Hydrobiologia **180**: 17–33, 1989.
J. L. Kaster (ed.) Aquatic Oligochaete Biology.

Phylogeny and origins of Enchytraeidae

Kathryn A. Coates
Department of Invertebrate Zoology, Royal Ontario Museum, 100 Queen's Park, Toronto, Ontario, Canada M5S 2C6

Key words: Enchytraeidae, Achaetinae, phylogenetics, *Marionina*

Abstract

Phylogenetic analyses of Achaetinae (Enchytraeidae: Oligochaeta) and other bisetate enchytraeids indicate that the achaetines include the earliest species of Enchytraeidae but that Achaetinae is not monophyletic. The earliest species of bisetate enchytraeid now extant are restricted to South America, Africa, and India. As this part of the enchytraeid lineage is ancestral to other enchytraeid taxa, it is suggested that Enchytraeidae may have arisen in South America or a contiguous Southern land mass.

Less than 50% of the 11 genera of Enchytraeidae considered are supported by the results of these analyses as evolutionary or phylogenetic groups. Five are substantiated as monophyletic: *Achaeta, Lumbricillus, Fridericia, Randidrilus,* and *Enchytronia*. In addition to most of the achaetine genera, *Marionina* is shown to be in great need of revision. Its recognition is a continuing source of confusion to taxonomic resolution of Enchytraeidae.

Introduction

'Traditional classifications generally called "natural" or "evolutionary" are often considered "multi-purpose systems", expressing both phenetic and cladistic relationships. The extent to which classifications are based on one or the other is entirely up to the individual taxonomist and afterwards it is not deducible from the resulting system. Nevertheless, these are the systems generally used for all kinds of evolutionary considerations. It is exactly their dual foundation on both phenetic and cladistic information which makes them, however, totally unsuitable for such considerations (Bremmer & Wanntrop, 1978, p. 328).'

Review of evolutionary studies on aquatic oligochaetes (Coates, 1987a) revealed that up to 1986 phylogenetic methods had rarely been employed to establish unambiguous classifications of this group. Moreover, the family Enchytraeidae has also been given less attention in terms of classical taxonomic studies than other families of aquatic oligochaetes. The most recent subfamilial classification of Enchytraeidae is by Cernosvitov (1937). It is very rarely used, primarily because the subfamilial relationships of Enchytraeidae are not well-supported and, as a consequence, the subfamilial classification is not very illuminating to taxonomic problems. Traditional and cladistic methods were previously employed to evaluate the taxonomy and phylogenetic position of *Propappus* (Coates, 1986, 1987b). That genus has been regarded as the stem group for Enchytraeidae because of its unique characteristics within the family and because those same characteristics were shared with members of wholly aquatic oligochaete families.

The major results following from the reassessment of *Propappus* were the recognition of this as the sole genus of a new family Propappidae and the recognition of some possible outgroups/sister groups to Enchytraeidae. For the analytical methodology that I employ some criterion for evolutionary rooting is required and a frequently adopted and well-defended criterion for this polarity decision is an outgroup (Stevens, 1980; Wiley, 1981; Kluge & Strauss, 1985).

A bisetate condition, two setae in each of four setal bundles per segment, was also recognized as potentially the primitive state for Enchytraeidae. Nevertheless, although this state may underlie much of the evolution of the oligochaetes, there is no evidence in my analyses for or against it as the ancestral state of all oligochaetes. In Enchytraeidae, the subfamily Achaetinae (Cernosvitov, 1937) includes most enchytraeid species with two setae per bundle as well as *Achaeta* with no setae. About ²/₃ of the species of Achaetinae are only found in the Southern Hemisphere, with more than ¹/₃ known only from South America (Table 1). This subfamily and other bisetate enchytraeid species were analysed by cladistic methods to determine the validity of Achaetinae as a monophyletic group and the possibilities of recognizing it as the most primitive clade in Enchytraeidae.

Phylogenetic analysis also enables a close look at characters and hypothesized homologies underlying the existing classification of Enchytraeidae.

Table 1. Distribution of Achaetinae. Abbreviations: SA, South America; IND, India; AUST, Australia; EUR, Europe; NA, North America; AF, Africa, COSMO, Cosmopolitan

Genus	Distribution of species	Number of species (northern)
Achaeta	SA, IND, AUST, EUR, NA	25 (16)
Hemienchytraeus	SA, AF, IND, EUR	13 (1)
Guaranidrilus	SA, EUR, NA	12 (2)
Tupidrilus	SA	4 (0)
Stercutus	COSMO, AF	2 (1)
Aspidodrilus	AF	1 (0)

Materials and methods

The methods for my analyses can be broken down into two components, data gathering plus taxon recognition and phylogenetic analysis.

Data gathering

Characters

Characters were determined by reference to the literature and traditions therein (including Cernosvitov, 1937; Nielsen & Christensen, 1959; Kasprzak, 1984) and through examination of specimens. Both two-state and multistate characters were included (Table 2). Through additive binary recoding (Sokal & Sneath, 1963; Farris *et al.*, 1970) 129 binary characters were determined.

Taxa

Type material was borrowed from: British Museum (Natural History) (BMNH); National Museums of Canada, National Museum of Natural Sciences (NMCIC); United States National Museum, Smithsonian Institution (USNM); National Museum of Ireland (NMI); Zoological Museum, University of Helsinki (HUZM); Museum National d'Histoire Naturelle, Paris (MNHN); Zoologisches Museum, Museum fur Naturkunde der Humboldt Universitat, Berlin, DDR (MNHU); and Royal Ontario Museum, Invertebrate Zoology (ROMIZ). Other material was obtained from the collections of K. Kossmagk-Stephan, S. Piper, H.R. Baker, N.P. Finogenova, C. Erséus, S. Byers, R.J. Diaz, Saudi Arabian Tetra Tech Inc. (Tetra Tech), and EVS Consultants Ltd. The abbreviations bracketed are used in Table 3.

Specimens were examined and photographed

Table 2. List of characters and putative transitions (bracketed) used for analyses of the bisetate and achaetine Enchytraeidae.

Char #	Description	#NC	
		Lit.	Mat.
1–4	seminal vesicles (sperm sacs) (single 1000, absent 1100, paired 1010, paired and lobed 1011)	20	0
5–9	penial bulb, invaginations and internal development of associated glands at the male pore (simple pore 10000, separate glands at pore 11000, compact glandular bulb 11100, glandular bulb with an external muscle layer 11110, bulb with muscle layer and bilobed 11111)	13	0
10	accessory, medial penial bulb (absent, present)	0	0
11–14	epidermal modifications, folds or protrusions at male pores (simple pore 1000, pore on an external papilla 1100, male pore invaginated 1010, male pore invaginated with epidermal flap over external opening 1011)	11	0
15	sperm funnel collar (narrow, expanded)	4	0
16–19	vas deferens, relative length and extent (short 1000, medium 1100, long but contained in XII and XIII 1110, very long 1111)	7	0
20	vas deferens, coiling pattern (not coiled, coiled in a spiral)	4	0
21	vas deferens, terminal modifications (not modified, dilated)	0	1
22	copulatory glands (present in several segments, lacking)	40	1
23	copulatory papillae or pads (absent, present)	39	1
24	female gonoduct, location of pore (in septal groove, posterior)	57	10
25–29	spermathecae, presence and condition of ental attachment (paired, free extending beyond segment of origin 10000, absent 11000, paired, free, restricted to one segment 10100, paired, attached to oesophagus in segment of origin 10010, paired, attached via fused ental ducts 10011)	0	0
30	spermatheca, glands at ectal pore (absent, present)	3	0
31–33	spermatheca, ectal duct (naked 100, swollen but without free glandular cells on surface 110, with free superficial glands 111)	3	0
34–36	spermatheca, non-glandular modifications of ectal duct (consistent diameter 100, dilated in segment of origin 110, dilated and bent at about 90 degrees 111)	5	0
37	spermatheca, ampullar wall protrusions (smooth, with protrusions)	3	0
38	spermatheca, ampullar diverticula (simple, with diverticula)	3	0
39–42	distribution and organization of sperm in spermathecal ampulla ('bunches' 1000, encapsulated 1100, rings 1010, rings embedded in ampullar walls 1011)	9	0
43	nephridia, preseptal part (funnel only, funnel plus some of canal)	6	0
44	nephridia, postseptal part (lobed with little interstitial tissue, compact)	4	0
45	nephridia, origin of efferent duct (anterior or ventral, terminal or just subterminal)	4	1
46	nephridia, terminal modification of the efferent duct (unmodified, expanded)	16	0
47–51	dorsal setal bundles, distribution (present in all segments 10000, absent from all segments 11000, absent from segment II 10100, absent from midbody segments and sometimes II 10110, absent from all posterior segments and sometimes II 10111)	0	0
52–55	ventral setal bundles, distribution (present in all segments 1000, absent from all segments 1100, absent from a few segments around the clitellum 1010, most absent 1011)	0	0
56–59	setal 'glands', distribution (present dorsally and ventrally 1000, present dorsally, ventrally and laterally 1100, present dorsally 1010, absent 1011)	0	0
60–65	dorsal setal bundles, numbers of setae (two to three throughout 100000, two to three in anterior segments, two posteriorly 110000, two throughout 111000, two anteriorly, one posteriorly 111100, one or two throughout 111110, one throughout 111111)	17	14
66–69	ventral setal bundles, numbers of setae (two to three throughout 1000, two throughout 1100, two anteriorly, one posteriorly 1110, one throughout 1111)	16	7
70	setal shape (sigmoid, straight)		
71–73	setae, relative lengths over body (more or less equal throughout 100, short anteriorly, longer posteriorly 110, short anteriorly, shorter in midbody segments, longer posteriorly 111)	35	8
74	dorsal coelomic pores (absent, present)	0	0
75	head pore location (the presence of a head pore is an autapomorphy of the enchytraeids in this analysis so that even though two states were considered this was not polarized by an outgroup comparison) (anterior at tip of prostomium, posterior not at tip)	4	4

Table 2. (continued)

Char #	Description	#NC	
		Lit.	Mat.
76–80	clitellar gland cells, pattern (scattered 10000, in regular transverse rows 11000, in regular longitudinal rows 11100, only hyaline cells dorsally 11110, gland cells reduced to only a few large, lateral cells 11111)	10	2
81–83	epidermal pads (no pads 100, pads paired, lateral 110, epidermal pads all dorsal, a stripe 101)	55	5
84–88	pharyngeal peptonephridia (absent 10000, paired, unbranched 11000, paired and branched 11100, unpaired, enlarged at base 10010, unpaired, enlarged at base and branched terminally 10011)	1	0
89–91	oesophageal appendages in IV (absent 100, laterally paired in posterior of IV 110, single, dorsal 101)	0	0
92–94	oesophageal appendages in V (absent 100, dorso-laterally paired 110, single dorsal 101)	0	0
95–98	oesophageal appendages in VI (absent 1000, single, dorsal 1100, single dorsal and single ventral 1110, paired, dorso-laterally 1101)	0	0
99–101	transition from oesophagus to intestine, location (postclitellar 100, clitellar 110, preclitellar 111)	36	7
102–105	gut diverticula, often at the oesophageal-intestinal transition (absent 1000, single, dorsal in IX 1100, paired in VII 1010, paired in X 1011)	1	0
106	septa, thickened in preclitellar body region (absent, present)	53	5
107	pharyngeal glands, secondary glands i.e. pharyngeal glands less compact (present, only compact primaries)	5	0
108	dorsal pharyngeal gland lobe on 4/5, fusion (not fused, fused)	6	0
109	dorsal pharyngeal gland lobe on 5/6, fusion (not fused, fused)	6	0
110–112	dorsal pharyngeal gland lobe on 6/7, fusion (not fused 100, fused 110, absent 101)	7	0
113–115	origin of dorsal blood vessel (preclitellar 100, intraclitellar 110, postclitellar 111)	2	0
116	anterior bifurcation of dorsal blood vessel, location (prostomial, pharyngeal)	50	1
117–119	brain form, extent of medial fusion (deeply cleft at posterior margin 100, with curved indentation at posterior margin 110, entire 111)	7	1
120–123	habitat (freshwater 1000, terrestrial 1100, littoral 1010, subtidal 1011)	2	0
124	sperm funnel (simple, glandular preseptal funnel)	0	0
125	ovaries in XII (present, absent)	0	0
126	ovaries in XIII (present, absent)	0	0
127	tips of setae (in this analysis bifid is autapomorphic for the Propappus outgroup) (bifid, simple-pointed)	16	7
128	setal nodulus (nodulate, annodulate)	16	7
129	testes in X (in this analysis present is autapomorphic for the haplotaxid outgroup) (present, absent)	3	0

with bright-field illumination on a compound microscope. Drawings for future reference were made through use of a drawing tube. Specimens of 45 of 110 bisetate and achaetine enchytraeid species considered for inclusion in the phylogenetic analyses (Table 3) were examined. Data for the other enchytraeid species were determined from original descriptions and from redescriptions of type and/or new material. The seven species listed in Table 3 as a genus name followed by "xxx are undescribed species. The indication following the quotes is usually of a collection location. No names are recommended or suggested here for these species.

Type specimens of South American species described by Righi, Bittencourt, and Christoffersen could not be borrowed (G. Righi & M.L. Christoffersen, pers. comm.). Similarly, material of species described by Kasprzak could not be obtained (Kasprzak, pers. comm.). Enquiries to various institutions (as indicated in Reynolds & Cook, 1976) regarding species described by Lasserre (1964), Prabhoo, Dozsa-Farkas, Michaelsen (1907), Graefe, and Righi, Ayres, and Bittencourt received no response. The type materials of Indian species described by Dash and Thambi and of European species described by Nielsen and Christensen can no longer be located

Table 3. Species list for phylogenetic analyses of the bisetate and achaetine enchytraeids; indicating all species considered and synonymies found prior to analyses. Taxa designated by a genus followed by "xxx are undescribed. # NC indicates the occurrence of unavailable or logically noncodeable binary characters; species codes are as used in all figures; the source of information, literature or material, is given with an indicationof the material used; and an indication is given of whether the species was included in phylogenetic analyses. For abbreviations of material locations see Materials and Methods.

Species (Synonyms)	# NC	Code	Lit./Mat.	Included
Achaeta aberrans	29	Aab	L	+
Achaeta affinis	50	Aaf	L	+
Achaeta bulbosa	28	Abu	L	+
Achaeta camerani	22	Aca	L	+
Achaeta christenseni	32	Ach	L	+
Achaeta danica	37	Ada	L	+
Achaeta eiseni	32	Aei	L	+
Achaeta hallensis	16	Aha	M MNHU 10667–10668	+
Achaeta indica	33	Ain	L	+
Achaeta iridescens	23	Air	L	+
Achaeta littoralis	22	Ali	M MNHN AAH1, AS31	+
Achaeta maorica	62		L	–
Achaeta "minuta	16	A"m	M Piper	+
Achaeta nielseni	34	Ani	L	+
Achaeta nurmineni	52		L	–
Achaeta parva	34	Apa	L	+
Achaeta piti	35	Api	L	+
Achaeta segmentata	37	Asg	L	+
Achaeta seminalis	29	Ase	L	+
Achaeta silvatica	19	Asi	M HUZM	+
Achaeta "tjarno	17	A"t	M Baker/Coates	+
Achaeta "vancouver	16	A"v	M Byers EVS	+
Achaeta vesiculata	30	Ave	L	+
Enchytronia annulata	17	Ean	L	+
Enchytronia christenseni	13	Ech	L	+
Enchytronia parva	0	Epa	M	+
(Marionina diverticulata			HUZM)	
(Marionina magnaglandulosa			HUZM 6724372)	
(Enchytronia minor			MNHU 10620)	
Fridericia bisetosa	22	Fbi	L	+
Fridericia bulbosa	17	Fbu	L	+
Fridericia nielseni	0	Fni	M MNHU 10622	+
Fridericia renatae	0	Fre	M MNHU 10623	+
Guaranidrilus athecatus	22	Gah	L	+
Guaranidrilus atlanticus	6	Gat	L	+

Table 3. (continued)

Species (Synonyms)	#NC	Code	Lit./Mat.	Included
Guaranidrilus cernosvitovi	0	Gce	M BMNH 1949.3.1.957, 960	+
Guaranidrilus europeus	2	Geu	M BMNH 1978.39.2	+
Guaranidrilus finni	6	Gfi	L	+
Guaranidrilus glandulosus	0	Ggl	M BMNH 1949.3.1.949-953	+
Guaranidrilus joanae	6	Gjo	L	+
Guaranidrilus mboi	6	Gmb	L	+
Guaranidrilus oiepe	7	Goi	L	+
Guaranidrilus oregonensis	0	Gor	M USNM 118243-118245 ROMIZ I1226-1228	+
Guaranidrilus rarus	1	Gra	M BMNH 1949.3.1.954-955	+
Guaranidrilus sawayai	7	Gsa	L	+
Hemienchytraeus africanus	0	Haf	M BMNH 1949.3.1.705, 712, 714-715	+
Hemienchytraeus bifurcatus	22	Hbi	L	+
Hemienchytraeus brasiliensis	48		L	–
Hemienchytraeus cipoensis	10	Hci	L	+
Hemienchytraeus guineanus	17	Hgu	L	+
Hemienchytraeus inversus	7	Hin	L	+
Hemienchytraeus khallikotosus	36	Hkh	L	+
Hemienchytraeus mauriliae	18	Hma	L	+
Hemienchytraeus rixae	12	Hri	L	+
Hemienchytraeus shirensis	17	Hsh	L	+
Hemienchytraeus solimoensis	16	Hso	L	+
Hemienchytraeus stephensoni	1	Hst	M BMNH 1949.1.4.56-60	+
(Enchytraeus rangoonensis			BMNH 1933.2.23.321)	
Hemienchytraeus theae	21	Hth	L	+
Hemifridericia parva	14	Hfp	L	+
Lumbricillus buelowi	0	Lbu	M Kossmagk-Stephan	+
(Lumbricillus nielseni			HUZM)	
Lumbricillus crymodes	0	Lcr	M	+
(Enchytraeus crymodes			BMNH 1933.5.25.1271-1275, 1279-1282)	
Lumbricillus dubius	17	Ldu	L	+
Lumbricillus eudioptus	51		L	–
Lumbricillus knoellneri	0	Lkn	M Kossmagk-Stephan	+
(Lumbricillus cervisiae			Kossmagk-Stephan)	
(Lumbricillus christenseni			BMNH 1965.12.1-22)	

Table 3. (continued)

Species (Synonyms)	#NC	Code	Lit./Mat.	Included
Lumbricillus muscicolus	36	Lmu	L	+
?Marionina aberrans	19	Mab	M Finogenova	+
Marionina achaeta	27	Mac	L	+
Marionina “arabia1	0	M“1	M Tetra Tech/Coates	+
Marionina “arabia3	1	M“3	M Tetra Tech/Coates	+
Marionina “arabia8	0	M“8	M Tetra Tech/Coates	+
Marionina argentea	3	Mar	M Kossmagk-Stephan Tetra Tech/Coates	+
Marionina canadensis	14	Mca	M NMCIC 1900-2858a-c	+
Marionina charlottensis	0	Mch	M Coates	+
Marionina clavata	23	Mcl	L	+
Marionina communis	18	Mco	L	+
Marionina craggi type M	13	McM	M NMCIC 1900-2856a	+
Marionina craggi type H	11	McH	M NMCIC 1900-2856a	+
Marionina ecuadoriensis	28	Mec	L	+
Marionina elgonensis	13	Mel	M BMNH 1949.3.1.726	+
Marionina elongata	22	Meo	L	+
Marionina filiformis	13	Mfi	L	+
Marionina glandulifera	7	Mgl	M Kossmagk-Stephan Coates	+
Marionina graefei	6	Mgr	M Kossmagk-Stephan	+
Marionina indica	58		L	–
Marionina klaskisharum	6	Mkl	M NMCIC 1982-0074-0075 USNM 073893-4	+
Marionina macfadyeni	53		L	–
Marionina mesopsamma	18		L	+
Marionina minutissima	0	Mmi	M NMI 106.1974	+
Marionina neroutsensis	0	Mne	M Coates	+
Marionina normanni	94		L	–
Marionina oligosetosa	6	Mol	M Kossmagk-Stephan	+
Marionina patua	15	Mpa	L	+
Marionina pituca	20	Mpi	L	+
Marionina preclitellochaeta	6	Mpr	M Kossmagk-Stephan Coates	+

Table 3. (continued)

Species (Synonyms)	#NC	Code	Lit./Mat.	Included
Marionina simillima	19	Msi	L	+
Marionina sjaelandica	0	Msj	M Kossmagk-Stephan Coates	+
Marionina southerni	0	Mso	M Kossmagk-Stephan Coates	+
Marionina subterranea	6	Msu	M Kossmagk-Stephan Coates	+
Marionina swedmarki	0	Msw	M Erseus	+
Marionina tica	18	Mti	L	+
Marionina "tkommi	1	M"t	M Piper	+
Marionina vesiculata	21	Mve	L	+
Marionina weilli	14	Mwi	L	+
Marionina welchi	1	Mwe	M USNM 43479-80 Coates	+
Randidrilus codensis	6	Rco	M USNM 43476-43478	+
Randidrilus westheidei	0	Rwe	M Kossmagk-Stephan	+
Stercutus ugandensis	11	Sug	L	+
Tupidrilus gei	9	Tge	L	+
Tupidrilus lacteus	9	Tla	L	+
Tupidrilus marcusae	7	Tma	L	+
Tupidrilus wilsoni	5	Twi	L	+
Propappus glandulosus	1	Pgl	M BMNH 1949.3.1.226-228 ROMIZ Coates	+
Metataxis brinkhursti	1	Hbr	M USNM 050876	+

(M.C. Dash & B. Christensen, pers. comm., respectively).

Certain species were considered to be synonymous for these phylogenetic analyses (Table 3). All of *Marionina diverticulata*, *M. magnaglandulosa*, and *Enchytronia minor* are junior synonyms of *E. parva*. *Lumbricillus nielseni* is a junior synonym of *L. buelowi*; and *L. cervisiae* and *L. christenseni* are junior synonyms of *L. knoellneri*. Further remarks on these can be found in Coates (1987a).

Six species in included genera, *Achaeta neotropica*, *A. bohemica*, *A. brevivasa*, *Enchytronia hellenica*, and *Hemifridericia varanensis*, were not considered either because they were not known to me prior to the analyses (the latter two species) or because type materials were not received.

Systematic analysis

The hypothesized polarities of characters were based on outgroup comparisons. Transitions of multistate characters, with more than one state of a transition series unique to the achaetine and other Enchytraeidae, were informed by my preceding analyses (Coates, 1986, 1987b) and systematic literature. In the list of characters (Table 2), hypothesized transitions are indicated in parentheses generally from plesiomorph through apomorph states but the additive binary codes given indicate the precise transitions used. The outgroup species were *Propappus volki* and *Metataxis brinkhursti.*

Twenty-eight binary and 26 multistate characters were determined (Table 2). Fully linear transformations were not hypothesized for all multistate characters so that those 26 function in the phylogenetic analyses as 42 character transitions.

A final data set including 103 taxa and 129 binary characters (Coates, 1987a, Table 20) was analysed, in whole or in part, using WAGNER and FWAGNER programs of the PHYSYS computer system (Mickevich & Farris, 1982). Programs that provided fit measures and the location on the cladogram of optimized character state transitions were applied to the results of all of the above. These were LFIT and DIAGNOSE. A consistency index was also calculated for each binary character using DIAGNOSE. Where the results of WAGNER or FWAGNER analyses included more than a single 'shortest' tree, consensus trees were generated by NELSON and ADAMS programs and these were compared to the parent set of trees.

Results of data collection

Characters

Character data were not available or not logically codeable (NC) for several characters, for many species included in the analyses (Table 2). The absences of some reproductive structures in what may be parthenogenetic forms were not assumed to be homologous (i.e. originating from the same ancestral state) and were considered logically noncodeable. Phylogenetic analyses using parsimony might allow a more complete exploration of the relationships of the parthenogenetic species, and of probable character transitions and adaptive character state origins.

Taxa

As is clear from the discussion of the character data, the state of each character included could not be determined for each species analyzed (Table 3). Missing data, as expected, was primarily a problem for species surveyed through the literature. The condition of some type material also prevented the determination of every character for the 'specimens observed' component of included taxa.

Setate species for which data were obtained only from the literature were excluded from my analyses if the number of characters without data exceeded 38. This is greater than the mean number plus one standard deviation of missing characters for all literature species. The phylogenetic relationships resolved for species with such large numbers of missing data could not be considered reliable. The mean and standard deviation for species lacking setae were treated separately because these species were logically NC for all setal number, setal shape, and relative setal lengths character transitions (Table 2). For species lacking both dorsal and ventral setae (*Achaeta* and some *Marionina*) this would total 16 NC's. Asetate species were excluded with numbers of NC characters, logically and missing, greater than 45. An exception was made for

Achaeta affinis (Table 2), which also lacks spermathecae and thus gains additional logically NC characters.

Results of systematic analyses (Fig. 1)

Informative characters

The male reproductive system (characters number 1–23) of enchytraeids seems generally to have been underestimated as far its complexity. As yet, the question of whether seminal vesicles (1–4) are the same as testis sacs has no single answer because of a broad usage of the terms. The testis sacs of *Lumbricillus* may indeed be distinct from seminal vesicles or sperm sacs of other Enchytraeidae and a paired and lobed condition is unique for at least some of its species.

The structure of penial bulbs (5–10) and the development of these requires further observation as the hypothesized tendencies to increased complexity is congruent with the cladograms but the details of transitions are incongruent. Similarly, coiling of vasa deferentia and presence of copulatory glands have distinct distributions, although much information regarding the latter is missing.

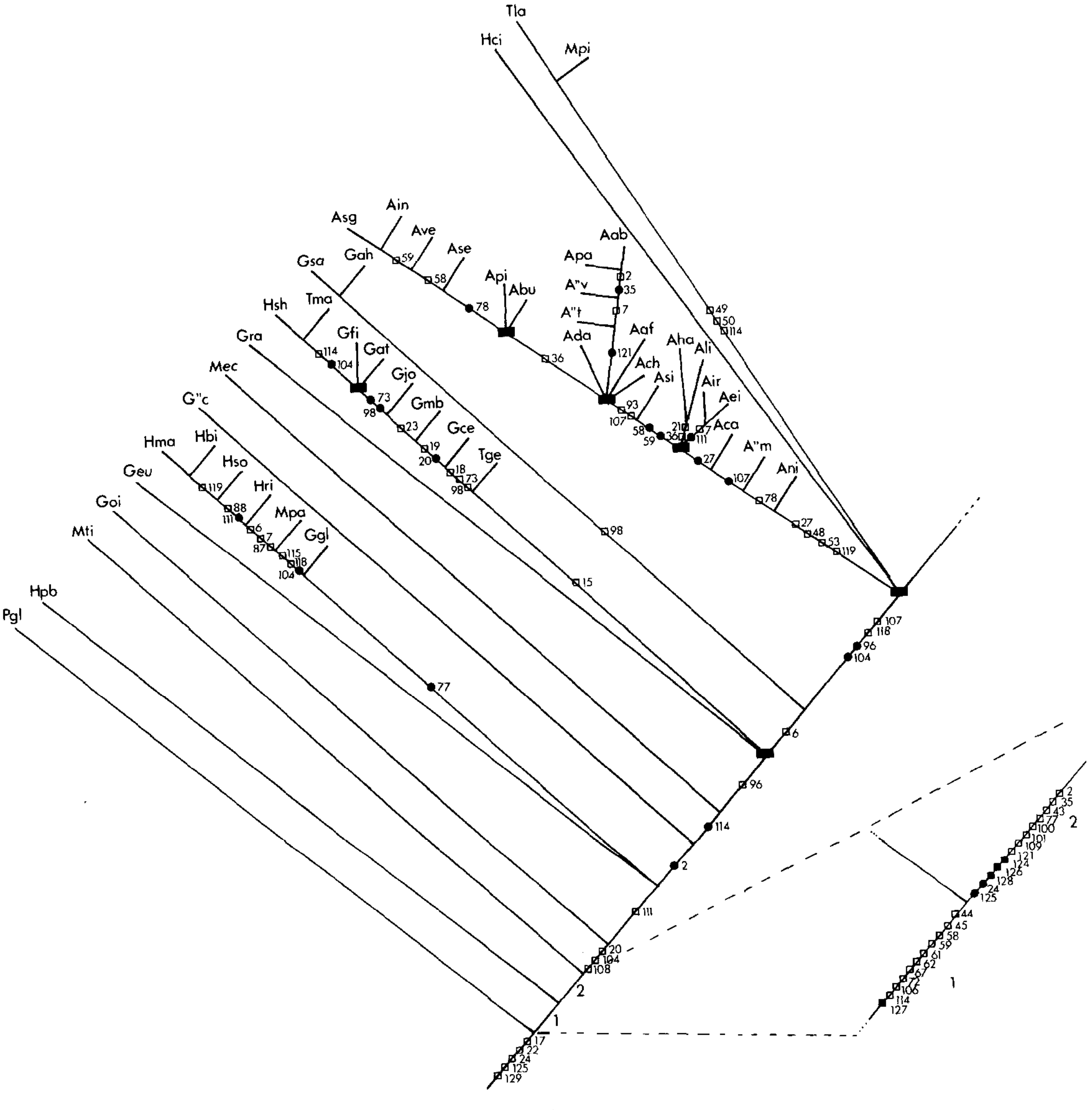

Fig. 1a.

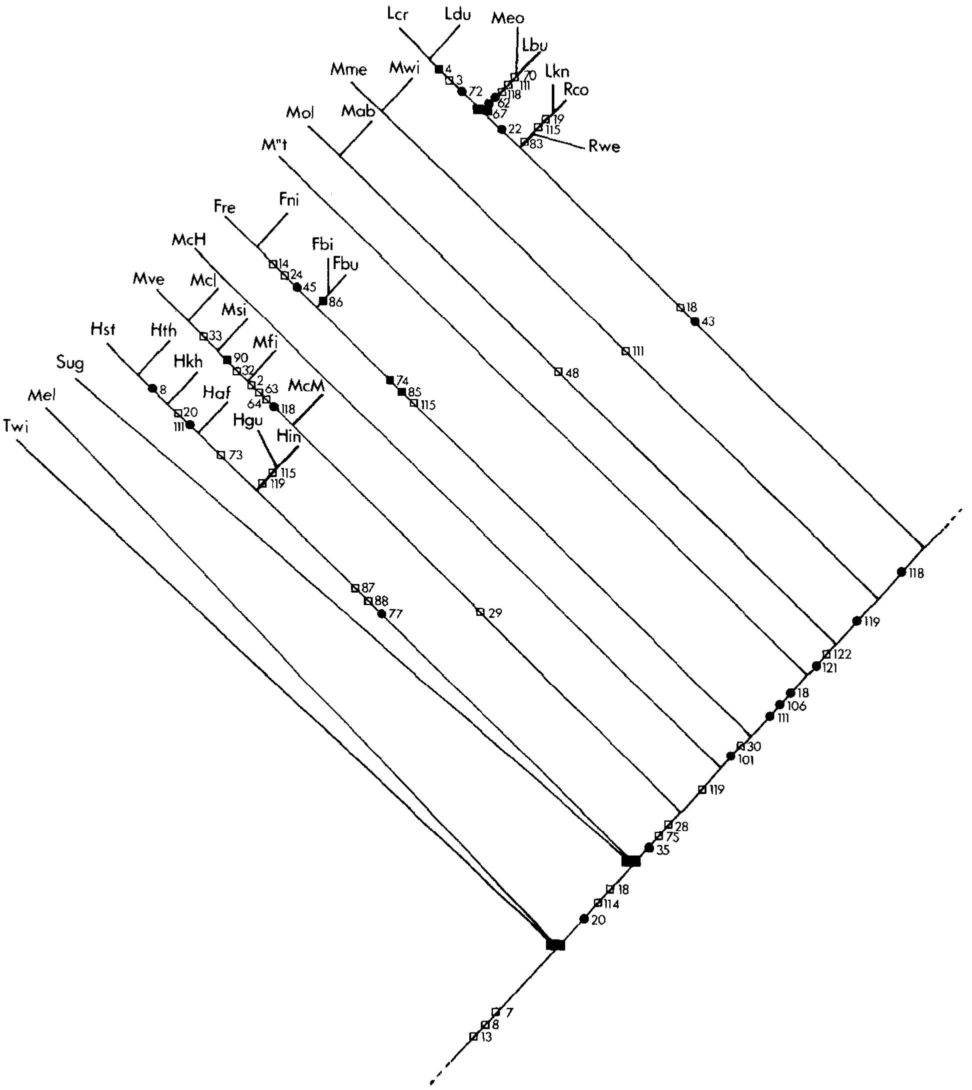

Fig. 1b.

Epidermal modifications at the male pore (11–14), modifications of the vasa deferentia at the pores (21), and copulatory papillae and pads (22–23) are generally too poorly defined or known to positively contribute to phylogenetic analyses.

Characteristics of the sperm funnel collar (15) and the length of vasa deferentia (16–19) are not clearly distinguished and might be investigated as physiological variations. States of the latter character, which is meristic, might be better recognized through analyses of frequency distributions.

In the female reproductive system (24–42), spermathecae (25–42) offer some characters that are easily recognized and for which information is consistently available (Table 2). Spermathecal form (25–29, 34–38), glandular distributions on the duct (30–33), and sperm distributions in the ampullae (39–42) all show clear patterns. The absence of spermathecae, however, is not phylogenetically interpretable, in most cases, prior to analyses.

External, glandular modifications (30–33) of the spermathecal duct originated after the achaetines. Nonglandular modifications, expansions and bends (34–36), are characteristic of

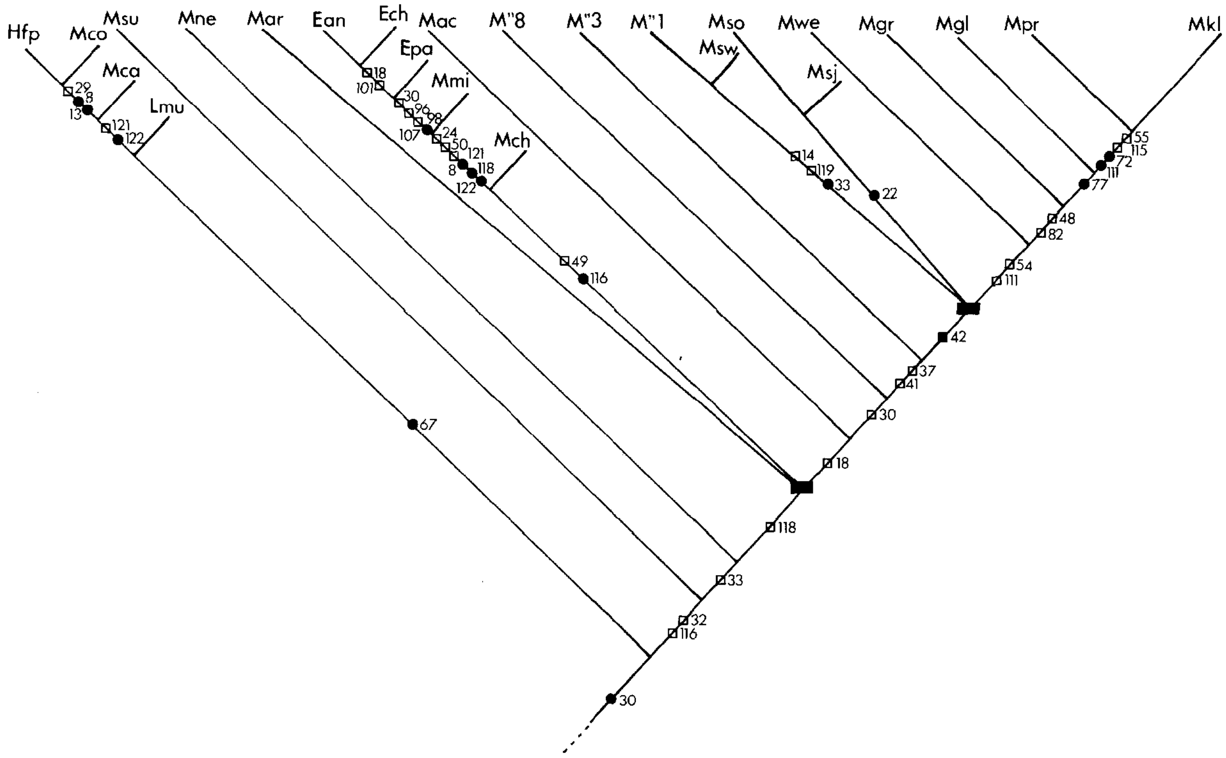

Fig. 1c.

Fig. 1. Cladogram resolved by the WAGNER program of PHYSYS for 103 taxa × 129 characters; CI = 19.3. Character numbers (Table 2) accompany indications of character state transitions; ■, unique 0 → 1 (fully congruent for this analysis), □, 0 → 1, ●, 1 → 0. Character state changes of terminal taxa are not indicated for clarity of presentation. Redundant characters have been omitted.

the achaetines, in fact, the bent condition is restricted to *Achaeta*. Spermathecae attached to the oesophagus by ental ducts (28) characterize the 'higher Enchytraeidae'.

Ampullar diverticula and protrusions (37–38) might be more useful in further investigations including more enchytraeid genera with these modifications. Various ampullar protrusions and the presence of sperm rings in them characterize a group of littoral *Marionina*.

Some nephridial characteristics (43–46), may serve as synapomorphies for all the enchytraeids, others are poorly understood, such as the origin and terminal expansion of the efferent duct. Large preseptals (43) in some nominal species of *Marionina* are not homologous to large preseptals found in more plesiomorph, but not sister, taxa.

The distribution and numbers of setae in dorsal and ventral bundles (47–55, 60–69) offer reliable phylogenetic characteristics. All setal states in enchytraeids derive from setal bundles with two more or less straight, simple-pointed setae in all locations. The complete absence of setae, either dorsally, ventrally or both, is not obviously the culmination of a linear tendency. Complete absence of setae first originates in *Achaeta* and is found in only a few more derived taxa. The absence of most but not all ventral setae is characteristic here of some *Marionina* but is also known in *Grania*. Multisetate is not a single characteristic, with one origin, and the patterns and forms of setae within bundles of multisetate taxa require closer examination.

Setal glands (56–59) are unique to *Achaeta*. A diversified terminology should be investigated for similarly named structures in taxa outside Enchytraeidae.

A head pore (75) is characteristic of all Enchytraeidae but its origins are very unclear. Dorsal coelomic pores (74) of Enchytraeidae are characteristic only for *Fridericia*.

In view of the apparent origin of all clitellar gland cell distributions (76–80) from transverse banding, it would be informative to observe the sequence of changes in patterns during maturation. Other epidermal glandular modifications (81–83) (thought to be adhesive glands) considered here are poorly known but certain states are characteristic, locally, for *Randidrilus* and for some nominal species of *Marionina*.

Pharyngeal peptonephridia (84–88) are probably good distinguishing characteristics, however, not all species with paired peptonephridia were included and basally paired peptonephridia have, possibly, two very distinct origins. Paired peptonephridia, at least those considered here, have origins independent of unpaired peptonephridia. A more specific terminology should be used in order to distinguish these pharyngeal appendages. Such a terminology could only be developed in conjunction with further structural studies.

Gut modifications called oesophageal appendages (89–98) appear to have originated a number of times, in different segmental locations. Appendages in IV (89–91) are diagnostic of one clade of nominal species in *Marionina*; appendages in V (92–94) are diagnostic for *Achaeta*; appendages in VI (95–98) may characterize one clade of nominal species of *Guaranidrilus* and do characterize *Enchytronia*, but the historical origins of appendages in VI for the latter and possibly their structures are different from the former taxon.

The location of gut transitions (99–101) are very poorly discriminated. Only records of postclitellar intestinal origins are coincident with previously and presently recognized genera. This character can be recognized a priori, by the limits on possible states, as a character type prone to parallelism or convergence (R.W. Murphy, pers. comm.).

Paired gut diverticula (102–105) were restricted for these analyses to species of the nominal genus *Guaranidrilus*, but nevertheless were not resolved here to be a synapomorphy of one taxon. Gut diverticula of somewhat different form are found in species of other enchytraeid genera, including *Henlea*, and these diverticula are unlikely to be homologous to those in any nominal species of *Guaranidrilus*.

The states of thickened septa (106) in various segments were very poorly discriminated as well as poorly known. Details of how much thicker and how many thicker were not employed in the recognition of states because of the large amount of missing data for the character.

The states of the condensation of pharyngeal glands (107–112) require reanalysis. It seems likely that two different states are confounded as 'secondary glands' (107), one characteristic of the outgroup and early enchytraeids and the other derived from a compact state. Dorsal fusion of pharyngeal glands is characteristic for all enchytraeids, and no clear pattern emerged for the absence of fusion at any or all of the bearing septa.

Information on the origin of the dorsal blood vessel (113–115) regarding intraspecific variation and variation at the generic level is probably inaccurate both with regards to specific variation and actual location. As the location of the gut transition, this character may be determined a priori as prone to homoplasy. The anterior bifurcation of the dorsal blood vessel (116) is more difficult to mistake but much information is missing about this character. A posterior, suprapharyngeal bifurcation has only been reported in nominal, littoral species of *Marionina*.

The form of the posterior margin of the brain (117–119) was largely incongruent with generic level taxa, although species of *Achaeta* consistently have truncate posterior brain margins. Character states are matters of degree, from fused to unfused, and as such difficult to delimit. As well, intraspecific and interspecific variation are poorly analyzed.

From an original wet terrestrial habitat, it seems that enchytraeids have invaded many habitats (120–123) more than once. Derived habitats are generally characteristic of genera or other higher-level, subfamilial taxa, for example, *Lumbricillus*, its immediate plesiomorph sisters, and more apomorph enchytraeids are characteristically littoral.

The characters used were often unambiguously diagnostic at specific ranks although homoplasious (sometimes with very low individual consistencies) overall. For instance, the presence of setal glands clearly distinguishes *Achaeta* but a fully parsimonious resolution of the distributional states within *Achaeta* was not found. Similarly, certain modifications of the spermathecal ectal duct distinguish the apomorph part of the enchytraeid lineage from the achaetines but there is homoplasy within the apomorph lineage. States of a single character cannot distinguish all genera or subfamilies either in phylogenetic analyses or for classifications following an ordering convention (Wiley, 1981).

The analytical methods used can only reveal patterns of shared character states inherent in the data assembled and, of these, only the most parsimonious that a particular algorithm can find. Resolution of some of the problems of character recognition and discrimination, character type, and other sources of homoplasy such as hybridization might increase the clarity and parsimony of interpreted phylogenies, although the patterns detected would not necessarily change.

Supraspecific taxa

From the results of 3 analyses (Coates, 1987a) 9 multispeciate clades of equal rank (Fig. 1) (more or less the same as genus), under the constraints of an ordering criterion (Wiley, 1981), could be recognized within the taxa used. Some of these clades are not yet well-supported by unambiguous synamorphies, even though they may have been consistently supported by the same homoplastic characters. The relationships of consistent clades were more similar for two full data analyses than for a third analysis employing only a fraction of the characters. Of all the results, those for the full data set (Fig. 1) with an outgroup including two taxa, rather than one, were preferred on the basis of goodness-of-fit measures (C.I. = 19.3 and F = 87.4) and because the root was better established by two outgroup taxa. A single tree was obtained in this analysis so that consensus tree comparison was not applicable.

The ordered, multispeciate clades resolved are (Fig. 1):

1. terrestrial or semi-aquatic, seminal vesicle absent, head pore anterior, preseptal of nephridia including some of canal, anterior blood vessel bifurcation prostomial, spermathecal ectal duct dilated, vasa deferentia coiled; within lineage: dorsal blood vessel origin postclitellar, some with basally unpaired peptonephridium.
   Marionina patua, Hemienchytraeus rixae, H. solimoensis, H. bifurcatus, and *H. mauriliae*.
2. seminal vesicle single; within lineage: gut diverticula paired at 7/8, oesophageal appendages in VI.
   Guaranidrilus rarus, G. sawayai sp. dub. (fide Coates & Diaz, 1988, = *G. rarus*), *G. athecatus, G. cernosvitovi, G. mboi, G. joanae, G. atlanticus, G. finni*, and, possibly, *Tupidrilus gei, T. marcusae*, and *Hemienchytraeus shirensis* sp. dub.
3. **Achaetinae: Achaeta** free glands at spermathecal pore; within lineage: dorsal and ventral setae absent, some with expansion at dilation on spermathecal ectal duct, some with setal glands, some with oesophageal appendages in V.
   A. nielseni sp. dub., *A.* "minuta, *A. camerani, A. eiseni, A. iridescens, A. littoralis, A. hallensis, A. silvatica, A. christenseni* sp. dub., *A. affinis, A. danica, A.* "tjarno, *A.* "vancouver, *A. parva, A. aberrans, A. bulbosa, A. piti, A. seminalis, A. vesiculata, A. indica* sp. dub., *A. segmentata* sp. dub. (*A. maorica* sp. dub., *A. nurmineni* sp. dub., *A. neotropica, A. brevivasa*, and *A. bohemica* sp. dub. probably belong here as well.)
4. **Hemienchytraeus** compact, muscular penial bulb with invaginated male pores, vasa deferentia not coiled; within lineage: with basally unpaired pharyngeal peptonephridium.
   H. stephensoni, H. inversus sp. dub., *H. guineanus* sp. dub., *H. africanus, H. khallikotosus* sp. dub., and *H. theae* sp. dub.
5. spermathecae attached to oesophagus in V, head pore posterior, spermathecal ectal duct not dilated; within lineage: spermathecae with fused ental ducts.
   Marionina vesiculata, M. clavata, M. filiformis, and *M. simillima*.

6. **Fridericinae: *Fridericia*** glands at ectal pore of spermatheca; within lineage: with paired pharyngeal peptonephridia, with dorsal coelomic pores.
   F. bulbosus, F. bisetosa, F. renatae, and *F. nielseni.*
7. **Enchytraeinae (part): *Lumbricillus*** littoral; within lineage: preseptal of nephridia including funnel only, some with multiple setae, some with paired and lobed seminal vesicles.
   L. buelowi, L. crymodes, L. dubius, Randidrilus codensis, R. westheidei, and *L. knoellneri.*
8. **Enchytraeinae (part): *Hemifridericia*** without glands at spermathecal ectal pore; within lineage: setal bundles with 3 setae, most terrestrial, some with fused spermathecal ental ducts.
   H. parva, Marionina communis, and possibly *M. canadensis.*
9. **Enchytraeinae (part):** anterior bifurcation of dorsal blood vessel pharyngeal, some with small spermathecal diverticula and embedded sperm rings.
   Marionina subterranea, M. neroutsensis, M. argentea, Enchytronia parva, E. christenseni, E. annulata, M. minutissima, M. charlottensis, M. achaeta, M. "arabia8, *M.* "arabia3, *M.* "arabia1, *M. swedmarki, M. sjaelandica, M. southerni, M. welchi, M. graefei, M. glandulifera, M. preclitellochaeta*, and *M. klaskisharum.*

Species incertae sedis and species dubia

Other taxa are incertae sedis with respect to a multispeciate group. Some of these unresolved taxa are also species dubia, due primarily to inadequate taxonomic information. Species incertae sedis are: *Marionina aberrans, M. oligosetosa, M. weilli, M. mesopsamma, M. elgonensis, M. elongata, M. pituca, M. ecuadoriensis, M. tica*, and the undescribed *M.* "tkommi; *Lumbricillus muscicolus* and *L. eudioptus*; *Tupidrilus lacteus* and *T. wilsoni* (generic type); *Hemienchytraeus cipoensis*; *Stercutus ugandensis*; and *Guaranidrilus glandulosus* (generic type), *G. europeus, G. oregonensis*, and *G. oiepe*. Species that are both dubia and incertae sedis include: *Marionina macfadyeni, M. indica, M. normanni*, and *M. craggi* type M as well as *M. craggi* type H; and *Hemienchytraeus brasiliensis.*

There is no recent phylogenetic analysis to which the clades found here can be compared. Cernosvitov's (1937) classification anticipated some of these but large numbers of species have been described since and the discussion of generic relationships made by Cernosvitov indicated that his subfamilies, especially Achaetinae, and some genera were not conceived as monophylies.

Phylogenetic analyses eventually should allow evolutionary investigations of Enchytraeidae to proceed within a theoretically consistent framework and allow the logical reclassification of species in nominal genera such as *Marionina*. The resolution of phylogenetic groups and relationships among nominal species of *Guaranidrilus, Hemienchytraeus* and *Tupidrilus* requires a more localized analysis of character distributions and determination of probable sources of homoplasy in characters such as basally unpaired peptonephridia and paired gut diverticula (Coates, this volume). Such sources of homoplasy may be found in the reproductive speciation mechanisms of these taxa.

Discussion

Results of the analyses of Achaetinae indicate that: Achaetinae is not monophyletic; the taxa originally classified in Achaetinae may include the earliest species of the family; and, thus, Enchytraeidae originated in and were widespread in a southern land mass. Of the 11 enchytraeid genera included, 6 appear to be substantially monophyletic, although fully congruent characters have not been clearly established; the rest require formal reclassification. Of the achaetine genera, only *Achaeta* is monophyletic; of the nonachaetine genera, *Marionina* is most fractured and requires revision before species can be placed with any confidence within that assemblage. Male duct characters of Enchytraeidae are amongst the most genealogically informative as presently recognized and require careful examination in taxonomic studies.

A reclassification of all Enchytraeidae is not proposed because it is felt there is not enough stability or corroboration of the relationships found; and because not all of Enchytraeidae was analysed. There is evidence for substantial homoplasy in character data for Enchytraeidae as they are currently employed. Unambiguous synapomorphies were not found for all taxa which are otherwise supported by homoplasies.

Problems with the data used for the analyses are recognized at four levels. Existing higher phylogenies of Oligochaeta and Annelida have not been hypothesized in ways that allow a clear recognition of major, underlying assumptions. In the absence of these indications, it is difficult to recognize better supported sister groups or even closer outgroups. As well, the relationships between potential outgroups is minimally resolved.

The higher level phylogeny for enchytraeids and propappids determined (Coates, 1987b) was limited in its consistency due especially to a general lack of information about haplotaxid species. Information on all species of that group is important because Haplotaxidae, as it was recognized in Coates (1987b), is not monophyletic and probably includes the independent sisters to Tubificidae and Enchytraeidae.

Species of Enchytraeidae are not consistently well-distinguished and several synonymies and indistinguishable taxa were encountered for the small number of species (103) considered in my analyses. Even recently, numerous species have been very poorly described and are not determinable to a generic level.

Finally, several attributes of different taxa that were recognized as states of unit characters may not be homologous. Some of this homoplasy (some due to analogical terminology) seemed likely prior to my analyses but differences could not be demonstrated with any convincing structural or developmental evidence. However, all the incongruence in my data cannot be simply set aside as bad interpretations of characters. Within some taxa, for example *Achaeta*, individual character distributions, karyological data not used in the analyses (Nielsen & Christensen, 1959; Christensen, 1961), and apparent (Fig. 1) species relationships suggest that auto- and allopolyploidy have been the basis of some phyletic diversification. In such cases, reticulation and unresolvable multifurcations will be the true pattern of species relationships (Funk, 1985).

Reconstructing a part of the evolutionary history of Enchytraeidae was viewed as a complex problem. It had not been approached before with a fully described evolutionary logic and with a methodology that could process simultaneously a large amount of data. The cladograms that I have obtained provide grounds for assessing what we thought we already knew, such as which characters are good evolutionary predictors, what are the genera of Enchytraeidae, and that reticulate evolution or hybridization is an uncommon mode of speciation in animals.

Acknowledgements

Many thanks are given to all those individuals and their various home institutions who loaned and gave me specimens. This work was undertaken as part of the research for my Ph.D. program and was supported by Ocean Ecology, Institute of Ocean Sciences, Fisheries and Oceans, Canada; the University of Victoria; and the Department of Invertebrate Zoology, Royal Ontario Museum.

References

Bremer, K. & H.E. Wanntrop, 1978. Phylogenetic Systematics in Botany. Taxon 27: 317–329.

Cernosvitov, L., 1937. System der Enchytraeiden. Bull. Assoc. Russe Rech. Sci. Prague, 5: 262–295.

Christensen, B., 1961. Studies on cyto-taxonomy and reproduction in Enchytraeidae. With notes on parthenogenesis and polyploidy in the animal kingdom. Hereditas, 47: 387–450.

Coates, K.A., 1986. Redescription of the oligochaete genus *Propappus*, and diagnosis of the new family Propappidae

(Annelida: Oligochaete). Proc. Biol. Soc. Wash., 99: 417–428.

Coates, K.A., 1987a. Phylogenetic analysis of some Enchytraeidae (Annelida: Oligochaeta): parsimony analysis of structural characters. University of Victoria. Ph.D. dissertation. Nov. 1987.

Coates, K.A., 1987b. Phylogenetics of some Enchytraeidae (Annelida: Oligochaeta): a preliminary investigation of relationships to the Haplotaxidae. Hydrobiologia 155: 91–106.

Coates, K.A., 1989. Preliminary investigations of hybridization/reticulate evolution in *Guaranidrilus* (Enchytraeidae: oligochaeta). Hydrobiologia (this volume) 180: 35–45.

Coates, K.A. & R.J. Diaz, 1988. Description of *Guaranidrilus oregonensis* (Oligochaeta: Enchytraeidae) from North America, with additional comments on the genus. Proc. Biol. Soc. Wash., 101: 773–783.

Farris, J.S., A.G. Kluge & M.J. Eckardt, 1970. A numerical approach to phylogenetic systematics. Syst. Zool., 19: 172–191.

Funk, V.A., 1985. Phylogenetic patterns and hybridization. Ann. Missouri Bot. Gard., 72: 681–715.

Kasprzak, K., 1984. Generic criteria in Enchytraeidae (Oligochaeta) family. Biologia (Bratislava), 39: 163–172.

Kluge, A.G. & R.E. Strauss, 1985. Ontogeny and Systematics. Ann. Rev. Ecol. Syst., 16: 247–268.

Lasserre, P., 1964. Notes sur quelques oligochètes Enchytraeidae, présent surs les plages du Bassin d'Arcachon. Proc.-verb. Soc. Linn., Bordeaux, 101: 87–91.

Michaelsen, W., 1907. Oligochaeta. Fauna Südwest-Australiens. Ergeb. Südwest-Australischen Forsch., 1905 1: 117–232.

Nielsen, C.O. & B. Christensen, 1959. Studies on Enchytraeidae. 7. Critical revision and taxonomy of European species. Nat. Jutl., 8-9: 1–160.

Mickevich, M.F. & J.S. Farris, 1982. Phylogenetic analysis system (PHYSYS). Fortran V software system of cladistic and phenetic algorithms.

Reynolds, J.W. & D.G. Cook, 1976. Nomenclatura Oligochaetologica. The University of New Brunswick, Fredericton.

Sokal, R.R. & P.H. Sneath, 1963. Principles of animal taxonomy. W.H. Freeman and Co., San Francisco.

Stevens, P.F., 1980. Evolutionary polarity of character states. Ann. Rev. Ecol. Syst., 11: 333–358.

Wiley, E.O., 1981. Phylogenetics. The theory and practice of phylogenetic systematics. John Wiley and Sons, New York.