TÜBİTAK

Research Article

Turk J Zool
2012; 36(1): 85-94
© TÜBİTAK
doi:10.3906/zoo-1002-70

Microtaxonomy of fragmenting *Enchytraeus* species using molecular markers, with a comment on species complexes in enchytraeids

Rut COLLADO[1], Eva HASS-CORDES[2], Rüdiger M. SCHMELZ[1,*]

[1]University of A Coruña, Science Faculty, Department of Animal Biology, Plant Biology, and Ecology, Rua da Fraga, 10, E-15008 A Coruña - SPAIN

[2]University of Osnabrück, Department of Biology/Chemistry, Barbarstr. 11, 49076 Osnabrück - GERMANY

Received: 22.02.2010

Abstract: Populations of *Enchytraeus* that reproduce by fragmentation are distributed worldwide, but their species-level taxonomy is unresolved because morphological differences are inconclusive. Therefore, we compared the isozyme and RAPD-PCR patterns of 5 populations of fragmenting enchytraeids from widely distant localities (Japan, Iran, Greece, and Brazil). Among these populations, 3 were identified (*E. japonensis*, *E. bigeminus*, and *E. dudichi*) and 2 were unidentified. Multiple isozyme bands suggested polyploidy in all investigated populations. In all RAPD-PCR algorithms except 1, the fragmenting group formed a cluster separate from 2 nonfragmenting *Enchytraeus* species. All 5 populations were clearly separable on the levels of protein and DNA, but the unidentified Greek populations clustered more closely with *E. japonensis*. It remains unknown whether the fragmenting group in *Enchytraeus* is monophyletic or polyphyletic. Although every investigated population may deserve species rank, a consistent classification is impossible at present.

Key words: Cryptic species, molecular diversity, Enchytraeidae, Oligochaeta, Clitellata, Annelida, *Enchytraeus bigeminus* group

Introduction

Enchytraeids have different modes of reproduction (Christensen, 1994). Apart from amphimixis, reproduction by fragmentation (architomy) is characteristic of some species in *Buchholzia*, *Cognettia*, and *Enchytraeus*. Fragmenting *Enchytraeus* spp. are present worldwide, although records are scarce (Figure 1, Table 1). To date, 4 fragmenting species of *Enchytraeus* have been described: *E. fragmentosus* Bell, 1959; *E. bigeminus* Nielsen & Christensen, 1963; *E. japonensis* Nakamura, 1993; and *E. dudichi* Dózsa-Farkas, 1995. Reports of fragmentation in a fifth species of *Enchytraeus*, *E. variatus* Bouguenec & Giani, 1987 (Bouguenec and Giani, 1989), were considered doubtful by Schmelz and Collado (2010).

The taxonomy of fragmenting *Enchytraeus* is difficult. According to Schmelz et al. (2000), *E. fragmentosus*, *E. bigeminus*, and *E. japonensis* are morphologically indistinguishable in the fragmenting stage. Sexual stages of *E. bigeminus* and

* E-mail: enchytrailer@telefonica.net

Figure 1. World distribution of fragmenting *Enchytraeus* spp. as presently known. The geographical origin of the cultures of *E. fragmentosus* (No. 1) and *E. bigeminus* (No. 4) is unknown; bold-lined circles: origin of cultures investigated in this study.

Table 1. World records of fragmenting *Enchytraeus* spp. For location numbers, see Figure 1.

Loc. No.	Country	Habitat	as	Reference
1	USA (Iowa?)	Compost?	*E. fragmentosus*	Bell (1959)
2	Brazil, Amazon	Rain forest	*E.* sp.	Schmelz (unpublished)
3	Brazil, Paraná	Rain forest area, partly degraded	*E.* sp.	Römbke et al. (2005, 2007)
4	Denmark?	? (Laboratory culture)	*E. bigeminus*	Nielsen and Christensen (1963)
5	Germany	Former sewage field	*E.* cf. *bigeminus*	Heck (1995)
5	Germany	Urban grassland, sewage field	*E. bigeminus*	Heck et al. (1999)
5	Germany	Pasture	*E. bigeminus*	Jänsch and Römbke (2003)
5	Germany	Compost	*E. bigeminus*	Schmelz (unpublished)
5	Germany	Forest soil	*E. bigeminus*	Schmelz (unpublished)
6	France	Compost	*E. bigeminus*	Bouguenec and Giani (1987)
7	Hungary	Greenhouse	*E. dudichi*	Boros and Dózsa-Farkas (2007)
8	Greece	Field	*E. fragmentosus*	Vavoulidou et al. (1999)
9	Sao Tomé Island	?	*E. dudichi*	Boros and Dózsa-Farkas (2007)
10	Iran	Garden soil	*E. dudichi*	Dózsa-Farkas (1995)
10	Iran	Garden soil	*E. bigeminus*	Dózsa-Farkas (1995)
11	Japan	Crop field	*E. japonensis*	Nakamura (1993)

E. japonensis differ morphologically by one trait only (Table 2). Christensen (1980) found abnormally high chromosome numbers in *E. bigeminus* (c. 144 = c. 8n, c. 160 = c. 10n), which means that various levels of polyploidy further complicate the picture. Schmelz et al. (2000) compared protein patterns (isozyme and total protein) of *E. bigeminus* and *E. japonensis* taken from 2 long-run laboratory cultures and found marked differences that justified the maintenance of *E. bigeminus* and *E. japonensis* as separate species. However, *E. fragmentosus* had to be invalidated because its sexual organs are insufficiently known and type or other reference material is missing.

With these results, it seems necessary to apply molecular methods for a correct identification of specimens. However, even with molecular methods, the process of identification is not straightforward. How similar should specimens be in order to belong to the same species? Which degree of difference, calculated as genetic distance, would justify the recognition of a new species?

To address these questions, we added 3 more populations of fragmenting enchytraeids to the study. One of them was a descendant of the original culture of *E. dudichi* from Iran (Dózsa-Farkas, 1995). The 2 other populations of unidentified fragmenting *Enchytraeus* sp. originated from research sites of the Agricultural University of Athens, Greece (Vavoulidou et al., 1999; identified there as *Enchytraeus fragmentosus*), and from rain forest soils in the Amazon near Manaus, Brazil (Schmelz, unpublished). Fragmenting specimens of the Greek culture were indistinguishable from those of *E. bigeminus* and *E. japonensis*, whereas *E. dudichi* and the Brazilian culture differed from the rest in the coelomocyte texture. *E. dudichi* further differed from the rest in a higher number of segments and more chaetae per bundle. A morphological comparison of the 5 fragmenting cultures is given in Table 2.

Specimens were taken from cultures raised in the laboratory. We investigated isozyme patterns and RAPD-PCR fingerprints. We wanted to know

Table 2. Comparison of the different populations of fragmenting *Enchytraeus* species used in this study. Data from different sources; bold: peculiar traits.

	E. bigeminus	*E. japonensis*	*E.* sp. "Greece"	*E. dudichi*	*E.* sp. "Manaus"
Mean segment number	ca. 45, up to 80	ca. 45, up to 80	ca. 40	**ca. 60, up to 128**	ca. 35, up to 59
Mean body length	3-10 mm	3-10 mm	3-6 mm	ca. 14 mm	ca. 5 mm
Dorsal blood vessel origin	X-XIII	X-XIII	X-XIII	XIV-XVI (-XXIII)	XII-XIII (-XX)
No. chaetae per bundle	2	2	2	**lateral 2, 3 ventral 3, (4)**	2
Additional glands near male pores	**present**	absent	unknown	absent	unknown
Coelomocyte vesicles	globular	globular	globular	**irregular**	**irregular**
Coelomocyte aggregations	pale	pale	pale	**grey**	**brownish**
Origin and loc. no. (Figure 1)	Unknown[1] Loc. 4?	Japan[2] Loc. 11	Greece[3] Loc. 8	Iran[4] Loc. 10	Brazil[5] Loc. 2

[1]Identification based on morphology (additional glands near male pores in sexual specimens).
[2]Strain of original culture (Nakamura, 1993).
[3]Soil sample (taken by E. Vavoulidou) from crop field, Thessaloniki, Greece (Vavoulidou et al., 1999).
[4]Strain of original culture (Dózsa-Farkas, 1995) kindly provided by K. Dózsa-Farkas.
[5]Soil sample (taken by J. Römbke) from rain forest near Manaus, Amazon, Brazil (unpublished).

the ways in which the new populations added to the molecular diversity of the fragmenting group in *Enchytraeus* and whether patterns could be detected that might help to delimit species taxa within the fragmenting group.

Material and methods

The study was carried out at the laboratory of Systematic Zoology, University of Osnabrück. Table 2 gives an overview of the materials used and their geographic origin. All specimens were taken from mass cultures raised in the laboratory on soil substrate in petri dishes for at least 1 year. The size of the starter populations was unknown; in *E. japonensis*, it was one specimen. Morphological investigation was carried out on specimens in vivo and as stained whole mounts. Further details were taken from the literature. Sexual stages were unknown in the Greek population and could not be induced with the known methods (Myohara et al., 1999). The culture from Manaus was variable in the beginning regarding the chaetal pattern, but homogeneous when molecular analyses started. Specimens with parasites or commensals (e.g., nematodes, ciliates) were not found. Isozyme patterns were analyzed following the methods of Schmelz (2003) and Schmelz et al. (2000), based on the protocol established by Brockmeyer (1991), using isoelectric focusing on polyacrylamide gels (PAGIF). Due to their small body size, animals were processed entirely. Before processing, worms were maintained in water until defecation was completed. A total of 4 isozyme patterns were analyzed: esterases (EST), malate dehydrogenase (MDH), glucose phosphate isomerase (GPI), and phosphoglucomutase (PGM). In different gel runs, 8 to 16 specimens per culture were compared. *E. crypticus*, a species with known banding patterns (Brockmeyer, 1991), was used as a reference in order to allow comparison with previous studies (Schmelz et al., 2000). Isozyme patterns were compared as a whole with respect to identity or nonidentity. The RAPD-PCR procedure applied here was described by Westheide and Haß-Cordes (2001). From each fragmenting culture, 3 specimens were used. Furthermore, 2 specimens each from cultures of *E. luxuriosus* and *E. albidus*, 2 nonfragmenting species that differ widely in their morphology, were analyzed, in order to assess the genetic distances among the fragmenting cultures. *E. luxuriosus* is able to reproduce uniparentally, probably by self-fertilization (Schmelz and Collado, 1999), and *E. albidus* is obligately amphimictic (Brockmeyer, 1991). The 8 used primers yielded 169 different fragments. The fingerprints were transferred into a binary matrix and analyzed with UPGMA, WPGMA, complete linkage, single linkage, and neighbor joining.

Results

Isozymes: All specimens belonging to the same culture had identical banding patterns; in other words, no within-population variation was detected. Figure 2 shows a zymogram of the esterase patterns. All cultures differed among each other in at least 3 of the 4 isozyme systems tested. Differences refer to patterns as a whole and not to single presumptive loci. Only the GPI-patterns of *E. japonensis* and *E.* sp. "Greece" were identical; in a few further cases, differences between cultures were small and present only in the weak bands, while the strong bands were identical (Table 3). Esterase patterns were especially complex (Figure 2); fragmenting cultures had up to twice as many bands (8-10) as the nonfragmenting *E. crypticus* (4 bands). MDH patterns had up to 4 bands. Due to within-culture homogeneity, an analysis in terms of populational genetics (number of loci,

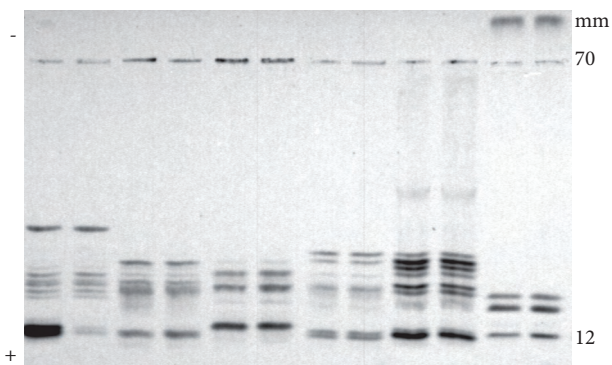

Figure 2. Esterase patterns of fragmenting *Enchytraeus* spp. together with those of nonfragmenting *E. crypticus* as a reference. All specimens belonging to the same culture had identical banding patterns; therefore, only 2 specimens of each culture are shown. Bands at mm 70 are artifactual, residues of the applicator slot; PAGIF, separation distance of 9 cm.

Table 3. Congruence of isozyme banding patterns after IEF among all 5 fragmenting *Enchytraeus* cultures. Investigated isozymes: EST, PGM, GPI, MDH; brackets: patterns similar but not identical (patterns completely different in all other cases); EST patterns were idiosyncratic for each culture.

Enchytraeus	*dudichi*	*japonensis*
"Manaus"	(MDH)[1]	
bigeminus	(GPI)1	
"Greece"		GPI, (MDH)[1], (PGM)[2]

[1]Strongly staining band at same position, weak bands differing.
[2]Double band in *E. japonensis* and single band in *E.* sp. 1 "Greece," both at same position.

distribution of alleles, etc.) was not carried out.

RAPD-PCR fingerprinting (Figure 3): With one exception, specimens from the same culture clustered together, separate from the rest. One specimen of the *E. dudichi* culture constantly clustered at the base of the *E. japonensis* culture; this may be due to a contamination of the original culture, which, according to Dózsa-Farkas (1995), also contained *E. bigeminus*. This specimen was removed from the analysis. All 7 cultures formed a cluster of their own. Calculated genetic distances among the fragmenting cultures ranged from 0.75 to 0.91 (Table 4). Genetic distance was lowest between the cultures of *E. japonensis* and *E.* sp. "Greece." These 2 clustered together in all 5 types of analysis, with bootstrap values between 52 (complete linkage) and 70 (neighbor joining). In 4 of the 5 cluster methods used, fragmenting and nonfragmenting cultures were separate from each other, forming 2 distinct clusters.

Discussion

PAGIF and RAPD-PCR showed the same results. All 5 cultures of fragmenting *Enchytraeus* spp. were distinct and separate from each other, but the Greek

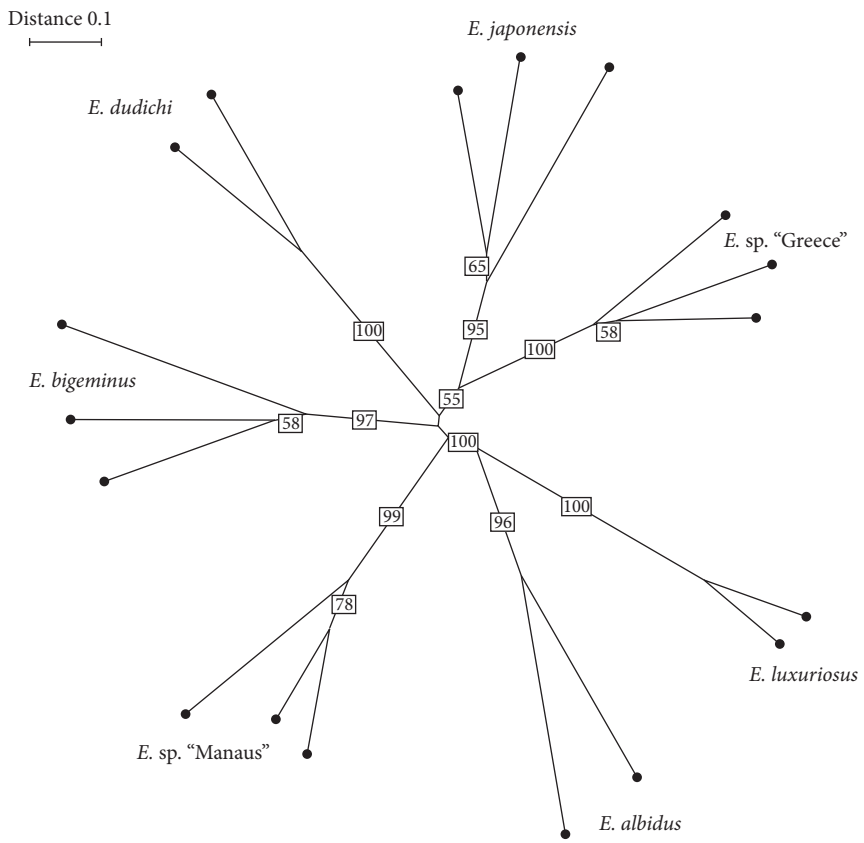

Figure 3. Neighbor-joining tree diagram of genetic distances of fragmenting *Enchytraeus* spp., together with the 2 nonfragmenting species, *E. luxuriosus* and *E. albidus*, as calculated from RAPD fingerprints.

Table 4. Mean distances (in %, top) among investigated populations of fragmenting and nonfragmenting *Enchytraeus* spp., with standard deviations (bottom).

Enchytraeus	*japonensis*	*dudichi*	"Greece"	"Manaus"	*bigeminus*	*luxuriosus*	*albidus*
japonensis	50						
dudichi	90	67					
"Greece"	75	80	39				
"Manaus"	84	92	84	39			
bigeminus	91	90	82	88	53		
luxuriosus	90	97	96	88	91	58	
albidus	88	90	97	88	92	88	24

Enchytraeus	*japonensis*	*dudichi*	"Greece"	"Manaus"	*bigeminus*	*luxuriosus*	*albidus*
japonensis	6.24						
dudichi	14.54	23.15					
"Greece"	6.84	7.56	5.32				
"Manaus"	7.93	2.40	7.39	11.67			
bigeminus	5.62	4.95	6.37	5.78	7.45		
luxuriosus	3.01	4.78	3.66	9.78	4.38	-	
albidus	5.09	8.11	2.89	7.02	7.08	2.61	-

culture was more similar to *E. japonensis* than to any other culture. Furthermore, in the RAPD-PCR analysis, fragmenting and nonfragmenting cultures were sorted into 2 different clusters (Figure 3). Finally, multiple isozyme banding patterns (Figure 2) indicated polyploidy not only in *E. bigeminus* (see Christensen 1980), but also in the other 4 fragmenting populations.

The molecular differences among all 7 cultures suggest species status for each of them, the Greek culture included. Note that RAPD-PCR differences among the fragmenting cultures are in the range of the distance between the 2 nonfragmenting species (Table 4); in a few cases, they are even higher. The status of *E. luxuriosus* and *E. albidus* as 2 different species is beyond doubt; they are morphologically clearly different and belong to 2 different subgroups in the genus (Schmelz and Collado, 2010). Hence, using the distance of *E. luxuriosus* and *E. albidus* as a yardstick, all 7 cultures deserve species rank.

The strong differences in isozyme patterns suggest reproductive isolation of all populations rather than intraspecific heterogeneity. The latter cannot be discarded, however, because we compared genetically homogenized cultures (clones, in fact) and not field populations. For example, it seems possible that the EST variations between *E. japonensis* and the Greek culture (Figure 2) represent 2 differently heterozygous versions of the same gene pool, fixed and multiplied by fragmentation. An extension of this type of interpretation to the other cultures, however, would be difficult to harmonize with the almost complete lack of agreement of isozyme patterns between cultures; note that with PAGIF-IEF, differences of band positions of less than 1 mm are diagnostic, when reproducible. Furthermore, to ascribe all morphological variations listed in Table 2 to intraspecific variability would contradict current taxonomic evidence (e.g., Schmelz, 2003). Finally, the karyological heterogeneity found in *E. bigeminus* (Christensen, 1980) and the above-mentioned genetic

distances calculated from the RAPD-PCR patterns favor interspecific over intraspecific variability. Nevertheless, assessments of the variability of field populations remain a desideratum.

Reproductive isolation may be questioned as an adequate criterion in populations or species with architomy (= fragmentation) as the basic mode of reproduction. However, all available evidence in the literature (Vena et al., 1969; Christensen, 1973, 1980; Dózsa-Farkas, 1996; Myohara et al., 1999; Tadokoro et al., 2006; Sugio et al., 2008) suggests that sexual reproduction is part of the reproductive cycle of populations of fragmenting *Enchytraeus* spp.

Schirmacher et al. (1998) found, with the same RAPD methods, an exceptionally low genetic distance (0.17) between laboratory strains of 2 morphologically identical species, *Enchytraeus crypticus* Westheide & Graefe, 1992 and *E. variatus* Bouguenec & Giani, 1987. The respective distances between these 2 species and the morphologically clearly distinguishable species *E. doerjesi* Westheide & Graefe, 1992 (0.83 and 0.84) were in the range of distances between the fragmenting cultures calculated in this study.

The group of fragmenting *Enchytraeus* spp. is probably a profuse swarm of cryptic, but nonetheless good, biological species. There is slight morphological variation among the investigated cultures; notably, *E. dudichi* is distinguished by the numerical pattern of chaetae (Table 2). However, the triad of *E. bigeminus*, *E. japonensis*, and the Greek culture demonstrates that each distinguishable morphospecies in the group may include several cryptic species. Christensen (1980) detected 2 cytologically incompatible karyotypes (c. 8n and 10n) in the original culture of *E. bigeminus* and did not note any morphological differences. Morphological identification is further hampered by 3 factors: 1) the large intraspecific variability of traits, partly inducible by the changing of culture conditions (Schmelz et al., 2000); 2) traits that are restricted to sexual stages; and 3) the slightness of differences, for example, in the coelomocyte texture. Therefore, light microscopic morphology does not seem to be reliable to delimit taxa within the group.

Fragmentation together with polyploidy seems to be responsible for the large submorphological diversity in fragmenting *Enchytraeus* spp. Christensen (1980, 1994) emphasized that fragmentation in *Enchytraeus* is a reproductive strategy of its own and not an escape mechanism to meet chromosome incompatibilities; despite polyploidy, gametogenesis in *E. bigeminus* is entirely normal. It seems, therefore, that 2 factors, each of them alone with the potential to create taxic diversity, come together here to create a prolific breeding chamber of species.

It may be difficult to distinguish species taxa within this complex. Even molecular methods are not straightforward, because the degree of intraspecific variation of fragmenting *Enchytraeus* is unknown. The genetic homogeneity of a laboratory culture is not a reference for field populations. Therefore, the next step toward a taxonomy of fragmenting *Enchytraeus* should be the assessment of the variability of field populations and a search for evidence of interbreeding.

A further question is the origin of the large diversity of fragmenting *Enchytraeus* discovered in this study. Fragmenting *Enchytraeus* may have evolved from a single population and then spread and diversified around the globe, or fragmentation may have evolved several times independently out of local nonfragmenting populations. Our RAPD results suggest the former (see the clustering of fragmenting populations in Figure 3), without excluding the latter. A combination of both processes is possible, as well. Under all scenarios, the origin would be the same: one or several species of the *Enchytraeus buchholzi* group, as defined by Schmelz and Collado (2010), distributed worldwide. The question regarding monophyly or polyphyly should be addressed by comparing sympatric pairs of fragmenting and nonfragmenting species from several localities.

For future microtaxonomic studies, a set of different analytical methods, such as karyology, isozymes, and DNA sequences, seems recommendable. Data should be objective and transferrable, not comparative and restricted to one study; this excludes RAPD fingerprints but includes chromosomes, DNA-sequences, and, with limitations, isozymes. Of course, light microscopic scrutiny remains indispensable.

With the present state of knowledge, we refrain from delimiting new species taxa within the group of fragmenting *Enchytraeus* species. As a pragmatic approach and pending further evidence, populations

with bisetose worms may be collectively designated as "*Enchytraeus bigeminus* sensu lato," following the work of Schmelz and Collado (2010), and worms with 3 or more chaetae may be identified as "*Enchytraeus dudichi* sensu lato." Furthermore, the informal classificatory designation of "*E. bigeminus* group" may be used for all specimens or populations of fragmenting *Enchytraeus*.

Outlook: species complexes in enchytraeids and taxonomy

Among enchytraeids, the taxonomically complicated situation in fragmenting *Enchytraeus* is by no means exceptional. There is ample evidence that the morphological level is insufficient to recognize species taxa in this family (Table 5). Different cytotypes and the hermaphroditism of the group, which favors

Table 5. Literature evidence for species complexes in enchytraeids apart from the *Enchytraeus bigeminus* group. Mor: evidence based on morphology, Cyt: evidence from chromosome numbers, Prot: evidence from isozyme or total protein patterns, DNA: evidence from DNA sequences.

	Mor	Cyt	Prot	DNA	References
Achaeta affinis Niels. & Christ.					Graefe (unpublished), Schmelz and Collado (2010)
Achaeta bohemica Vejd.	+				Healy (1979), Graefe (unpublished), Schmelz and Collado (2010)
Achaeta danica Niels. & Christ.	+				Graefe (unpublished), Schmelz and Collado (2010)
Achaeta eiseni Vejd.	+				Rota (1994, 1995), Schmelz and Collado (2010)
Buchholzia fallax Mich.		+			Christensen (1980)
Cernosvitoviella atrata (Bretsch.)	+				Chalupský (1992), Rota and Healy (1999)
Cognettia clarae Bauer	+				Schmelz and Collado (2010)
Cognettia sphagnetorum (Vejd.)	+	+			Christensen (1980), Chalupský (1992), Schmelz and Collado 2010
Enchytraeus albidus Henle	+			+	Parapar et al. (2009), Erséus et al. (2009)
Enchytraeus buchholzi Vejd.	+	+	+		Christensen (1980), Chalupský (1992), Rota (1995), Schmelz and Collado (2010)
Enchytraeus lacteus Niels. & Christ.		+			Christensen (1980)
Enchytraeus coronatus Niels. & Christ.		+			Christensen (1980)
Enchytronia parva Niels. & Christ.	+				Chalupský (1992), Rota (1995)
Fridericia alata Niels. & Christ.	+				Schmelz (2003)
Fridericia aurita Issel	+		+		Schmelz (2003)
Fridericia bisetosa (Lev.)	+	+	+		Christensen (1980), Schmelz (2003)
Fridericia connata Bretsch.	+	+	+		Christensen (1980), Schmelz (2003)
Fridericia galba (Hoffm.)	+	+	+		Christensen (1980), Christensen et al. (1992), Schmelz (2003)
Fridericia gamotheca Issel	+				Schmelz (2003)
Fridericia glandifera Friend	+				Schmelz (2003)
Fridericia hegemon (Vejd.)	+				Schmelz (2003)
Fridericia isseli Rota	+				Schmelz (2003)
Fridericia maculata Issel	+				Schmelz (2003), Schmelz and Collado (2010)
Fridericia maculatiformis Dózsa-F.	+				Schmelz (2003)
Fridericia minor Friend = *F. gracilis* v. Bülow	+				Rota and Healy (1999), Schmelz (2003)
Fridericia peregrinabunda Mich.	+				Schmelz (2003)
Fridericia perrieri (Vejd.)	+		+		Schmelz (2003)
Fridericia ratzeli (Eisen)	+	+	+		Christensen (1980), Schmelz (2003)
Fridericia semisetosa (Dózsa-F.)	+				Schmelz (2003)
Fridericia singula Niels. & Christ.	+				Schmelz (2003)
Fridericia striata (Lev.)			+		Christensen et al. (1989)
Hemifridericia parva Niels. & Christ.		+			Christensen (1980)
Henlea perpusilla Friend		+			Christensen (1980)
Lumbricillus lineatus Müller		+	+		Christensen (1980)
Lumbricillus rivalis Lev.			+		Christensen (1980)
Marionina appendiculata Niels. & Christ.	+				Parapar et al. (2009)
Marionina argentea (Mich.)	+				Chalupský (1992)
Marionina spicula (Leuckart)		+			Christensen (1980)

various types of nonamphimictic reproduction such as self-fertilization, parthenogenesis, and fragmentation, may be held responsible. However, even species with ordinary amphimictic reproduction may harbor cryptic species. More and more cryptic species diversity in oligochaetes is being uncovered, and examples are available in the review by Erséus and Gustafsson (2009). This phenomenon, widely distributed over the entire animal kingdom (Knowlton, 2000; Bickford et al., 2006; Pfenninger and Schwenk, 2007), is increasingly gaining attention, and it challenges traditional taxonomy. Our suggestion here is a more flexible use of the species category and a more extended use of a sensu lato approach for the delimitation of binomial species taxa. Molecular species should only be recognized when their identification and that of neighboring species is unambiguous, and when there is a necessity to distinguish between them, for example in the case of pathogens, model organisms, or ecotoxicological test species.

Acknowledgments

Thanks to W. Westheide (University of Osnabrück, Germany) for providing laboratory facilities, to Klára Dózsa-Farkas (Eötvös Loránd University of Budapest, Hungary) for sending a sample culture of *E. dudichi*, to M. Myohara (National Institute of Agrobiological Sciences, Tsukuba, Japan) for providing a sample culture of *E. japonensis*, to Jörg Römbke (ECT Oekotoxikologie GmbH, Flörsheim, Germany) for sending the soil samples that contained the Brazilian strain, and to E. Vavoulidou (NAGREF, Soil Science Institute of Athens, Greece) for providing the soil sample that contained the Greek strain of fragmenting *Enchytraeus*. The constructive criticism of 2 anonymous referees improved the manuscript.

References

Bell, A.W. 1959. *Enchytraeus fragmentosus*, a new species of naturally fragmenting oligochaete worm. Science 129: 1278.

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K. and Das, I. 2006. Cryptic species as a window on diversity and conservation. Trends Ecol. Evol. 22: 149-155.

Boros, G. and Dózsa-Farkas, K. 2007. Preliminary investigations of the enchytraeid fauna in Hungarian greenhouses. Folia Fac. Sci. Univ. Masaryk. Brun., Biol. 110: 135-140.

Bouguenec, V. and Giani, N. 1987. Deux nouvelles espèces d'*Enchytraeus* (Oligochaeta, Enchytraeidae) et redescription d'*E. bigeminus* Niel. & Chr. Remarques sur le genre *Enchytraeus*. Annal. Limnol. 23: 9-22.

Bouguenec, V. and Giani, N. 1989. Biological studies upon *Enchytraeus variatus* Bouguenec & Giani 1987 in breeding cultures. Hydrobiologia 180: 151-165.

Brockmeyer, V. 1991. Isozymes and general protein patterns for use in discrimination and identification of *Enchytraeus* species (Annelida, Oligochaeta). J. Zool. Syst. Evol. Res. 29: 343-361.

Chalupský, J. 1992. Terrestrial Enchytraeidae (Oligochaeta) and Parergodrilidae (Polychaeta) from Sweden, with description of a new enchytraeid species. Zool. Scr. 21: 133-150.

Christensen, B. 1973. Density dependence of sexual reproduction in *Enchytraeus bigeminus* (Enchytraeidae). Oikos 24: 287-294.

Christensen, B. 1980. Annelida. In: Animal Cytogenetics, 2 (ed. B. John), Gebrüder Bornträger, Berlin.

Christensen, B. 1994. Annelida-Clitellata. In: Reproductive Biology of Invertebrates, Vol. VI, part B. Asexual Propagation and Reproductive Strategies (eds. K.G. Adiyodi and R.G. Adiyodi), Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, pp. 1-23.

Christensen, B., Hvilsom, M. and Pedersen, B.V. 1989. On the origin of clonal diversity in parthenogenetic *Fridericia striata* (Enchytraeidae, Oligochaeta). Hereditas 110: 89-91.

Christensen, B., Hvilsom, M. and Pedersen, B.V. 1992. Genetic variation on coexisting sexual diploid and parthenogenetic triploid forms of *Fridericia galba* (Enchytraeidae, Oligochaeta) in a heterogeneous environment. Hereditas 117: 153-162.

Dózsa-Farkas, K. 1995. *Enchytraeus dudichi* sp. n., a new fragmenting Enchytraeus species from Iran (Enchytraeidae, Oligochaeta). Opusc. Zool. Budapest 27-28: 41-44.

Dózsa-Farkas, K. 1996. An interesting reproduction type in enchytraeids (Oligochaeta). Acta Zool. Acad. Sci. Hung. 42: 3-10.

Erséus, C. and Gustafsson, D. 2009. Cryptic speciation in clitellate model organisms. In: Annelids in Modern Biology (ed. D.H. Shain), Wiley-Blackwell, Hoboken, NJ, pp. 31-46.

Healy, B. 1979. Records of Enchytraeidae (Oligochaeta) in Ireland. J. Life Sci. R. Dubl. Soc. 1: 39-70.

Heck, M. 1995. Enchytraeidenzönosen als Indikatoren belasteter Flächen in der Region Berlin. Newsletter on Enchytraeidae 4: 69-77.

Heck, M., Achazi, R.K. and Schmelz, R.M. 1999. Investigations on enchytraeid populations in urban forests and green lands of Berlin. In: Newsletter on Enchytraeidae, No. 6, Proceedings of the 3rd International Symposium on Enchytraeidae, Osnabrück, Germany (eds. R.M. Schmelz and K. Sühlo), Universitätsverlag Rasch, Osnabrück, Germany, pp. 127-155.

Jänsch, S. and Römbke, J. 2003. Ökologische Charakterisierung ausgewählter Enchytraeenarten hinsichtlich relevanter Bodeneigenschaften. Z. Umweltchem. Ökotox. 15: 95-105.

Knowlton, N. 2000. Molecular genetic analyses of species boundaries in the sea. Hydrobiologia 420: 73-90.

Myohara, M., Yoshida-Noro, C., Kobari, F. and Tochinai, S. 1999. Fragmenting oligochaete *Enchytraeus japonensis*: a new material for regeneration study. Dev. Growth Differ. 41: 549-555.

Nakamura, Y. 1993. A new fragmenting enchytraeid species, *Enchytraeus japonensis* from a cropped Kuroboku soil in Fukushima, Northern Japan (enchytraeids in Japan 5). Edaphologia 50: 37-39.

Nielsen, C. O. and Christensen, B. 1963. The Enchytraeidae. Critical revision and taxonomy of European species. Supplement 2. Nat. Jutland. 10: 1-19.

Parapar, J., Martínez-Ansemil, E., Caramelo, C., Collado, R. and Schmelz, R. M. 2009. Polychaetes and oligochaetes associated with intertidal rocky shores in a semi-enclosed industrial and urban embayment, with the description of two new species. Helgol. Mar. Res. 63: 293-308.

Pfenninger, M. and Schwenk, K. 2007. Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. BMC Evolut. Biol. 7: 121-126.

Römbke, J., Collado, R. and Schmelz, R.M. 2005. Oligochaetes (Clitellata) of the Mata Atlântica (Parana, Brazil): first results of the SOLOBIOMA project. Proc. Estonian Acad. Sci. Biol. Ecol. 54: 302-309.

Römbke, J., Collado, R. and Schmelz, R.M. 2007. Abundance, distribution, and indicator potential of enchytraeid genera (Enchytraeidae, Clitellata) in secondary forests and pastures of the Mata Atlântica (Paraná, Brazil). Acta Hydrobiol. Sin. 31 Suppl.: 139-150.

Rota, E. 1994. Enchytraeidae (Oligochaeta) of western Anatolia: taxonomy and faunistics. Boll. Zool. 61: 241-260.

Rota, E. 1995. Italian Enchytraeidae (Oligochaeta). I. Boll. Zool. 62: 183-231.

Rota, E. and Healy, B. 1999. A taxonomic study of some Swedish Enchytraeidae (Oligochaeta), with descriptions of four new species and notes on the genus Fridericia. J. Nat. Hist. 33: 29-64.

Schirmacher, A., Schmidt, H. and Westheide, W. 1998. RAPD-PCR investigations on sibling species of terrestrial *Enchytraeus* (Annelida: Oligochaeta). Biochem. Syst. Ecol. 26: 35-44.

Schmelz, R.M. 2003. Taxonomy of *Fridericia* (Oligochaeta, Enchytraeidae). Revision of species with morphological and biochemical methods. Abh. Naturw. Ver. Hamburg (NF) 38: 415 + 73 fig.

Schmelz, R.M. and Collado, R. 1999. *Enchytraeus luxuriosus* sp. nov., a new terrestrial oligochaete species (Enchytraeidae, Clitellata, Annelida). Carolinea 57: 93-100.

Schmelz, R.M. and Collado, R. 2010. A guide to European terrestrial and freshwater species of Enchytraeidae (Oligochaeta). Soil Organisms 82: 1-176.

Schmelz, R.M., Collado, R. and Myohara, M. 2000. A taxonomical study of *Enchytraeus japonensis* (Enchytraeidae, Oligochaeta): Morphological and biochemical comparisons with *E. bigeminus*. Zool. Sci. 17: 505-516.

Sugio, M., Takeuchi, K., Kutsuna, J., Tadokoro, R., Takahashi, Y., Yoshida-Noro, C. and Tochinai, S. 2008. Exploration of embryonic origins of germline stem cells and neoblasts in *Enchytraeus japonensis* (Oligochaeta, Annelida). Gene Expres. Pat. 8: 227-236.

Tadokoro, R., Suglo, M., Kutsuna, J., Tochinai, S. and Takahashi, Y. 2006. Early segregation of germ and somatic lineages during gonadal regeneration in the annelid *Enchytraeus japonensis*. Current Biol. 16: 1012-1017.

Vavoulidou, E., Römbke, J., Sidiras, N., Bilasis, D. and Tsigou, R. 1999. Effects of three different soil cultivation and fertilisation treatment on earthworms and enchytraeids. In: Newsletter on Enchytraeidae, No. 6, Proceedings of the 3rd International Symposium on Enchytraeidae, Osnabrück, Germany (eds. R.M. Schmelz and K. Sühlo), Universitätsverlag Rasch, Osnabrück, Germany, pp. 91-100.

Vena, J.A., Hess, R.T. and Gotthold, M.L. 1969. Attainment of sexuality in *Enchytraeus fragmentosus* Bell under laboratory conditions. Experientia 25: 761.

Westheide, W. and Haß-Cordes, E. 2001. Molecular taxonomy: description of a cryptic *Petitia* species (Polchaeta: Syllidae) from the island of Mahé (Seychelles, Indian Ocean) using RAPD markers and ITS2 sequences. J. Zool. Syst. Evol. Res. 39: 103-111.