow continuously and are sharply pointed. The distal spines are slightly longer than the proximal ones (from 14–12 μm to 7 μm). In squeezed animals it can be seen that the spines of the proximal part of the cirrus are slightly isolated from the others. The ejaculatory duct does not penetrate the cirrus and is much longer than in the former species.
In *A. spinosa* (Figs. 7B and E, 8) the copulatory

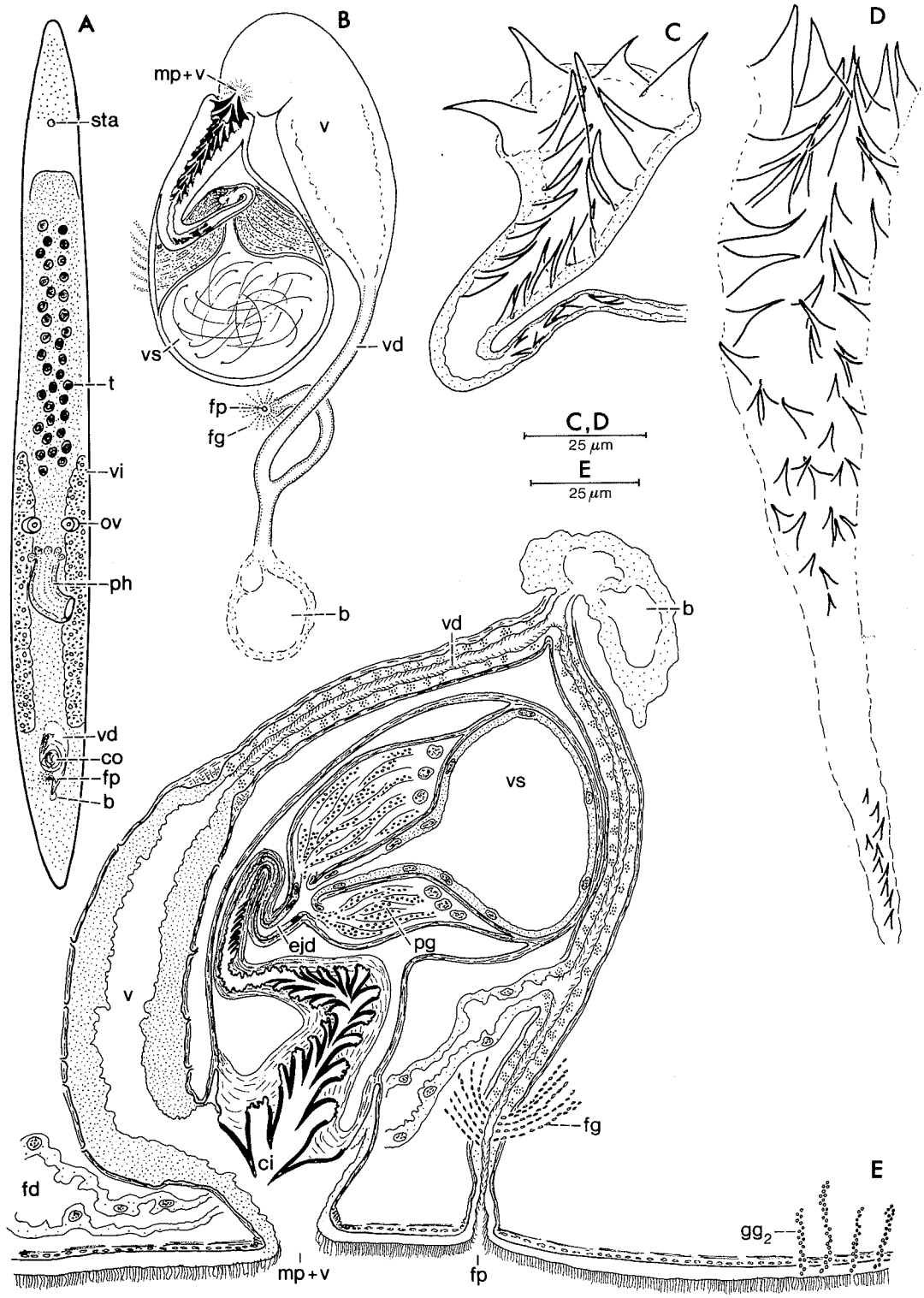

Fig. 7. Archilopsis spinosa: A. Habitus. B. Copulatory organs from living animal. C and D. Cirrus (from squeezed animals). E. Reconstruction of the genital organs, from semi-thin sections (seen from the left).

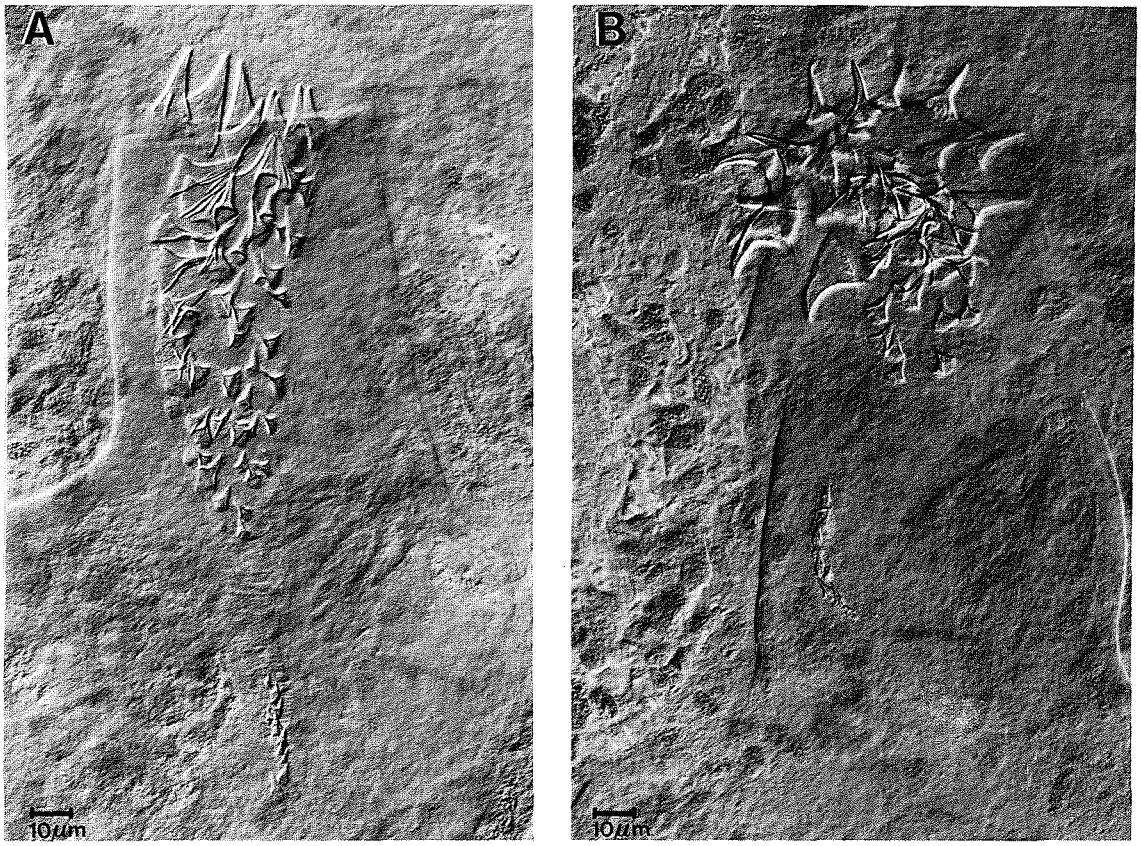

Fig. 8. Archilopsis spinosa: Photographs of the cirrus from the Sylt population (Germany), from squeezed animals.

bulb is about 180 µm long and the cirrus about 90 µm. The cirrus gradually broadens from proximal to distal, while the spines become larger (from 3–4 µm up to 17–20 µm). In the most distal part of the ejaculatory duct an isolated row of 9–13 very small spines occurs at one side of the lumen (cfr. Jensen 1878, p. 70, Fig. 7c). All spines have the same shape, broad at the basis and gradually narrowing to the pointed tip. The ejaculatory duct is extremely long (about 230 µm including the section with the small spines) in this species.

Female genital organs. (Fig. 1E, 3E, 5E, 7E). The ovaries are situated ventro-laterally in front of the pharynx. The vitellaria are dorso-laterally stretched from about half way the row of testes to the level of the copulatory bulb. The ovovitelloducts fuse behind the pharynx to form the common female duct which has a high nucleated and non-called epithelium. This duct then runs to the female pore. In front of the copulatory bulb the duct widens, forming a bursa of the resorbiens type with a strongly vacuolated epithelium. In all four species a vagina internal starting in the male atrium is present. It continues as a 'vaginal duct' over the copulatory bulb to the female (oviposition) pore where it is joined by the common female duct. This pore is surrounded by female glands. The epithelium of the vagina is high, not ciliated, with insunk nuclei and strongly eosinophilous (secretory epithelium?). The vaginal duct is lined with a ciliated epithelium with insunk nuclei. Both vagina and vaginal duct are surrounded by a well developed muscular layer. A postpenial bursa with an epithelium of the resorbiens type opens into the vaginal duct above the copulatory bulb.

Table 2. Biometrical data of some species characteristics of the *Archilopsis* species.

	A. marifuga	*A. spinosa*	*A. arenaria*	*A. unipunctata*
Length of animals	2–3 mm	3–4 mm	2.5–3 mm	3–4 mm
Length of the copularoty bulb	± 150 μm	± 180 μm	± 100 μm	± 150 μm
Length of the cirrus	± 55 μm	± 90 μm (distal part)	± 40 μm	± 75 μm
Stylet:				
– length	–	–	27–37 μm	40–45 μm
– breadth	–	–	10–13 μm	27–30 μm
Length of the cirrus spines:				
– proximal spines	7 μm	3–4 μm	4 μm	5–7 μm
– distal spines	12–14 μm	17–20 μm	7 μm	18–22 μm 20–21 μm (Maristo, 1938) 20–25 μm (Luther, 1960 Fig. 29 G,H)
Number of specimens used for measurements	10	12	30	12

Its epithelium is connected with the gastrodermis and in some places there is only a thin layer of tissue between the gut lumen and the bursa lumen (in *A. marifuga* this bursa lies more to the anterior, the bursal tissue being stretched over the vaginal duct nearly as far as the posterior end of the copulatory bulbus; this situation is probably the result of contraction (Fig. 5E))

B. Karyology

All four species show the same diploid number 2n = 10 (Fig. 9). The diploid chromosome complement is formed by 5 parts of homologous chromosomes gradually decreasing in length, the shortest chromosome being approximately 2/3 of the longest one. All four species have almost the same total karyotype length and the corresponding chromosome pairs have about the same relative and absolute length (Tables 3 and 4).

None of the four species showed any appreciable difference among the various populations investigated; in the case of both *A. unipunctata* and *A. marifuga* a remarkable homogeneity was present in the populations of the same species on either side of the Atlantic ocean.

There are no significant differences between the karyotype of *A. marifuga* and *A. spinosa* (Table 4). All chromosomes are clearly acrocentric with values of the centromeric index more or less markedly lower than 12.5 (the upper border of the acrocentric class, according to Levan *et al.*, 1964).

In *A. arenaria* and *A. unipunctata* four chromosomes out of five (pairs no. 1,3,4,5) are acrocentric (c.i. < 12.5), chromosome pair no. 2 is submetacentric in both species (c.i. 34.22 in *A. arenaria* and 36.19 in *A. unipunctata* see Table 3). With this value the second chromosome of *A. unipunctata* lies close to the border between metacentric and submetacentric (classification according to Levan *et al.*, 1964). These differences between *A. arenaria* and *A. unipunctata* are not significant.

For all the species the haploid number n = 5 was ascertained in spermatocytes I and II. The chiasma frequency observed in spermatocytes I is low, in accordance with was already reported for other Monocelididae (Curini-Galletti *et al.*, 1984; Curini-Galletti *et al.*, 1985).

Diagnosis and occurrence of the *Archilopsis* -species

Archilopsis Meixner, 1938: Monocelididae with pharynx in the posterior body half, ovaries in front of the pharynx. Copulatory organ of the type with spiny cirrus with or without a central stylet.

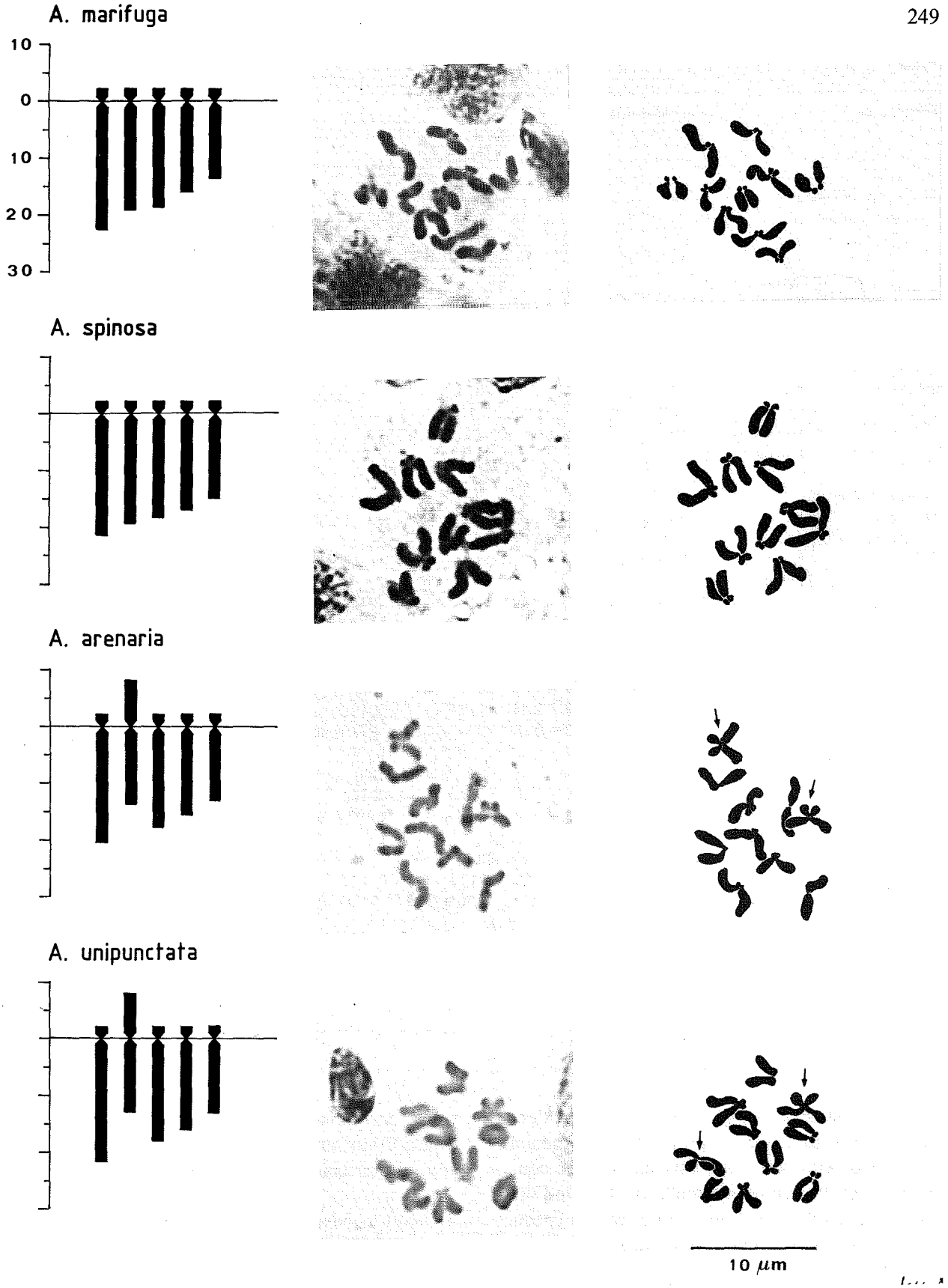

Fig. 9. Idiograms, plates and drawing of the karyotypes from spermatogonial mitosis.

Table 3. Karyometric data from the five chromosomes of the haploid set of *Archilopsis unipunctata* and *Archilopsis arenaria*.

Population		Chromosome					Haploid karyotype length (μm)
		1	2	3	4	5	
Archilopsis unipunctata							
Passamaquoddy	r.l.:	24.07 ± 1.71	21.86 ± 2.16	20.69 ± 1.32	17.98 ± 1.14	15.39 ± 1.06	
bay (Canada)	c.i.:	≦12.5	37.50 ± 2.26	≦12.5	≦12.5	≦12.5	13.02 ± 0.92
	size (μm)	3.13 ± 0.42	2.82 ± 0.24	2.68 ± 0.28	2.38 ± 0.25	2.01 ± 0.14	
	nomencl.:	t	m/sm	t	t	t	
Kristieneberg	r.l.:	23.19 ± 0.77	21.25 ± 1.92	20.37 ± 0.94	18.87 ± 0.77	16.32 ± 0.63	
(Sweden)	c.i.:	≦12.5	34.88 ± 2.22	≦12.5	≦12.5	≦12.5	14.09 ± 1.74
	size (μm)	3.26 ± 0.36	3.00 ± 0.51	2.86 ± 0.37	2.65 ± 0.33	2.30 ± 0.31	
	nomencl.:	t	sm	t	t	t	
means	r.l.:	23.63	21.55	20.53	18.42	15.85	
	c.i.:	≦12.5	36.19	≦12.5	≦12.5	≦12.5	13.55
	size (μm)	3.19	2.91	2.77	2.51	2.15	
	nomencl.:	t	sm	t	t	t	
Archilopsis arenaria							
De panne	r.l.:	24.52 ± 1.80	21.19 ± 1.09	20.90 ± 1.06	17.99 ± 0.79	15.39 ± 1.17	
(Belgium)	c.i.:	≦12.5	33.56 ± 3.07	≦12.5	≦12.5	≦12.5	12.59 ± 0.63
	size (μm)	3.08 ± 0.21	2.67 ± 0.26	2.63 ± 0.21	2.27 ± 0.27	1.94 ± 0.26	
	nomencl.:	t	sm	t	t	t	
Oostende	r.l.:	23.03 ± 1.05	22.52 ± 0.49	21.25 ± 0.53	17.90 ± 1.40	15.35 ± 0.41	
(Belgium)	c.i.:	≦12.5	35.14 ± 2.50	≦12.5	≦12.5	≦12.5	13.97 ± 2.02
	size (μm)	3.21 ± 0.41	3.11 ± 0.47	2.97 ± 0.38	2.25 ± 0.49	2.16 ± 0.33	
	nomencl.:	t	sm	t	t	t	
Zwin	r.l.:	23.12 ± 1.47	21.19 ± 1.25	20.38 ± 0.49	18.85 ± 1.14	16.60 ± 1.49	
(Belgium)	c.i.:	≦12.5	33.27 ± 2.43	≦12.5	≦12.5	≦12.5	11.34 ± 0.95
	size (μm)	2.63 ± 0.33	2.43 ± 0.28	2.34 ± 0.20	2.10 ± 0.20	1.84 ± 0.17	
	nomencl.:	t	sm	t	t	t	
Sylt	r.l.:	23.02 ± 1.03	21.04 ± 1.03	20.5 ± 0.46	18.8 ± 0.57	16.63 ± 0.86	
(Germany)	c.i.:	≦12.5	35.19 ± 3.30	≦12.5	≦12.5	≦12.5	11.09 ± 1.77
	size (μm)	2.55 ± 0.38	2.33 ± 0.37	2.28 ± 0.39	1.99 ± 0.55	1.84 ± 0.31	
	nomencl.:	t	sm	t	t	t	
Roscoff	r.l.:	22.39 ± 1.08	21.90 ± 2.13	20.42 ± 0.95	18.67 ± 0.90	16.54 ± 1.01	
(France)	c.i.:	≦12.5	33.96 ± 3.22	≦12.5	≦12.5	≦12.5	11.24 ± 1.26
	size (μm)	2.58 ± 0.33	2.47 ± 0.39	2.30 ± 0.25	2.10 ± 0.25	1.85 ± 0.19	
	nomencl.:	t	sm	t	t	t	
means	r.l.:	23.22	21.57	20.69	18.44	16	.10
	c.i.:	≦12.5	34.22	≦12.5	≦12.5	≦12.5	12.05
	size (μm)	2.81	2.60	2.50	2.14	1.93	
	nomencl.:	t	sm	t	t	t	

Prostate glands discharge in the ejaculatory duct via two prostate ducts. Internal vagina prepenial associated with the male atrium. Vagina connected with the common females duct behind the copulatory bulb by a vaginal duct with a (postpenial) bursa. Karyotype: five pairs of homologous chromosomes gradually decreasing in length. Four out of five pairs are acrocentric, the second pair being submetacentric or acrocentric.

Archilopsis unipunctata (Fabricius, 1826): Cirrus about 75 μm long, continuously widening from

Table 4. Karyometric data from the five chromosomes of the haploid set of *Archilopsis marifuga* and *Archilopsis spinosa*.

Population		Chromosome					Haploid karyotype length (μm)
		1	2	3	4	5	
Archilopsis marifuga							
Zwin	r.l.:	25.58 ± 1.65	22.03 ± 0.70	19.58 ± 0.93	17.47 ± 0.88	15.39 ± 1.00	
(Belgium)	c.i.:	≦12.5	≦12.5	≦12.5	≦12.5	≦12.5	13.21 ± 2.39
	size (μm)	3.35 ± 0.64	2.83 ± 0.56	2.61 ± 0.48	2.34 ± 0.45	2.08 ± 0.47	
	nomencl.:	t	t	t	t	t	
Ambleteuse	r.l.:	23.96 ± 1.09	21.23 ± 0.38	20.28 ± 0.83	18.43 ± 0.49	16.09 ± 1.57	
(France)	c.i.:	≦12.5	≦12.5	≦12.5	≦12.5	≦12.5	13.91 ± 3.56
	size (μm)	3.33 ± 0.09	2.95 ± 0.99	2.80 ± 0.62	2.56 ± 0.62	2.27 ± 0.47	
	nomencl.:	t	t	t	t	t	
Passamaquoddy	r.l.:	23.95 ± 1.04	21.56 ± 0.54	19.65 ± 0.86	18.37 ± 0.76	16.49 ± 0.68	
Bay (Canada)	c.i.:	≦12.5	≦12.5	≦12.5	≦12.5	≦12.5	12.24 ± 0.43
	size (μm)	2.93 ± 0.14	2.63 ± 0.10	2.40 ± 0.12	2.26 ± 0.15	2.02 ± 0.12	
	nomencl.:	t	t	t	t	t	
means	r.l.:	24.49	21.61	19.84	18.09	15.99	
	c.i.:	≦12.5	≦12.5	≦12.5	≦12.5	≦12.5	13.12
	size (μm)	3.20	2.82	2.60	2.39	2.12	
	nomencl.:	t	t	t	t	t	
Archilopsis spinosa							
Sylt	r.l.:	23.05 ± 0.99	21.49 ± 0.70	20.23 ± 0.46	18.62 ± 0.61	16.61 ± 1.04	
(Germany)	c.i.:	≦12.5	≦12.5	≦12.5	≦12.5	≦12.5	13.63 ± 1.28
	size (μm)	3.14 ± 0.25	2.93 ± 0.26	2.76 ± 0.27	2.54 ± 0.26	2.27 ± 0.30	
	nomencl.:	t	t	t	t	t	
Roscoff	r.l.:	22.88 ± 0.80	21.37 ± 0.77	20.18 ± 0.58	18.47 ± 0.91	17.10 ± 1.07	
(France)	c.i.:	≦12.5	≦12.5	≦12.5	≦12.5	≦12.5	13.73 ± 2.51
	size (μm)	3.15 ± 0.66	2.94 ± 0.60	2.77 ± 0.49	2.53 ± 0.40	2.34 ± 0.42	
	nomencl.:	t	t	t	t	t	
means	r.l.:	22.96	21.43	20.20	18.54	16.85	
	c.i.:	≦12.5	≦12.5	≦12.5	≦12.5	≦12.5	13.68
	size (μm)	3.14	2.93	2.76	2.53	2.30	
	nomencl.:	t	t	t	t	t	

proximal to distal end. Distally with a girdle of large spines (18–25 μm long), proximal spines much shorter (5–7 μm). Spines mostly rounded at the tip. Stylet (only visible in well squeezed animals or scctioned animals); 40–45 μm long and 27–30 μm broad. Pore indices: 4–2–6.

Karyotype: 4 pairs of acrocentric chromosomes, and one pair submetacentric (the second).

Occurrence: A long list of localities for this species is given by Luther (1960). The identification of the species is, however, doubtful in numerous cases (see discussion below) and only findings after 1938 (description of Meixner and Maristo) may be unambiguously accepted. From these findings it can be concluded that *A. unipunctata* has a North-Atlantic and Baltic (Karling, 1974) distribution and never occurs on open beaches with high dynamic. *A. unipunctata* is most probably absent in the Mediterranean since it has never been found there after 1938. *A. unipunctata* is a eurytope and euryhaline species, found on macrophytes and higher plants, in sand varying from coarse sand with gravel to fine sand with silt and detritus in places which are more or less protected (lagoones, estuaries, closed seas etc.).

Archilopsis arenaria sp.n.: Cirrus about 40 μm long, with stylet about 10–13 μm broad and

27–37 μm long. Cirrus narrows from proximal to distal. Spines of the cirrus almost uniform, triangular, the distal spines longer (7 μm) than the proximal ones (4 μm). Pore indices 3–2–8.

Karyotype: 4 pairs of acrocentric chromosomes, one pair submetacentric (the second).

Occurrence: This species has been found in fine to medium sand in the mediolitoral of beaches along the Belgian coast, near the littoral station of Sylt and in front of the Biological Station of Roscoff, in exposed and in protected but genuinely marine localities (Table 1). This species is recently mentioned by Ax & Armonies (1987) for the Canadian east coast.

Type locality: Belgium North-Sea coast, De Panne, mediolittoral, fine sand, August 1981.

Holotype: One whole mount (paratype: one specimen serially sectioned, Heidenhain's hemotoxyline-eosine).

N.B.: This species has been reported as *Archilopsis unipunctata* by P. Martens (1984a) and as *Archilopsis* sp. by E. Martens (1986).

Archilopsis marifuga sp.n.: Cirrus about 50–55 μm long with a constriction in the proximal third, without a stylet. Distal spines larger (12–14 μm) then the proximal ones (about 7 μm). Ejaculatory duct long without isolated spines nor forming a stylet within the cirrus. Pore indices: 4–2–6.

Karyotype: 5 pairs of acrocentric chromosomes.

Occurrence: This species was found in rather fine sand with silt and detritus, always in a more or less protected area, generally in brackish water. For known localities see Table 1.

Type locality: estuary of the Slack, Ambleteuse (France), fine sand with mud, May 1979.

Holotype: One whole mount (paratype: different specimens serially sectioned, Masson's Triplestain).

N.B.: This species has been reported as *Archilopsis unipunctata* by Martens and Schockaert (1981).

Archilopsis spinosa (Jensen, 1878): Cirrus large, about 90 μm long without a stylet, gradually narrowing from distal to the proximal. Distal spines about 17–20 μm long. Half way in the long ejaculatory duct there is a row of isolated small spines. Pore indices: 4–2–5.

Karyotype: 5 pairs of acrocentric chromosomes.

Occurrence: This species was found by us in sand with silt and detritus on sandflats in marine localities. Due to possible confusion with the other species, it is known with certainty only from type locality (Herlo, Norway, on *Fucus*: Jensen 1878) and from localities reported in Table 1. The reports of Ax (1951) and Sopott (1972) are probably referred to this species. This species is also recently mentioned by Ax & Armonies (1987) for the Canadian east coast but under the provisional name *A. inopinata*.

Discussion

A. Taxonomy

From the descriptions it is clear that four different species can be discerned within the genus *Archilopsis*. They differ in a number of morphological characteristics: size shape and distribution of cirrus-spines, presence or absence of a stylet within the cirrus (some biometrical data are presented and compared in Table 2), and in their karyology (Tables 3 and 4).

Which of these four species is to be regarded as *A. unipunctata* (Fabricius, 1826)? Of all known descriptions, those of Maristo (1938), Luther (1960) and Karling (1974) deal with the same species under different names. Non of them exactly mentions the presence of a stylet but this can easily be overlooked. On the other hand the drawings of Luther (1960 Fig. 29 G,I and J) all show an everted cirrus with at the top a well lined penis papilla which might be a weakly developed hard structure. On Karling's photograph (1974 Fig 176) it is possible to recognize the stylet (see Fig. 2G). Also Meixner (in his second and unpublished part of 'Die Tierwelt der Nord- und Ostsee') draws a straight tube at the top of the exerted cirrus.

From these interpretations as well as from our

observations (including the material from the Swedisch Museum) it must be pointed out that *A. unipunctata* has a stylet within the cirrus which can be protruded by everting the cirrus. This obviously weakly developed hard structure has the same basic constriction as the stylet of other Monocelididae (own observations).

Jensen (1878) described *Monocelis spinosa* as characterized by an extremely long ejaculatory duct provided with some isolated small spines. Three of the populations we have studied perfectly fit Jensen's description, so that we consider the taxon *Archilopsis spinosa* (Jensen, 1878) as valid.

No taxa are available for the other two species, and we need to consider them as new: *Archilopsis arenaria* sp.n. and *Archilopsis marifuga* sp.n.

'*Archilopsis unipunctata*' described by E. Martens & Schockaert (1981) does not present a stylet. We have investigated the same material as well as living animals from the same locality (see Table 1). They are here reported as *A. marifuga*.

In a preliminary revision of the Monocelididae, Karling (1966) has proposed to lump together a number of genera – among which *Archilopsis* – into the genus *Archiloa* de Beauchamp, 1910 mainly based on the presence of a copulatory organ of the conjuncta-duplex type with a spiny cirrus. From our present knowledge of a number of the genera concerned (own unpublished data) and from the underlaying descriptions, we suggest to maintain the genus *Archilopsis* Meixner, 1938 as a valid genus name. At least two synapomorphies can be indicated for the four species concerned: (1) the prostate glands which discharge into a pair of prostate ducts (see also E. Martens & Schockaert, 1981) and (2) the vagina interna continues in a long vaginal duct which is connected postpenially with a bursa and the female duct.

B. Relationships

From a morphological point of view the four *Archilopsis* species can be grouped two by two: *A. unipunctata* and *A. arenaria* on the one hand and *A. marifuga* and *A. spinosa* on the other hand. The first pair of species has a stylet within the cirrus, formed by a stabilized protrusion of the ejaculatory duct covered by a basal lamina derivation at its outer side (see E. Martens, 1986 for *A. arenaria*). A stylet of a similar origin is found in some *Duplominona* species (Ax & Ax 1977; P. Martens, 1984b) and in *Archilina endostyla* (Ax, 1959b). The presence of such a stylet is clearly an apomorphy, probably a parallelism in species not closely related, but it may be a synapomorphy within the genus *Archilopsis* (see also below).

The second pair of species, *A. marifuga* and *A. spinosa*, has some small proximal spines more or less separated from the other spines. In *A. marifuga* (see Fig. 5B) they lay less separated in the proximal part of the cirrus, while these clearly form an isolated group within the ejaculatory duct in *A. spinosa*. This characteristic is an apomorphy, maybe a synapomorphy for these two species (from outgroup comparison).

All four *Archilopsis* species have a chromosome set of $2n = 10$ with almost the same absolute karyotype length and relative chromosome lengths. Chromosomes 1,3,4,5 are acrocentric (c.i. < 12.5) in all species. Chromosome 2 is acrocentric in *A. marifuga* and in *A. spinosa* while the c.i. = 34.22 in *A. arenaria* and c.i. = 36.13 in *A. unipunctata* (these c.i. are not significantly different: $p > 0.05$). Since relative length of this second chromosome is the same in the four species, a pericentric inversion must underlie the differences in centromeric position. From a comparison with the karyotypes of other Monocelididae (Curini-Galletti *et al.*, 1985; Curini-Galletti *et al.*, 1988) it can be concluded that the set with 5 acrocentric chromosomes is the plesiomorph situation, and a submetacentric second chromosome due to pericentric inversion is an apomorphy, probably a synapomorphy for *A. arenaria* and *A. unipunctata*, which is congruent with the presence of the stylet within the cirrus.

The genus *Archilopsis* can thus be regarded as being composed of two sister-taxa, each of which consists of two sister-species (Fig. 10). Curiously enough, in each pair of species we find one (*A. unipunctata* and *A. marifuga*) in less dynamic habitats (even brackish water) and one (*A. arenaria* and *A. spinosa* respectively) in true marine habitat with higher dynamics. Apparently each

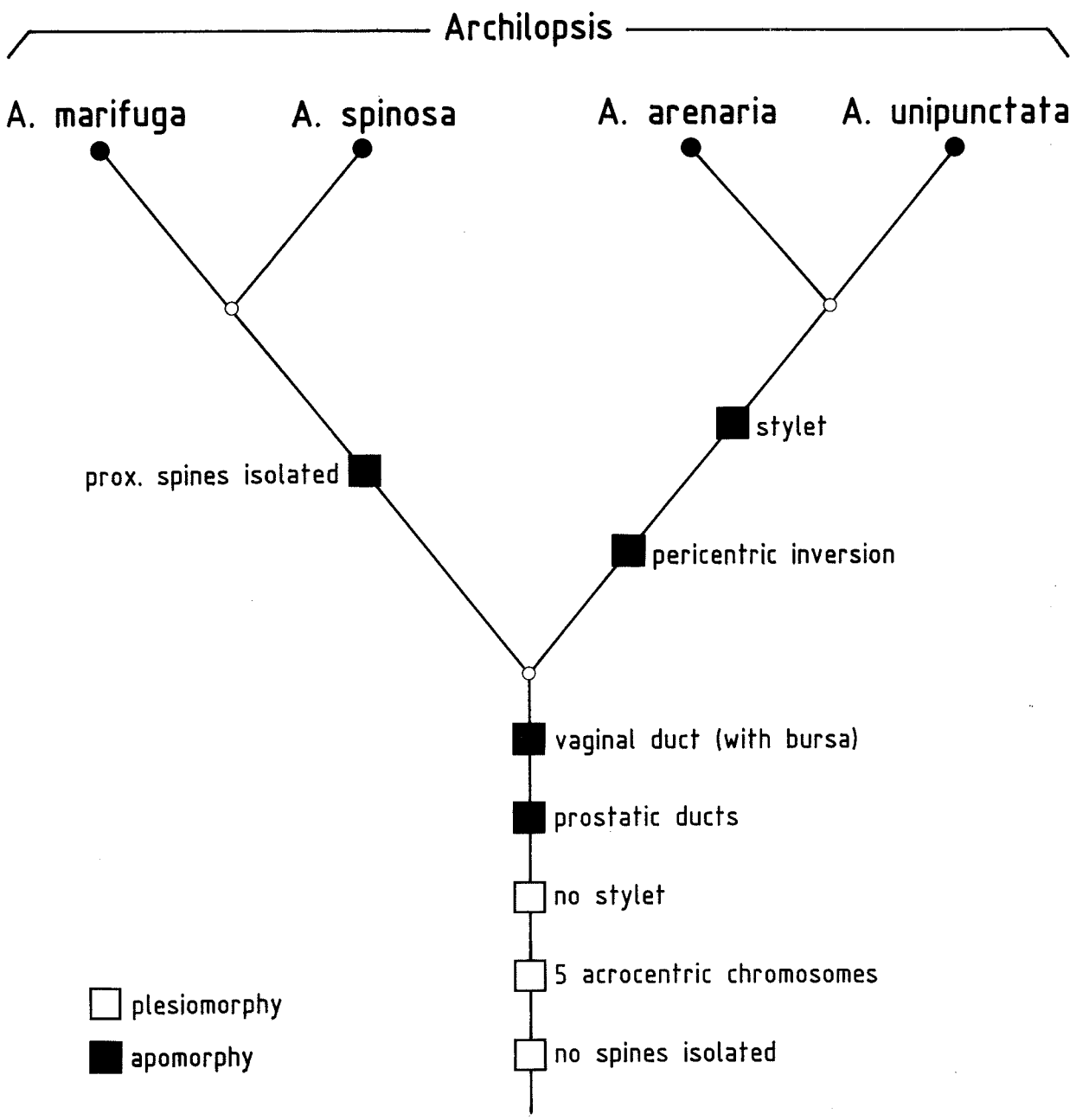

Fig. 10. Phylogenetic relationship within the genus *Archilopsis*.

species seems to be ecologically isolated from its sister-species while the two pairs of sister-species are clearly genetically separated.

With the data now available on the other Monocelididae with a duplex copulatory organ, we find it premature to discuss the relationship of the genus *Archilopsis* with the other genera.

Acknowledgements

The following persons are gratefully acknowledged for the hospitality, research facilities and/or loaning of material: Prof. Dr. M. Burt (Canada), Prof. Dr. P. Ax, Dr. B. Sopott, Dr. U. Noldt (Göttingen, Germany), Dr. K. Reise (Sylt,

Germany), Prof. Dr. P. Lasserre (Roscoff, France), Prof. Dr. T. Karling (Sweden) and Dr. E. Martens, N. Revis and P. Jouk (Belgium).

We also wish to thank Prof. Dr. E. Schockaert for the valuable discussions and critical reading of the manuscript and Prof. Dr. T. Karling for having addressed our attention to the taxon *A. spinosa* (Jensen, 1878). Ms. M. Uytterhaegen is acknowledged for reading the English of the manuscript and Mr. and Mrs. Withofs-Ieven and Mrs. H. Zurings for the technical assistance.

Abbreviations in the figures

b bursa
ci cirrus
co copulatory organ
ejd ejaculatory duct
fd female duct
fg female glands
fp female pore
gg glands
m mouth
mp male pore
ov ovary
pd prostatic duct
pg prostate glands
ph pharynx
s stylet
sd seminal duct
sta statocyst
t testes
v vagina
vd vaginal duct
vi vitellary
vs seminal vesicle

References

Ax, P., 1951. Die Turbellarien des Eulittorals der Kieler Bucht. Zool. Jb. Syst. 80: 277–378.

Ax, P., 1959a. Zur Kenntnis der Gattung Promonotus Beklemischev (Turbellaria, Proseriata). Zool. Anz. 163: 371–385.

Ax, P., 1959b. Zur Systematik, Ökologie und Tiergeographie der Turbellarienfauna in den kaspischen Brackwassermeeren. Zool. Jb. Syst. 87: 43–184.

Ax, P. & R. Ax, 1977. Interstitielle Fauna von Galapagos XIX. Monocelididae (Turbellaria, Proseriata). Mikrofauna Meeresboden 64: 1–44.

Ax, P. & W. Armonies, 1987. Amphiatlantic identities in the composition of the boreal brackish water community of Plathelminthes. A comparison between the Canadian and European Atlantic coast. Microfauna Marina 3: 7–80.

Claparède, E., 1861. Etudes Anatomiques sur les Annélides, Turbellariés, Opalines et Grégarines observés dans les Hébrides. Mém. Soc. Phys. & Hist. nat. de Genève 16(S): 71–16.

Curini-Galletti, M., L. Galleni & I. Puccinelli, 1984. Karyological analysis of Monocelis fusca, M. lineata (Monocelididae) and Parotoplana macrostyla (Otoplanidae). Helgoländer Meersunters. 37: 171–178.

Curini-Galletti, M., P. M. Martens & I. Puccinelli, 1985. Karyological observations on Monocelididae (Turbellaria, Proseriata): Karyometrical analysis of four species pertaining to the subfamily Minoninae. Caryologia 38: 67–75.

Curini-Galletti, M. C., I. Puccinelli & P. M. Martens, 1988. Karyotype analysis of ten species of the Monocelididae (Proseriata, Plathelminthes) with remarks on the karyological evolution of the family. Genetica: in press.

Fabricius, O., 1826. Fortsaettelse of Nye Zoologiske Bidrag VI. Nogle lidet bekjendte og tideels nye Flad-Orme (Planariae). Kongl. Danske Vidensk. Selskabs. Naturv. og Mathem. Afhandl. 2: 13–35.

Graff, L. von., 1882. Monographie der turbellarien I. Rhabdocoelida. Leipzig, 442 pp.

Graff, L. von., 1913. Platyhelminthes. Turbellaria II. Rhabdocoelida. Tierreich 35: 1–484.

Hyman, L. H., 1951. The invertebrates: Plathelminthes and Rhynchocoela. The acoelomate Bilateria. McGraw-Book Comp., New-York 763 pp.

Jensen, O., 1878. Tubellarier ved Norges vestkyst. Bergen.

Karling, T. G., 1966. Marine Turbellaria from the Pacific coast of North America IV. 1. Coelogynoporidae und Monocelididae. Ark. Zool. 18: 493–528.

Karling, T. G., 1974. Turbellarian fauna of the Baltic Proper. Identification, Ecology and Biogeography. Fauna fenn. 27: 1–101.

Levan, A., K. Fredga & A. A. Sandberg, 1964. Nomenclature for centrometric position on chromosomes. Hereditas 52: 201–220.

Luther, A., 1960. Die Turbellarien Ostfennoskandiens. I. Acoela, Catenulida, Macrostromida, Lecithoepitheliata, Prolecithophora, Proseriata. Fauna fenn. 7: 1–155.

Maristo, L., 1938. Beiträge zur Kenntnis der Monocelidinen (Turbellaria, alloeocoela). Annales Zool. Vanamo 8: 1–69, 1t.

Martens, E. E. & E. R. Schockaert, 1981. Observations on the ultrastructure of the copulatory organ of Archilopsis unipunctata (Fabricius, 1826) (Proseriata Monocelididae). Hydrobiologia 84: 277–285.

Martens, E. E., 1986. Comparative ultrastructure of copulatory organs having a stilet in the Proseriata (Turbellaria). Hydrobiologia 132: 165–173.

Martens, P. M., 1983. Three new species of Minoninae (Turbellaria, Proseriata, Monocelididae) from the North Sea, with remarks on the taxonomy of the subfamily. Zool. Scr. 12: 153–160.

Martens, P. M., 1984a. Comparison of three different extraction methods for Turbellaria. Mar. Ecol. Prog. Ser. 1: 229–23.

Martens, P. M., 1984b. Three new Duplominona species (Turbellaria, Monocelididae, Minoninae) from the Mediterranean. Cahiers de Biologie Marine XXV: 319–331.

Meixner, J., 1938a. Turbellaria (Strudelwürmer) I. Tierwelt N.-u. Ostsee 4b: 1–146.

Oersted, A., 1844. Entwurf einer systematischen Eintheilung der Plattwürmer. Copenhagen.

Papi, F., 1951. Über einen Methode die 'chitinösen' Gebilde des Genitalapparats bei Turbellarien zu färben und über die Bestachelung einiger Castrada-Arten. Mem. Soc. pro Fauna et Flora fenn. 26: 3–8.

Sopott, B., 1972. Systematik und Ökologie von Proseriaten (Turbellaria) der deutschen Nordseeküste. Mikrofauna Meeresboden 13: 1–72.