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Enzyme electrophoresis, genetic identity and description of a new genus and species of heteronemertean (Nemertea, Anopla) from northwestern Spain and North Wales

Alex D. Rogers [1], Juan Junoy [2], Ray Gibson [3] & John P. Thorpe [1]
[1] *Department of Evolutionary and Environmental Biology, University of Liverpool, Port Erin Marine Laboratory, Port Erin, Isle of Man, U.K.;* [2] *Departamento de Biologia Animal, Universidad de Alcala de Henares, 28871 Alcala de Henares, Madrid, Spain;* [3] *School of Natural Sciences, Liverpool Polytechnic, Byrom Street, Liverpool L3 3AF, U.K.*

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Abstract

The anoplan order Heteronemertea, particularly the genera *Cerebratulus*, *Lineus* and *Micrura*, contains a very large number of nominate species, many of which are inadequately described. As a consequence, systematic difficulties are encountered with the identification of many taxa in this group, especially those originally established primarily on the basis of their external features. The present paper concerns heteronemerteans collected from two locations, the Foz Estuary (north-western Spain) and Llandudno (North Wales). The Spanish collection included specimens identified as *Lineus longissimus* (Gunnerus), whilst samples from Llandudno contained large numbers of *Lineus viridis* (Müller); samples of a third similar but apparently undescribed species were found at both locations. Starch gel electrophoresis showed that samples of the apparent third species were genetically almost identical from each of the two locations, but were clearly different from the two described *Lineus* species. Histological studies of the unknown specimens revealed anatomical characters, including the unique feature of a proboscis epithelium ciliated throughout its length, which exclude it from any known heteronemertean taxon; it is accordingly placed in a new genus and species, for which the name *Riseriellus occultus* is proposed.

Introduction

The study of nemertean systematics is plagued by a multitude of inadequately described taxa, at and below the family level (Gibson, 1982a, 1985). Many of the older species were established primarily on the basis of external features, many of which are now considered to be taxonomically unreliable (Gibson, 1985; Gibson & Crandall, 1989) and also of little value in determining generic affinities. Even where species have been erected after histological investigation, descriptions of their anatomy are frequently either incomplete or emphasise characteristics whose taxonomic significance is difficult to assess. This is especially true when problems of intraspecific variability are considered (Friedrich, 1960; Berg, 1972; Sundberg, 1979, 1980), and it is highly likely that many nemertean 'species' are either complexes of different taxa or are synonymous with other forms.

The genus *Lineus* Sowerby, 1806, is the oldest established nemertean taxon. It contains more than 90 nominate species (Gibson, 1982b) and typifies many of the fundamental problems outlined above. During September 1990 nemerteans

of this genus were collected at Llandudno, North Wales, and in the Foz Estuary, north-western Spain. The collections included numerous examples of *Lineus longissimus* (Gunnerus, 1770) and *Lineus viridis* (Müller, 1774), as well as a third group of black to dark green heteronemerteans, in size and general appearance very similar to, but externally distinguishable from, both the *Lineus* species. Whether this third morphotype represented a separate species or was merely a variety of one or the other taxa was uncertain, since previous descriptions of the external features of both *Lineus longissimus* and *Lineus viridis* have been ambiguous. Histological differences between *Lineus* species are often small (Cantell, 1972, 1975) and may simply represent intraspecific variation; further, descriptions of the internal anatomy of species such as *Lineus longissimus* are frequently contradictory (Friedrich, 1935; Cantell, 1975, 1976). Preliminary studies on the systematic relationships between *Lineus longissimus*, *Lineus viridis* and the third morphotype found in Spain and Wales thus required a more objective approach, such as an investigation of enzyme variation by electrophoresis. This technique has been used to resolve taxonomic problems in many invertebrate groups (for reviews, see Ferguson, 1980; Thorpe, 1982, 1983; Ayala, 1983), including nemerteans (Cantell & Gidholm, 1977; Williams *et al.*, 1983; Sundberg & Janson, 1988). Enzyme electrophoresis was therefore employed to estimate the genetic distance between sympatric populations of *Lineus longissimus*, *Lineus viridis* and the third unknown heteronemertean morphotype; the results demonstrated conclusively that the unknown form was conspecific with neither of the lineids, and indicated that extensive histological investigations of specimens from both locations were needed to resolve the systematic status of this heteronemertean.

Materials and methods

Samples

Nine specimens of *Lineus longissimus* were collected from the Foz Estuary, Spain (approximately 43° 34′ N, 7° 14′ W) during September 1990, close to mean low water spring tide level. Ten examples of the third morphotype were also found at Foz, but these occurred much higher up the shore in mud banks, or amongst *Spartina* roots. Several additional specimens of the unknown taxon were obtained from the same location during May 1991. Collections made at Llandudno, also during September 1990, yielded 59 examples of *Lineus viridis* and 39 individuals apparently of the same unknown morphotype as those found at Foz. At Llandudno all samples were found on the upper shore beneath stones and rocks lying on damp, fine mud or silt. Further examples of the unknown species have subsequently been found (August 1991) at Rhosneigr and Trwyn du Point, Anglesey.

Representative living specimens of each of the three species were examined in detail under a binocular microscope, their maximum relaxed lengths and widths being recorded.

Electrophoresis

Horizontal starch gel electrophoresis was performed by standard methods (see Harris & Hopkinson, 1978; Ferguson, 1980; Richardson *et al.*, 1986) using 12.5% starch gels (Sigma Chemical Co. Ltd., Poole, Dorset, U.K.). Samples, were cut from the posterior ends of live specimens of each species and homogenized in a small volume of buffer (0.06M Tris/HCl pH 8.0). Two buffer systems were used:

Buffer system I (Ward & Beardmore, 1977): electrode buffer (30.29 g Tris, 11.98 g citric acid per litre, pH 8.0); gel buffer (192.5 ml electrode buffer diluted to 5 litres, pH 8.0).

Buffer system II (Williams *et al.*, 1983): electrode buffer (16.35 g Tris, 8.26 g citric acid, 0.46 g disodium salt of ethylene diamine tetra-acetic acid [Na_2EDTA] per litre, pH 7.1); gel buffer (16.35 g Tris, 9.035 g citric acid, 6.9 g Na_2EDTA per litre, diluted 1:15 with distilled water, pH 7.1).

Buffer system I was run at 140 V, 50 mA for 7 hours, buffer system II at 100 V, 50 mA for approximately 7 hours.

The following staining schedules, with the appropriate Enzyme Commission (E.C.) numbers indicated, were employed:

(i) Aminopeptidase A (APA) (E.C. 3.4.11.1) (Williams *et al.*, 1983): buffer system I; stain: 10 mg N-glycyl-L-leucine, 5 mg L-amino acid oxidase, 5 mg *o*-dianisidine, 10 mg horseradish peroxidase, 1 mg manganous chloride, 25 ml Tris HCl pH 8.0, 2% agar.

(ii) Glutamate-oxaloacetate transaminase (GOT) (E.C. 2.6.1.1) (Williams *et al.*, 1983): buffer system II; stain: 0.6 g Tris, 150 mg aspartic acid, 40 mg α-ketoglutaric acid, 20 mg polyvinylpyrolidone, 15 mg pyridoxal-5-phosphate, 40 ml distilled water. Incubate for 30 minutes, then add 100 mg Fast Blue RR salt in 10 ml distilled water.

(iii) Isocitrate dehydrogenase (ICD) (E.C. 1.1.1.42): buffer system II; stain: 80 mg isocitric acid, 285 mg magnesium chloride, 5 mg nicotinamide adenine dinucleotide phosphate (NADP), 5 mg MTT, 5 mg phenazine methosulphate (PMS), 25 ml 0.06M Tris HCl, pH 8.0.

(iv) Malate dehydrogenase (MDH) (E.C. 1.1.1.37): buffer system I; stain 100 mg L-malic acid, 970 mg Tris, 80 mg magnesium chloride, 15 mg nicotinamide adenine dinucleotide (NAD^+), 10 mg MTT, 1 mg PMS, 40 ml distilled water, 25 ml 2% agar.

(v) Mannose phosphate isomerase (MPI) (E.C. 5.3.1.9): buffer system II; stain: 40 mg mannose-6-phosphate, 10 mg NADP, 50 μl glucose-6-phosphate dehydrogenase, 40 u glucose phosphate isomerase, 5 mg MTT, 1 mg PMS, 10 mg magnesium chloride, 30 ml 0.06M Tris HCl, pH 8.0.

(vi) Octopine dehydrogenase (ODH) (E.C. 1.5.1.11) (Walsh & Somero, 1981): buffer system I; stain: 25 mg octopine, 15 mg NAD^+, 7 mg MTT, 2 mg PMS, 25 ml 0.06M Tris HCl, pH 8.0, 25 ml 2% agar.

(vii) 6-phosphogluconate dehydrogenase (6-PGDH) (E.C. 1.1.4.4): buffer system I; stain: 20 mg 6-phosphogluconate, 5 mg NADP, 5 mg MTT, 5 mg PMS, 10 mg magnesium chloride, 25 ml 0.06M Tris HCl, pH 8.0.

(viii) Phosphoglucose isomerase (PGI) (E.C. 5.3.1.9) (Harris & Hopkinson, 1978): buffer system I; stain: 20 mg fructose-6-phosphate, 5 mg NADP, 5 mg MTT, 5 mg PMS, 10 u glucose-6-phosphate dehydrogenase, 25 ml 0.06M Tris HCl, pH 8.0, 25 ml 2% agar.

(ix) Phosphoglucomutase (PGM) (E.C. 2.7.5.1): buffer system II; stain: 100 mg glucose-1-phosphate, 50 mg magnesium chloride, 10 mg NADP, 5 mg MTT, 5 mg PMS, 40 u glucose-6-phosphate dehydrogenase, 25 ml 0.06M Tris HCl, pH 7.1, 25 ml 2% agar.

Histological studies

Four specimens of the unknown morphotype, two from each location, were anaesthetized in 7.5% magnesium chloride, fixed in Bouin's fluid (made up in filtered seawater) and sectioned at 6 μm in 56 °C m.p. paraffin wax. Mallory's triple stain (Pantin, 1960) was used as standard.

Electrophoretic results and discussion

Allele frequencies for the ten enzyme loci which produced useful results for one or more of the species examined are presented in Table 1.

The results show that at nearly all loci there are large differences in allele frequency between sympatric samples of *Lineus longissimus* and the third morphotype from Spain, and between *Lineus viridis* and the unknown type from Llandudno, whereas between specimens of the form occurring both in Spain and North Wales differences in allele frequencies are very small. Over all the loci used, the genetic differentiation between the three species can be reduced to a single figure by using measures of genetic distance or similarity. Between *Lineus longissimus* and Spanish specimens of the new morphotype, and between *Lineus viridis* and Welsh examples of the new form, the value of Nei's (1972) genetic identity falls below 0.35 (Table 2). This indicates that not only are these likely to be separate species, but that they show genetic identity values lower than those usually found in congeneric comparisons (Ayala, 1975, 1983; Thorpe, 1982, 1983). However, similarly low values of genetic identity have been

Table 1. Allele frequencies at 11 loci for the species of heteronemerteans collected at Llandudno, North Wales, and in the Foz Estuary, northwestern Spain. Alleles are arranged in order of increasing mobility. Enzyme abbreviations are given in the materials and methods section.

Locus	Allele	*Lineus viridis*	*Lineus longissimus*	Unknown form	
				Llandudno	Foz
APA	1	0.000	0.940	0.000	0.000
	2	0.000	0.060	0.000	0.000
	3	1.000	0.000	1.000	1.000
GOT-2	1	0.000	0.000	1.000	1.000
	2	0.000	0.800	0.000	0.000
	3	0.000	0.200	0.000	0.000
	4	1.000	0.000	0.000	0.000
ICD-1	1	0.992	0.000	0.000	0.000
	2	0.000	1.000	0.000	0.000
	3	0.008	0.000	0.000	0.000
	4	0.000	0.000	1.000	1.000
ICD-2	1	0.052	0.000	0.000	0.000
	2	0.509	0.000	0.013	0.150
	3	0.431	0.833	0.154	0.300
	4	0.008	0.000	0.000	0.050
	5	0.000	0.167	0.820	0.450
	6	0.000	0.000	0.013	0.050
MDH-1	1	0.000	1.000	–	–
	2	0.008	0.000	–	–
	3	0.992	0.000	–	–
MDH-2	1	0.000	1.000	–	–
	2	0.297	0.000	–	–
	3	0.703	0.000	–	–
ODH	1	0.026	0.000	0.128	0.000
	2	0.000	0.000	0.115	0.250
	3	0.328	0.000	0.000	0.000
	4	0.000	0.944	0.757	0.750
	5	0.060	0.056	0.000	0.000
	6	0.224	0.000	0.000	0.000
	7	0.086	0.000	0.000	0.000
	8	0.233	0.000	0.000	0.000
	9	0.043	0.000	0.000	0.000
6-PGDH	1	0.000	–	0.743	0.700
	2	0.000	–	0.054	0.000
	3	0.000	–	0.176	0.300
	4	1.000	–	0.027	0.000
PGI	1	0.009	0.000	0.000	0.000
	2	0.966	0.000	0.000	0.000
	3	0.000	0.000	0.066	0.100
	4	0.025	0.000	0.000	0.000
	5	0.000	0.000	0.474	0.500
	6	0.000	0.100	0.000	0.000
	7	0.000	0.000	0.408	0.400
	8	0.000	0.900	0.000	0.000
	9	0.000	0.000	0.052	0.000
PGM	1	0.000	–	0.039	0.000
	2	0.000	–	0.132	0.150
	3	0.000	–	0.724	0.600
	4	0.000	–	0.079	0.250
	5	0.000	–	0.026	0.000
	6	0.042	–	0.000	0.000
	7	0.958	–	0.000	0.000

found between other species of nemerteans which, on morphological grounds, are considered congeneric (Williams *et al.*, 1983; Sundberg & Janson, 1988).

Distortion of gene frequencies due to inbreeding or the presence of clones is unlikely since there was substantial genetic variation within samples and two of the species showed good fits of allele frequencies to Hardy-Weinberg expectations (but note the statistical weakness of such tests on other than large sample sizes: Lewontin, 1958; Fairburn & Roth, 1980; Valenzuela, 1985). *Lineus longissimus* was not tested for fits of allele frequencies to Hardy-Weinberg expectations, since the sample size was too low.

Between specimens of the unknown morphotype from Spain and Wales the value of Nei's (1972) genetic identity is above 0.9. This value is within the range normal for conspecific populations and above the range expected for congeneric species, and indicates that the populations of this morphotype from the two geographically separate locations are very likely to be conspecific. The species represented by this morphotype thus apparently shows little genetic differentiation over a moderately large zoogeographical range. This suggests that it is likely to be sexually reproducing with a planktonic dispersive phase. Such a

Table 2. Pairwise comparisons based on isozyme data from *Lineus longissimus*, *Lineus viridis* and the unknown morphotype. Above diagonal, values for genetic distance; below diagonal, values for genetic identity (both calculated after Nei, 1972).

	L. longissimus	*L. viridis*	Unknown form	
			Llandudno	Foz
L. longissimus	+	2.364	1.386	1.280
L. viridis	0.094	+	3.912	2.847
Unknown (Llandudno)	0.250	0.020	+	0.018
Unknown (Foz)	0.278	0.058	0.982	+

suggestion is supported by the observations on one individual, which released tiny planktonic eggs into the surrounding seawater in a manner similar to that described for *Lineus lacteus* McIntosh, 1874, by Gontcharoff & Lechenault (1958).

Expected and observed values for mean heterozygosity per locus for these species were high (range 9.7–26.8%; Table 3), especially for *Lineus viridis* and the unknown form. Heterozygosity estimates for these two taxa are considerably higher than those typically found for most other eukaryotic species (generally about 5–15%; see Selander, 1977; Nevo, 1978; Nevo *et al.*, 1984) High values of heterozygosity have also been found previously in *Lineus torquatus* Coe, 1901 (Balakirev & Manchenko, 1984) and the hoplonemertean *Oerstedia striata* Sundberg, 1988 (Sundberg & Janson, 1988). These values are at variance with those of Williams *et al.* (1983), who described a complete absence of allelic diversity from *Lineus viridis*. This can partially be explained by the fact that these authors did not stain for ODH or ICD, the two enzymes which showed the largest allelic diversity in *Lineus viridis* during the present studies (observed heterozygosity for ODH = 0.775, for ICD = 0.553).

Table 3. Estimates of genetic variation in populations of *Lineus longissimus*, Lineus viridis and the unknown morphotype. H_e and H_o: mean expected and observed heterozygosities per locus respectively; $P_{(0.95)}$ and $P_{(0.99)}$: proportions of polymorphic loci with frequency of most common allele < 0.95 or < 0.99 respectively. n_e: mean effective number of alleles per locus.

	Lineus longissimus	*Lineus viridis*	Unknown form	
			Llandudno	Foz
H_e	0.126	0.178	0.217	0.268
H_o	0.097	0.145	0.163	0.254
$P_{(0.95)}$	0.375	0.273	0.636	0.545
$P_{(0.99)}$	0.375	0.545	0.636	0.545
n_e	1.625	2.454	2.500	2.128

It would seem that at least some species of nemerteans show heterozygosity values higher than most other groups of organisms (see Nevo, 1978; Nevo *et al.*. 1984). Such high values are apparently common in sea anemones and sponges (Solé-Cava & Thorpe, 1990, 1991). The reasons for such high levels of genetic variation are uncertain, but it has been suggested that genetic diversity may be related to environmental heterogeneity or predictability (Hedrick *et al.*, 1976). It may be of significance that, of the three species studied, *Lineus longissimus,* a predominantly subtidal taxon, had the lowest figures for heterozygosity. *Lineus viridis* and the new form are both intertidal taxa, and show exceptionally high values of heterozygosity. It could be argued that this difference occurs because the intertidal environment is far more heterogeneous and unpredictable than the subtidal, but it must be noted that in two similar species of *Oerstedia*, studied by Sundberg & Janson (1988), the subtidal species showed the greatest genetic diversity.

Comparison in external features between *Lineus longissimus*, *Lineus viridis* and the third morphotype

The external features of the unknown morphotype resemble both *Lineus longissimus* and *Lineus*

viridis, but close examination of living specimens reveals that all three taxa can be consistently distinguished from each other. Accounts of the external appearance of each of the three taxa are given below.

Lineus longissimus *(Gunnerus, 1770)*

Living specimens vary in length from 150–590 mm, in maximum width from 0.8–1.8 mm. Body width tends to remain uniform for up to about 70 mm from the tip of the head, but then gradually tapers posteriorly to end in a blunt tail. The body is typically somewhat dorsoventrally compressed.

The rather flattened head (Fig. 1A) is spatulate but appears short (length = twice width); it is characteristically slightly wider than the succeeding body region. The anterior tip of the head usually appears slightly bilobed. The dorsal cephalic surface is dark chocolate brown to black in colour, the anterior margins are white. Five pale and imprecisely defined longitudinal stripes occur on the dorsal cephalic surface, one median flanked on either side by two dorsolateral; the median stripe is typically white whereas the remaining stripes are pale brown. The cerebral ganglia may colour the posterior half of the head reddish. The ventral cephalic surface is usually paler than the dorsal and is marked by a single pale median stripe. Each lateral margin of the head bears a distinct horizontal cephalic furrow. There are 10–40 black or silvery-black eyes on each side of the head, forming an irregular patch near the anterior margin. In one specimen the ocelli were situated along the lower margin of the cephalic furrows. The slit-like mid-ventral mouth is long (5–6 mm) and commences just before or immediately after the posterior end of the cephalic slits.

The general body coloration is variable. The upper surface may be brown, dark chocolate brown or black; under artificial light it may appear maroon or exhibit a blue or purple iridescence. Five pale longitudinal stripes, continuous with those on the head, extend the full length of the body on the dorsal surface. The ventral surface may be the same colour as or slightly paler than the dorsal.

Lineus viridis *(Müller, 1774)*

Living specimens vary in length from 35–109 mm, in width from 0.4–1.5 mm, and exhibit a very variable body shape. Behind the cephalic region the body may be more or less uniform in width for up to about 60% of its length, then gradually narrow posteriorly to end in a finely pointed tail. Alternatively, the body width may increase behind the head for 7–26% of the body length, and

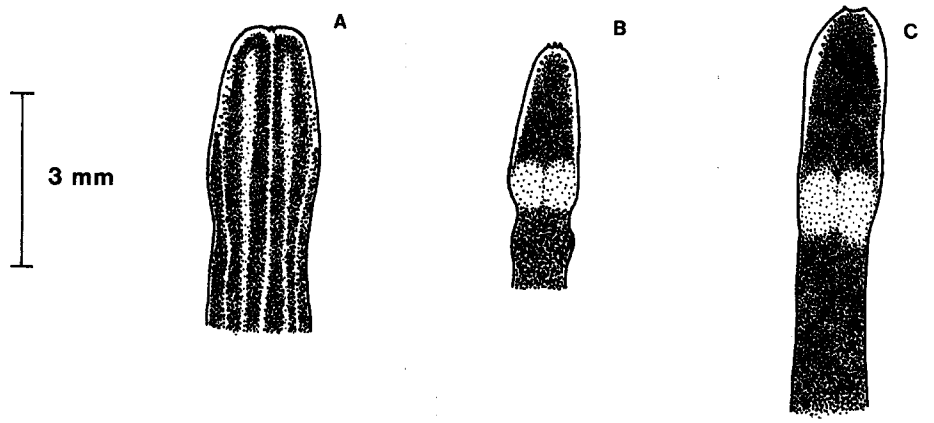

Fig. 1. The appearance of the heads, viewed in dorsal aspect, of A, *Lineus longissimus*, B, *Lineus viridis*, and C, the unknown morphotype, described in the present paper as *Riseriellus occultus* gen. et sp. nov.

then remain uniformly wide up to about 60% of its length before gradually narrowing to the tail. The body is compressed dorsoventrally, becoming progressively more flattened from the anterior to the posterior end.

The dorsoventrally flattened head (Fig. 1B) is generally spatulate, although its posterior portion is often markedly wider than the anterior to give it a spade-like shape. There is normally a shallow but distinct constriction between the head and the remainder of the body. The cephalic colour is variable, ranging from pale green through dark olive green to a deep bronze green or black. The cerebral ganglia are externally visible in the posterior part of the head as a pair of more or less distinct red patches. The margins of the head, which bear a single pair of lateral horizontal cephalic furrows, are pale. Two to eight black ocelli form an irregular and often asymmetrical dorsolateral row along either side of the anterior head region. The ocelli may be obscured by pigmentation, especially in darker coloured individuals. The small, mid-ventral and slit-like mouth commences just in front of or close behind the posterior end of the cephalic furrows. The oral margins are generally pale and often appear somewhat swollen.

The general body colour is also very variable. The dorsal surface may be black, deep bronze green, dark to light olive green or pale green. The colour intensity often shades from darker anteriorly to paler posteriorly. Pale annulations are normally distinguishable along the full length of the body and a pale mid-dorsal line may be present. In sexually mature specimens the gonopores show as an irregular dorsolateral row of white spots on each side of the body, commencing some distance behind the rear of the head. The ventral body surface is usually paler than the dorsal. When touched, the worms characteristically contract without coiling.

The new morphotype

Living specimens are 150–700 mm long and 1.0–1.3 mm wide. Their body width remains uniform for up to half the length, but then decreases posteriorly to terminate in a pointed tail.

The head (Fig. 1C) is spatulate and continuous with the succeeding body region, with no intervening constriction. In colour the head is very dark brown or green to black, with pale narrow lateral margins bearing a pair of horizontal cephalic slits. Three to eleven black ocelli form a dorsolateral row on each side of the anterior portion of the head. The posterior third of the head is often reddish due to the colour of the cerebral ganglia. The mid-ventral mouth begins far behind the brain lobes, approximately 1 mm beyond the rear end of the cephalic furrows; it is long, slit-like and possesses pale margins.

The body colour is variable. Anteriorly it may be black, greenish-black or dark olive, but the colour is nearly always lighter towards the posterior, shading to a dark or pale olive green or greenish-brown. The ventral surface may be similarly coloured or slightly paler. Under artificial light the body surface often shows a purple iridescence. Towards the posterior end of the body an irregular dorsolateral row of white spots on each side marks the position of the gonopores in sexually mature individuals. When touched, the worms tend to coil up into a tight spiral. The appearance of a complete specimen is shown in Fig. 2.

Description of the new taxon

Genus Riseriellus gen. nov.

Type species
Riseriellus occultus sp. nov.

Etymology
The genus is named in honour of Professor Nathan W. Riser as a tribute to his work on the morphology of nemerteans, in particular the Heteronemertea. The specific epithet, the Latin *occultus* (very secret), was chosen to indicate how the new taxon has previously been 'hidden' amongst *Lineus* species which externally it resembles.

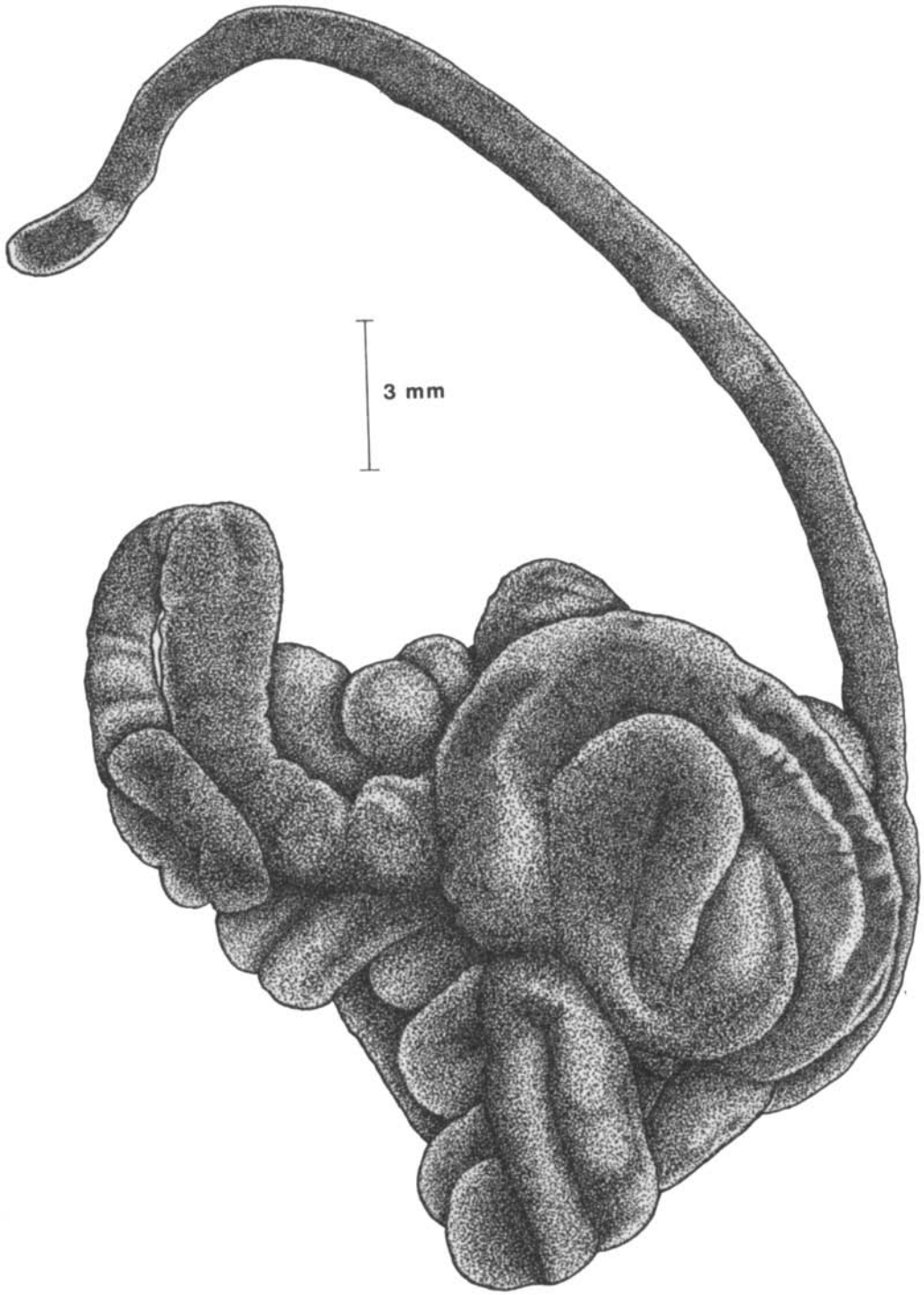

Fig. 2. The general appearance of a complete and somewhat contracted specimen of *Riseriellus occultus* gen. et sp. nov.

Diagnosis

Heteronemertea with a single pair of horizontal lateral cephalic slits; proboscis unbranched, containing two (outer circular, inner longitudinal) muscle layers and two muscle crosses; rhynchocoel circular muscles not interwoven with adjacent body wall inner longitudinal muscle fibres; dorsal fibrous core of cerebral ganglia forked only at rear into upper and lower branches; nervous system without neurochordal elements; foregut with neither somatic musculature nor subepithelial gland cell layer; dermis composed of well developed gland cell zone abutting directly against body wall outer longitudinal muscle layer, without distinct connective tissue stratum; body wall musculature without diagonal layer; caudal cirrus absent; intestinal diverticula indistinct; dorsoventral muscles missing from intestinal region; apical organ consisting of three separate sensory pits situated on tip of head; eyes present, arranged in row on either side of head; blood system comprising single spacious cephalic lacuna, foregut vascular plexus and three longitudinal vessels in intestinal region which are linked by pseudometameric transverse connectives; cephalic glands well developed but confined to dorsal half of head and not posteriorly reaching brain; excretory system situated between brain and mouth, collecting tubules penetrating post-cerebral blood lacunae; sexes separate.

Riseriellus occultus sp. nov.

Type specimens

The holotype, a female consisting of 168 slides of transverse sections, is deposited in the Liverpool Museum, William Brown Street, Liverpool L3 8EN, Registration Number 1992-7(01-168). (LIV); two unsectioned specimens are registered under number 1992. (LIV).

Type locality

Ría de Foz, north-western Spain (43° 34′ N, 7° 14′ W), first discovered by J. J. 20 September 1984 in seagrass beds (*Zostera noltii* Hornemann) in soft mud, upper shore.

Additional locations

Ría de Foz, in consolidated mud among roots of *Spartina* spp, upper shore, or in muddy sand with *Zostera noltii*; Llandudno, North Wales (53° 19′ N, 3° 49′ W), upper shore in damp fine mud or silt under stones and rocks; Rhosneigr, Anglesey (53° 15′ N, 4° 30′ W), upper shore in sandy-mud beneath boulders; Trwyn du Point, Anglesey (53° 18′ N, 4° 02′ W), upper shore in silty-sand, beneath boulders. In the Foz region recorded densities range from 6–131 worms m^{-2}; dominant associated fauna includes the polychaetes *Capitella capitata* (Fabricius), *Streblospio benedicti* Webster, *Pygospio elegans* (Claparède), *Heteromastus filiformis* (Claparède), *Alkmaria romijni* Horst, *Nereis* (= *Hediste*) *diversicolor* O. F. Müller and *Malacoceros* (= *Scolelepis*) *fuliginosus* (Claparède), the molluscs *Hydrobia ulvae* (Pennant) and *Scrobicularia plana* (da Costa), and the crustaceans *Melita palmata* (Montagu) *Idotea chelipes* (Pallas), *Chaetogammarus marinus* Leach, *Hyale nilssoni* (Rathke) and *Carcinus maenas* (Linnaeus), as well as unidentified species of Oligochaeta and Chironomidae. Mean grain sizes of sediments inhabited by the nemerteans are in the range 0.047–0.17 mm, whilst the silt-clay fraction varies from 16.8–73.15% and the organic content from 3.72–9.02%.

Body wall, musculature and parenchyma

The richly glandular epidermis, 25–30 μm in maximum thickness, closely agrees with the generalised heteronemertean form described by Norenburg (1985). Throughout most of the body it is dominated by large numbers of acidophilic rhabditoid glands (Fig. 3) which appear to correspond to the serous cells identified by Norenburg (1985). Rhabditoid density decreases markedly in the anterior ventral cephalic region, and the cells are completely missing from the epidermis on either side of the median furrow leading to the proboscis pore (Fig. 4); some reduction in their number is also evident in the posterior body regions. Throughout the body the epidermal basement lamina is thin but distinct.

Below the epidermis the subepidermal muscle layers (Figs 3, 5, 6) are well developed. The outer

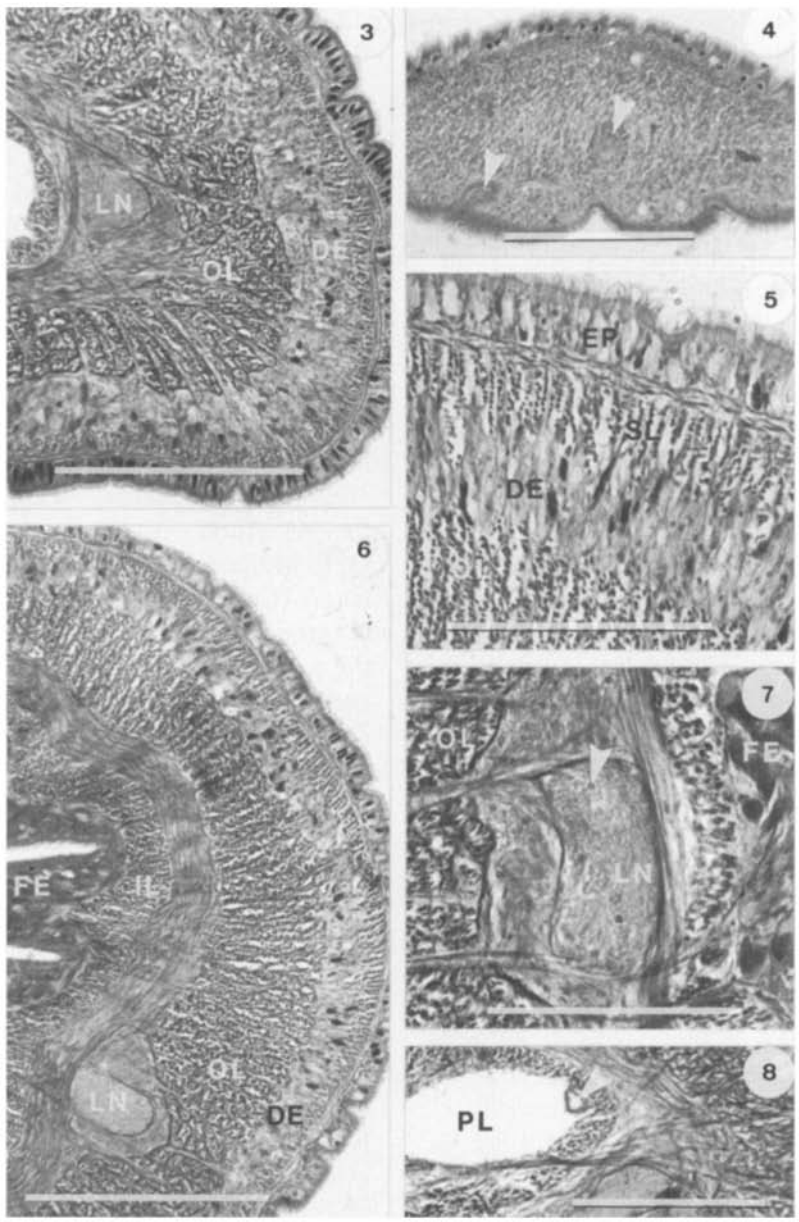

Fig. 3. Riseriellus occultus gen. et sp. nov. *3.* Transverse section between the brain and mouth to show the epidermal rhabditoid glands and body wall layers. Scale bar = 250 μm. *4.* Oblique transverse section near the tip of the head, showing two of the apical organ chambers (arrowheads) and absence of epidermal rhabditoid glands from the ventral surface. The ventral median furrow leads back to the proboscis pore. Scale bar = 200 μm. *5.* Part of the body wall in transverse section to show the structure of the epidermis, subepidermal muscle layers, dermis and outer portion of the main body wall outer longitudinal muscle layer. Scale bar = 100 μm. *6.* Transverse section through the foregut region to show the organisation of the body wall musculature. Scale bar = 250 μm. *7.* Transverse section through a lateral nerve cord in the foregut region, showing some of the radial muscle and connective tissue fibres which extend between the foregut wall and proximal portion of the epidermis. A myofibrillar (neuromuscular) strand in the fibrous tissue of the lateral nerve is indicated by an arrowhead. Scale bar = 100 μm. *8.* Transverse section through one of the post-cerebral lateral lacunae to show some of the horizontal transverse muscle bands extending peripherally above a lateral nerve cord. The arrowhead points to an excretory tubule running in the lumen of the blood lacuna. Scale bar = 200 μm. DE = dermis; EP = epidermis; FE = foregut epithelium; IL = body wall inner longitudinal muscle layer; LN = lateral nerve cord; OL = body wall outer longitudinal muscle layer; PL = post-cerebral blood lacuna; SL = subepidermal longitudinal muscle layer. All photomicrographs of sections stained with Mallory.

circular layer, 3–5 μm in maximum thickness, extends to the tip of the head. In contrast, the inner longitudinal fibres, particularly in the anterior ventral cephalic region, are barely distinguishable in front of the proboscis pore, whereas for most of the body length they are 15–30 μm deep (Fig. 5) and proximally abut directly against the well developed dermal gland cell layer.

The dermis is mostly 45–60 μm thick and composed entirely of gland cells (Fig. 5); a laminated connective tissue layer, typical of many heteronemertean taxa, is completely missing. Internally the dermal glands are bordered by the fibres of the main outer longitudinal body wall muscle layer (Fig. 5). Both bacillary and mucous dermal glands (Norenburg, 1985) are abundant, their distal portions in many places extending peripherally to discharge on the body surface. In the cerebral region and further forward the dermal gland cell layer becomes progressively reduced and, in front of the brain, it loses its integrity such that the glands cannot with certainty be distinguished from the cephalic and other subepidermal glands of the head. Behind the mid-foregut region the dermis is only about 20 μm thick and gradually decreases posteriorly.

The main body wall muscles comprise the typical heteronemertean arrangement of outer longitudinal, middle circular and inner longitudinal layers (Fig. 6), respectively 60–135 μm, 20–40 μm and 15–45 μm thick in the foregut region. There is no diagonal muscle layer. All three muscle layers are considerably reduced in thickness in the posterior portion of the body. The bundles of outer longitudinal muscle fibres are distinguishable from those of the subepidermal longitudinal layer by being enclosed in well developed connective tissue membranes which stain an intense blue colour with Mallory; the subepidermal muscles lack these membranes. Throughout the foregut region large numbers of radially oriented muscle and connective tissue fibrils (Fig. 7) extend peripherally from the wall of the gut, pass through the body wall layers and penetrate the extreme proximal portion of the epidermis. On either side of the mouth these radial muscle strands are reinforced by oblique muscles which have their origin at the outermost surface of the circular muscle coat.

The musculature between the mouth and brain is complex. Close in front of the mouth, where the anterior margins of the buccal chamber bulge forwards, several radial muscle fibrils merge to form thicker transverse bands of horizontal muscles which cross the body below the rhynchocoel. Laterally some of the fibres of these bands extend towards the body margins, passing either above (Fig. 8) or below the lateral nerve cords to terminate among the dermal gland cells. Further forwards, in front of the buccal region, the transverse muscle bundles split up to provide a meshwork of fibres criss-crossin the central part of the body between the lateral nerves and below the rhynchocoel. At this level the main circular muscle layer, which ventrally separates behind the mouth, remains incomplete but laterally gives off isolated fibres which merge with those of the meshwork. Close behind the brain, however, the circular layer for a short distance again forms a complete band of muscle fibres, but then begins to break up as it nears the rear of the cerebral sensory organs and in the cerebral ring is evident only as isolated fibres with a predominantly circular orientation.

The cephalic musculature consists of a loose meshwork of diagonal and oblique fibres, between which the various glands of the head are distributed (Fig. 9). These muscle fibres originate either in the body wall outer longitudinal layer, some of whose fibres turn obliquely inwards throughout the length of the head, or in the circular muscle layer which encloses the rhynchodaeum and cephalic blood lacuna. A layer of longitudinal muscle fibres, about 15–20 μm thick, is situated between the blood lacuna and circular muscles, the two muscle coats together forming a muscle cylinder similar to that described for many other heteronemerteans (Figs 9, 10).

Parenchymatous connective tissues are nowhere extensive; they show their greatest development in the intestinal region adjacent to the blood vessels and between these and the gut wall.

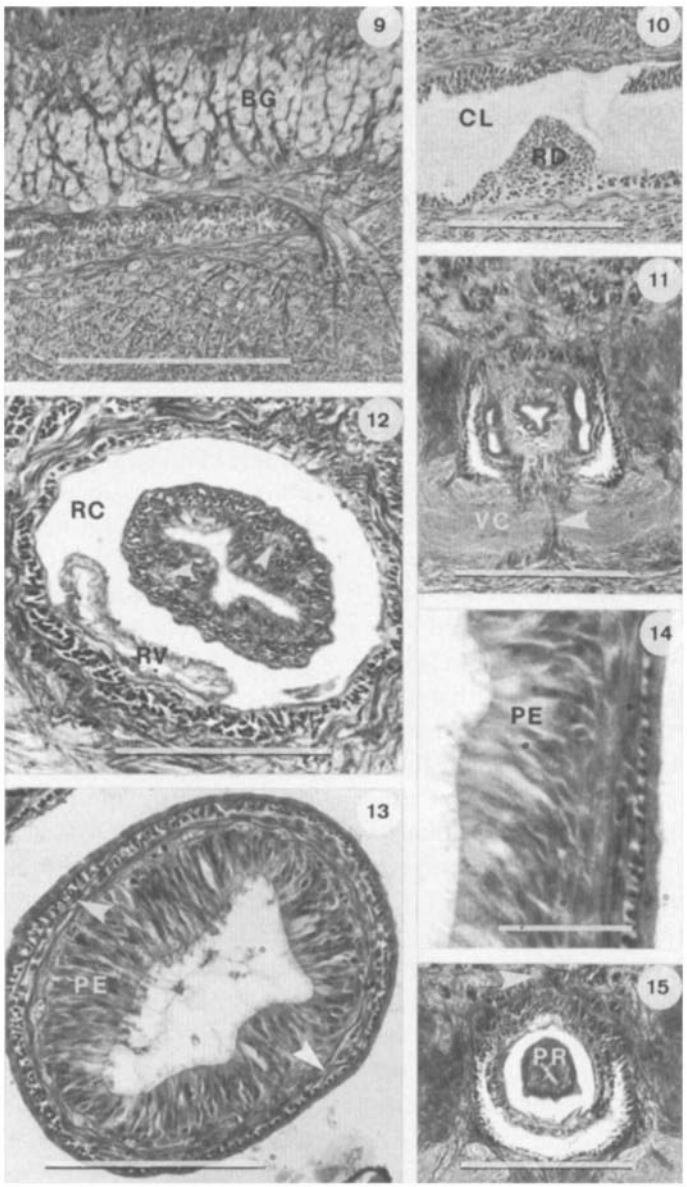

Fig. 4. Riseriellus occultus gen. et sp. nov. *9.* Transverse section through the cephalic region to show the basiphilic cephalic gland lobules and fibres of the central muscle cylinder. Scale bar = 250 μm. *10.* Transverse section through the middle cephalic region to show the single spacious blood lacuna and the rhynchodaeum. Scale bar = 200 μm. *11.* Transverse section through the brain region above the ventral cerebral commissure to show fibres leading to the proboscis insertion, indicated by an arrowhead, passing through the commissure. Scale bar = 200 μm. *12.* Transverse section to show the construction of the anterior portion of the proboscis, the two distinct muscle layers in the rhynchocoel wall and the rhynchocoelic villus. Arrowheads indicate the two large proboscis nerves. Scale bar = 100 μm. *13.* Transverse section to show the construction of the main portion of the proboscis. The position of the two muscle crosses are indicated by arrowheads. Scale bar = 100 μm. *14.* An oil immersion photomicrograph through part of the proboscis in longitudinal section to show the ciliation of its epithelium. Scale bar = 25 μm. *15.* Transverse section through the cerebral region to show the U-shaped blood channel arching below the rhynchocoel. The median dorsal nerve extending back from the dorsal cerebral commissure is indicated by an arrowhead. Scale bar = 200 μm. BG = basiphilic cephalic gland lobules; CL = cephalic lacuna; PE = proboscis epithelium; PI = proboscis insertion; PR = proboscis; RC = rhynchocoel; RD = rhynchodaeum; RV = rhynchocoelic villus; VC = ventral cerebral commissure. All photomicrographs of sections stained with Mallory.

Proboscis apparatus

The ventral, subterminal proboscis pore commences as a longitudinal invagination of the body wall, which internally expands to form an open tubular canal extending back for a short distance before closing off ventrally. For most of the cephalic length the rhynchodaeum appears as a simple tubular duct running below the blood lacuna, lined by a ciliated but non-glandular epithelium 10–12 μm thick, enclosed by longitudinal fibres of the cephalic muscle cylinder (Fig. 10). In the posterior part of the head, however, it begins to expand dorsoventrally, for a while partially protruding into the lumen of the blood channel, until the latter divides posteriorly to form a pair of spacious lacunae located on either side of the rhynchodaeum. The posterior portion of the rhynchodaeum is surrounded by a sphincter-like layer of circular muscles immediately in front of the proboscis insertion; the insertion is located in the brain ring above the ventral cerebral commissure (Fig. 11), muscle fibres leading to it from the body wall outer longitudinal muscle layer passing through a fenestration in the commissure and effectively splitting it into anterior and posterior portions.

The rhynchocoel reaches almost to the posterior tip of the body. Its wall contains separate longitudinal and circular muscle layers (Fig. 12), respectively some 7–8 μm and 15–18 μm in maximum thickness. The circular muscle layer is not interwoven with the fibres of the adjacent body wall inner longitudinal musculature, as in some heteronemertean genera.

The proboscis is unbranched and small; it is less than one-third the length of the body and has a maximum retracted diameter of about 180–200 μm. For most of its length it consists of a glandular epithelium 15–40 μm thick, a delicate nerve layer in which up to 10–12 distinct neural swellings can be distinguished, an outer circular muscle layer 4–6 μm in maximum thickness, an inner longitudinal muscle coat 6–8 μm across and an endothelium 4–5 μm deep (Fig. 13). There are two weakly developed muscle crosses extending from the circular musculature to the endothelial lining. The organisation of the proboscis musculature identifies the nemerteans as members of the family Lineidae, as defined by Gibson (1985). A short anterior region, extending from the proboscis insertion, presents a simpler construction. This region (Fig. 12) comprises an epithelium, 6–10 μm tall, in which there are fewer gland cells than in the main portion of the organ, a longitudinal muscle layer 6–20 μm thick in which two large nerves, 12–15 μm in diameter, can be distinguished, and an endothelium. An unusual feature of the proboscis, not reported for any other nemertean species, is that throughout its length the epithelium is ciliated (Figs 13, 14); basal bodies of the cilia can just be made out as minute dark spots under oil immersion. Riser (pers. comm.) reports that in all the lineid species he has examined, the proboscis epithelium contains proboscidial spines (Riser, 1990) but none have been found in the present species.

Blood system

The cephalic blood supply comprises a single spacious thin-walled median lacuna (Fig. 10), extending from near the tip of the head back to just in front of the proboscis insertion. As the rhynchodaeum posteriorly begins to expand dorsoventrally, the cephalic lacuna becomes increasingly constricted mid-dorsally and eventually divides into a pair of spacious lateral channels which flank the posterior portion of the rhynchodaeum. These lacunae become progressively smaller as they approach the brain and enter the cerebral ring. Near the front of the ventral cerebral commissure the lacunae meet ventrally to form a U-shaped duct (Fig. 15) which continues back beyond the commissure, at one point becoming temporarily subdivided by the ventral muscle fibres leading to the proboscis insertion (Fig. 11). Near the rear of the brain the duct separates medially to form two compressed channels, each of which arches dorsolaterally outwards over, but not bathing, the cerebral sensory organs. The inner ventral portions of these channels again meet medially, so that between the brain and mouth there are essentially three longitudinal blood spaces, the lateral pair on either side of the rhynchocoel being the larger (Fig. 8). Both these

and the median ventral channel are irregularly traversed by oblique and radial fibres derived from the median muscle meshwork; all three lacunae sometimes rejoin each other for a short distance, but their subdivision is such that in some sections there may appear to be four or five separate channels. Near the front of the mouth the various blood spaces fuse to form two spacious dorsolateral lacunae which, above the buccal region, begin to split up to form the origin of the well developed foregut vascular plexus (Fig. 16). Throughout the length of the foregut the dorsolateral lacunae flanking the rhynchocoel remain larger than any of the plexal branches.

The mid-dorsal blood vessel arises as a median dorsal branch of the U-shaped duct close to the rear of the ventral cerebral commissure. It immediately penetrates the floor of the rhynchocoel to form the rhynchocoelic villus (Figs 12, 17), which reaches back for most of the foregut length before emerging to continue to the end of the body.

In the intestinal region there are three spacious longitudinal vessels, irregularly linked by transverse channels arching around the intestinal wall. These vessels possess distinct walls in which occasional muscle fibres can be distinguished.

Nervous system

The brain is well developed; dorsal and ventral lobes are similar in volume but the ventral are much more elongate and extend some distance behind the dorsal, ending near the posterior limits of the cerebral sensory organs. Fibrous tissues of the dorsal ganglia are forked only at their rear into upper and lower branches. The dorsal cerebral commissure, about 15 μm in diameter, is located slightly anterior to the much thicker (90 μm) ventral commissure; an unusual feature of the latter is that it is effectively divided into anterior and posterior portions by muscle fibres leading to the proboscis insertion (Fig. 11). Most of the brain contains no trace of either an inner or outer neurilemma, but delicate connective tissue membranes can be distinguished in the posterior cerebral regions near the sensory organs and origins of the lateral nerve cords; there are no neurochord cells in the ganglionic tissues. The outer surface of the brain lobes is irregularly defined as a consequence of the intermeshed radial and oblique muscle fibres which freely penetrate and pass through the neuroganglionic cerebral components.

The lateral nerve cords lead directly from the rear of the ventral ganglionic lobes; they do not contain neurochords but isolated muscle fibrils (myofibrillae) can be discerned in their fibrous cores (Fig. 7). Throughout their length the lateral nerves possess thin but distinct inner and outer connective tissue neurilemmae.

The peripheral nervous system is well developed and generally similar to that of many other heteronemertean taxa. Large numbers of cephalic nerves lead forwards into the head and can be followed for some distance between the fibres of the cephalic muscle meshwork (Fig. 9). The neural layer extending around the outer surface of the body wall circular muscle coat (Fig. 6) contains a large mid-dorsal nerve, originating from the rear of the dorsal cerebral commissure. From the inner margin of each ventral ganglionic lobe a distinct nerve separates off, runs back some distance among the neuroganglionic tissues and then emerges to continue between the fibres of the median muscle meshwork. Behind the brain the two nerves, which lead to the foregut, are linked by several slender transverse connectives before they move apart to pass on either side of the mouth just below the 'salivary glands' which surround the oral aperture (Fig. 18). Behind the mouth the two nerves begin to branch profusely, forming a nerve network adjacent to the foregut epithelium; there is no post-oral commissure connecting the foregut nerves and the ultimate fate of the network could not be traced.

Alimentary canal

The long, slit-like ventral mouth commences about 1 mm beyond the posterior limit of the cephalic furrows, and is situated some distance behind the brain. It opens into a buccal region whose epithelium is surrounded by large numbers of subepithelial 'salivary glands' of several types (Figs 18, 19); below the ventrolateral buccal bor-

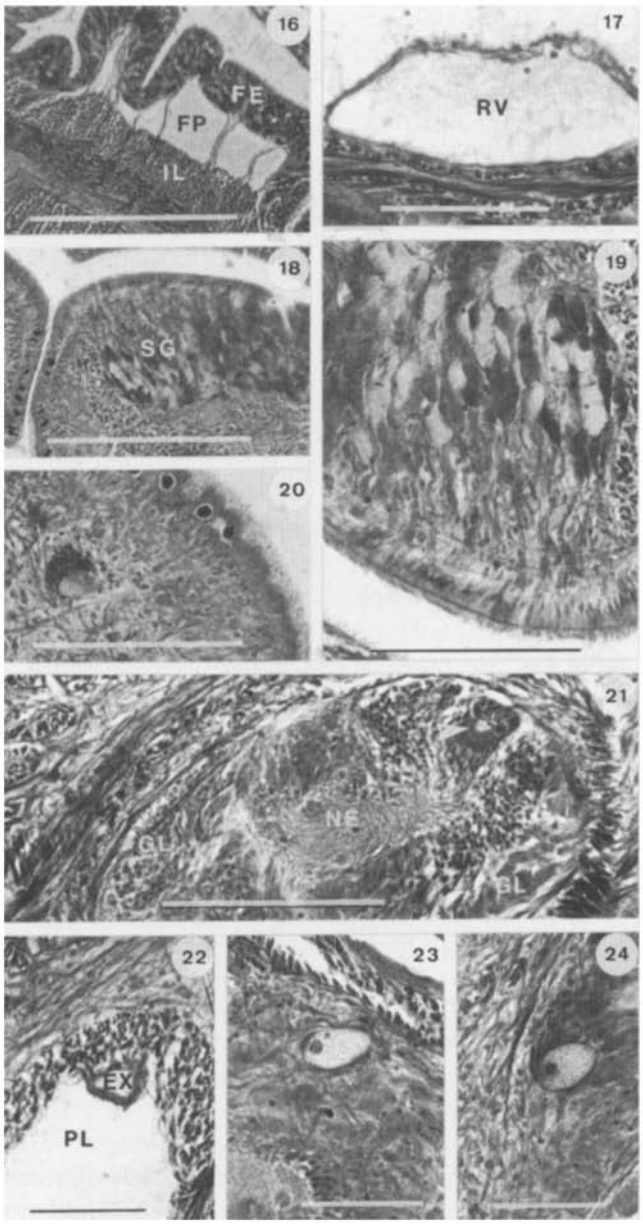

Fig. 5. Riseriellus occultus gen. et sp. nov. *16.* Part of the foregut region in transverse section to show branches of the vascular plexus. Scale bar = 250 μm. *17.* Transverse section through the posterior region of the rhynchocoelic villus. Scale bar = 50 μm. *18.* Transverse section through the mouth region to show the 'salivary glands' situated below the buccal epithelium. Scale bar = 250 μm. *19.* Enlargement of the 'salivary glands' to show the different types of constituent cells. Scale bar = 100 μm. *20.* Part of the head in transverse section to show one of the pigment cup ocelli. Scale bar = 100 μm. *21.* Transverse section through the middle portion of a cerebral sensory organ. The ciliated cerebral canal, in which major and minor canals can just be made out, is indicated by an arrowhead. Scale bar = 100 μm. *22.* Transverse section through one of the post-cerebral blood lacunae to show an excretory tubule running in its lumen. Scale bar = 50 μm. *23, 24.* Transverse sections through parts of the cerebral ganglia to show two of the parasites, possibly sporozoan, described in the text. Scale bars = 50 μm. EX = excretory tubule; FE = foregut epithelium; FP = branch of foregut vascular plexus; GL = glandular components of cerebral sensory organ; IL = body wall inner longitudinal muscle layer; NE = neural component of cerebral sensory organ; PL = post-cerebral blood lacuna; RV = rhynchocoelic villus; SG = 'salivary glands'. All photomicrographs of sections stained with Mallory.

ders these glands may extend 90 μm or more below the epithelium. The anterior portion of the buccal region, possibly as a consequence of contraction, bulges forwards, in sections appearing as a pair of large pouches reaching below the post-cerebral blood lacunae. A similar arrangement has been described for several other heteronemertean taxa.

The main foregut (Figs 6, 16) is lined by a richly glandular, ciliated and folded epithelium, up to 100 μm or more in maximum height. There is no subepithelial gland cell layer, nor somatic musculature, associated with the main foregut region.

The intestine is typically heteronemertean in appearance, its gastrodermal lining being up to 75–80 μm or more thick and containing enormous numbers of acidophilic glands. The lateral margins of the intestine are pouched but do not, as in many taxa, form distinct diverticula. Dorsoventral muscle bundles, typical of the intestinal region in many nemerteans, are completely missing from the present species. The anus opens at the posterior tip of the body.

Sensory organs

Three to eleven black ocelli are arranged in a dorsolateral row on either side of the anterior part of the head. Individual eyes are 30–35 μm in diameter and possess a simple pigment cup construction (Fig. 20). In sections the pigment shows as dense accumulations of minute dark brown granules.

The shallow lateral cephalic furrows extend from the tip of the head back to the rear cerebral region. As they pass the brain they begin to expand internally to form longitudinal chambers whose epithelium lacks gland cells but is densely ciliated. At about the level where upper and lower branches of the dorsal brain lobes separate, the inner wall of the chambers develops longer cilia (20–25 μm) and are associated with a zone of neuroganglionic tissue 25 μm or more deep. The ciliated cerebral canals lead obliquely inwards from this point, extending towards the upper branches of the dorsal cerebral lobes. As the canals reach the brain they meet further neuroganglionic masses and ventral accumulations of glandular tissue. Each canal, about 20 μm in diameter, then turns posteriorly to run along the outer dorsolateral border of the appropriate cerebral sensory organ (Fig. 21). The organs are intimately attached to the brain tissues and have a typically heteronemertean construction. Major and minor canals can clearly be distinguished in the posterior portions of each cerebral canal, just before they turn inwards to end amongst an accumulation of vacuolate glands. Each cerebral organ has a maximum diameter of about 120–150 μm.

Apical organ and cephalic glands

The apical sensory organ consists of three separate ciliated chambers, each about 45 μm in diameter, opening ventrally near the tip of the head (Fig. 4). The median chamber is in front of the ventrolateral pair and opens at the front of the proboscis pore furrow, the other two open on the ventrolateral cephalic surface slightly further back. The epithelium lining the chambers is 5–6 μm thick, densely ciliated and completely lacks gland cells.

Typical basophilic cephalic gland lobules are well developed and extensive, but are confined to the dorsal half of the head (Fig. 9) and commence behind the level of the proboscis pore. They form a more or less well defined mass of glands interspersed between the fibres of the cephalic muscle meshwork. Posteriorly the glands do not quite reach the anterior borders of the brain.

Excretory system

The thick-walled excretory tubules are located between the brain and mouth regions. They are 20–25 μm in diameter and mostly run immediately adjacent to the dorsal walls of the post-cerebral lateral blood lacunae. Up to two or three tubules can be distinguished on either side of the body. The posterior portions of the collecting tubules usually penetrate and may extend freely into the lumen of the blood lacunae (Figs 8, 22). A single efferent canal leads from the anterior region of the excretory system on either side of the body to open via a dorsolateral nephridiopore.

Reproductive system

The sexes are separate. Gonads are distributed in dorsolateral rows throughout most of the intestinal region of the body, but are missing from the extreme anterior portion. Testes of specimens collected during late September possess open gonoducts on their dorsolateral body surface. Each testis appears as a somewhat bilaterally compressed pouch lined by an undifferentiated epithelium up to 15 μm thick; in the lumen of many testes small numbers of spermatozoa can be distinguished and it appears that the animals had discharged their gonadal contents shortly before they were found.

Ovaries appear as large pouches containing masses of eggs, most of which are in a similar stage of development. More than 20 such eggs can be counted in any one ovary, and smaller numbers of immature ova can also be found attached to the ovarian wall. Larger eggs, 100 μm or more in diameter, contain a nucleus about 40 μm across in which a single nucleolus 10–12 μm wide is distinguishable. Females collected with mature males also possess open gonoducts, and in the laboratory one individual released eggs. The discharge of the eggs is very similar to that described for *Lineus lacteus* (Rathke) by Gontcharoff & Lechenault (1958); strings of eggs are released from the gonopores but quickly break up so that individual eggs are free. This differs strikingly from the egg-laying shown by *Lineus ruber* (O. F. Müller) and *Lineus viridis* (O. F. Müller), both of which lay their eggs in mucous sheaths attached to rock or algal surfaces.

Parasites

Several of the specimens examined contain parasites of various types. In the intestine gregariniform protozoans 45 μm or more long, containing a small nucleus in which two nucleoli are distinguishable, are often partially embedded in the gastrodermal wall. Other, possibly sporozoan, parasites are irregularly distributed in a variety of body tissues, including the brain, muscle layers and cephalic glands; these parasites are oval in shape, 30 μm long by 20 μm wide, and filled with a finely particulate cytoplasm in which a distinctly excentric acidophilic nucleus 5 μm wide is visible (Figs 23, 24). The parasites appear to be encapsulated in a thin but distinct membrane. A third type of parasite was found in smaller numbers in the epidermis and dermis; this appears as a large (60 μm or more diameter) more or less spherical cavity containing a membrane-bound amorphous mass of tissue in which no particular structure can be discerned. The appearance of these parasites bears some resemblance to the morula-like masses produced by the plasmodial generation of orthonectids, which are known to occur in a number of different invertebrates including nemerteans (Stunkard, 1982; Vernet, 1990).

Ecology

In the Foz Estuary the nemerteans are found in fairly compacted but wet muddy sediments in the upper shore zone, especially among the roots of *Spartina* or among *Zostera* beds, whereas at the same locality *Lineus longissimus* occurs at the mouth of the estuary under stones at low tide level. At Llandudno and on Anglesey *Lineus viridis* and the new species do not exhibit a similar spatial separation, both being found on the upper shore in silty mud beneath boulders.

Systematic discussion

Gibson (1985), in emphasising the need for complete descriptions of nemerteans, discussed the anatomy of the heteronemertean proboscis and proposed that all those genera with outer circular and inner longitudinal muscle layers should be grouped in the family Lineidae. Like many other authors (e.g., Wijnhoff, 1914), however, Gibson overlooked the significance of the nervous system in the determination of proboscis muscle layer homologies. Norenburg (1993) elegantly demonstrates that the evolution of the proboscis among heteronemerteans is more complex than previously appreciated and both discusses and illustrates several different proboscis morphotypes. His 'palaeotype', in which the two muscle layers (outer circular, inner longitudinal) are both subneural, is characteristic of the Lineidae as defined by Gibson, although Norenburg states that it seems more appropriate to consider the morpho-

logically diverse lineids as a suborder, the Lineiformes. The proboscis of the new species possesses a 'palaeotype' organisation, and on this basis the species is placed in the Lineidae *sensu* Gibson (Lineiformes *sensu* Norenburg).

Some 29 heteronemertean genera are known or

Table 4. Some of the morphological features commonly used for distinguishing between heteronemertean genera, summarised for those forms that are known to, or probably, possess a 'palaeotype' proboscis; data from Gibson (1990b), Gibson & Qi Sang (1991), Iwata (this volume) and Riser (this volume).

Genus	1	2	3	4	5	6	7	8
Amniclineus	0	0	0	c	+	0	0	+[a]
Antarctolineus	2	0	0	1	+	?	+	+
Apatronemertes	2	0	0	1	0	0	0	+[a]
Australineus	2	+	0	1	0	0	0	+
Corsoua	2	+	0	c	+	0	+	0
Craticulineus	0	+	+	c	0	0	0	0
Eousia	2	0	0	1	+	+	+	+
Euborlasia	1–2	+	0	c	0	0	+?	+
Flaminga	1	0	0	c?	+	+	0	0
Hinumanemertes	2	0	0	c + 1?	0	+	0	+
Kirsteueria	1	0	0	1	0	0	0	+
Lineopsella	0–2	0	0	c	0?	0	0?	+
Lineopselloides	0	0	0	c	0	0	+	+
Lineus	0–2	0– +	0	c/1	0	0	+	0– +
Micrella	2	0	0	?	0	+	0	+
Micrellides	2	0	0	c	0	?	0	+
Micrura	0–2	0– +	0	0/c/1	0	0– +	0	+
Micrurimorpha	2?	0	0	?	0	0	0?	+
Micrurinella	2	0	+	?	0?	?	0?	?
Neolineusz	0	0	0	?	0	0	+	+
Paralineopsis	0	0	0	0	0	0	+	0
Paralineus	2	0	0	?	0	0	0	0
Paramicrurinella	0	0	+	c	0	0	0	0
Planolineus	1	0	0	?	0?	?	?	?
Pontolineus	2	0	0	?	+	0?	0	0
Pussylineus	2	0	0	?	+	0	0?	?
Siolineus	2	0	0	1	0	0	0?	+
Tenuilineus	?	0	0	0?	0	0	+	+
Utolineus	0	0	0	c	+	0	+	+
Riseriellus gen. nov.	2	0	0	0	0	0	0	+

1 = Number of muscle crosses in proboscis.

2 = Dermis with (+) or without (0) distinct connective tissue layer between gland cells and body wall outer longitudinal musculature.

3 = Circular muscles of rhynchocoel wall interwoven between fibres of adjacent body wall inner longitudinal musculature (+) or quite separate (0).

4 = Foregut somatic musculature absent (0), circular (c) or longitudinal (1).

5 = Nervous system with (+) or without (0) neurochord cells.

6 = Caudal cirrus present (+) or absent (0).

7 = Foregut with (+) or without (0) subepithelial gland cell layer.

8 = Blood system in foregut region developed into plexus (+) or consiting of only t<wo vesels running in parenchyma (0).

[a] The foregut vascular plexus in *Amniclineus* (Gibson & Qi Sang, 1991) and *Apatronemertes* (Wilfert & Gibson, 1974) is intimately associated with components of the excretory system, the unusual arrangement in these taxa being interpreted as a consequence of their freshwater habits.

suspected to possess a 'palaeotype' proboscis (Gibson, 1985, 1990a, b; Gibson & Qi Sang, 1991; Iwata, this volume; Riser, this volume), although in a few (e.g., *Tenuilineus*) the proboscis is regarded as transitional to the 'heterotype' because it possesses a short additional outer longitudinal muscle layer just behind the proboscis insertion. Commonly several morphological features have been used as a means of distinguishing between the numerous heteronemertean genera and these are summarised for the 'palaeotype' group in Table 4.

The present specimens have a foregut vascular plexus and proboscis containing two muscle crosses, their rhynchocoel circular muscles are quite separate from the adjacent body wall inner longitudinal muscle fibres, they have no dermal connective tissue layer, neurochords nor caudal cirrus, and their foregut possesses neither somatic muscles nor subepithelial glands. Two further features of the present taxon have not been described for any other nemertean species; these are the ciliated nature of the proboscis epithelium, and the manner whereby the ventral cerebral commissure is divided by muscle fibres leading to the proboscis insertion. It is concluded that the combination of morphological characters shown by the nemerteans is such that they cannot be placed in any of the known heteronemertean genera with a 'palaeotype' proboscis and the new genus *Riseriellus* is accordingly established for them.

The morphological evidence used to establish the new genus and species is fully vindicated by the electrophoretic data discussed earlier; indeed, it was these biochemical investigations which first showed that the taxon, though in its external features very similar to both *Lineus longissimus* and *Lineus viridis*, was in fact significantly different from them. This is the first time that the two approaches have been used in conjunction with each other to establish a new nemertean taxon; other 'lineid' species are being similarly investigated and results will be reported in future publications.

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