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1 **First record of terrestrial *Enchytraeidae* (*Annelida: Clitellata*) in**
2 **Versailles palace's park, France**

3

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5

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13

14 **Abstract**

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France can be qualified as *terra incognita* regarding terrestrial enchytraeids because very little data has been recorded so far in this country. In spring and autumn 2016, enchytraeid communities were investigated in a loamy soil in a meadow located in the park of Versailles palace, France. In total, twenty four enchytraeid species were identified, belonging to six different genera. i.e., eleven *Fridericia* species, four *Enchytraeus* species, four *Achaeta* species, two *Buchholzia* species, two *Marionina* species and one *Enchytronia* species. According to the published data, this was one of the highest diversity found in a meadow in Europe.

Keywords: Enchytraeids; Potworms; Soil fauna; Annelids; *Oligochaeta*; Meadow

26 Introduction

27

28 Despite their key role in soils (Didden, 1993), enchytraeids (*Annelida: Clitellata*) are so far
29 poorly studied in many countries worldwide. To our knowledge, and except a few species recorded in
30 Schmelz and Collado (2010), no data have been published on enchytraeid communities in France, i.e.,
31 based on a literature search in the ISI Web of Knowledge, using the “All Databases” option, with the
32 formula: ‘(enchytr* or potworm*) and (France or French) in Topics’.

33

34 Although some studies assessed enchytraeid community structures in grasslands, meadows
35 and pastures, studying the influence of grazing (Schlaghamerský et al., 2007), liming (Davidson et al.,
36 2004), fertilization (Sokołowska and Seniczak, 2005) or pesticides (Clements et al., 1987; Forsters et
37 al., 1992; Martin, 1975), most of them only reported a total abundance of enchytraeids (e.g. Clements
38 et al., 1987; Francini et al., 2014; Van Vliet et al., 2006), without any details on species diversity. Yet,
39 several authors recommended to identify the individuals at least at the genus level, and if possible at
40 the species level (Pelosi and Römbke, 2016). Indeed, indicators at the genus or species level appeared
41 to be more sensitive to land use than total abundance. Moreover, taxonomic diversity provides key
42 information on the requirements and sensitivity of the different species to environmental conditions,
43 land use and management.

44

45 The objective of this study was to record the diversity of enchytraeids in a meadow in France
46 and to compare it with other data in Europe.

47

48 2. Material and Methods

49 2.1. Study site

50

51 Samples were collected in a meadow (about 1 ha, see Figure 1) located in the park of
52 Versailles palace, France (48°48'31''N, 2°05'26''E), which has not been cultivated since 1994. Before
53 1994, it was conventionally cultivated with e.g. barley, oats, beans, corn and wheat. The climate is
54 oceanic temperate, with a mean annual temperature of 11 °C and a mean annual rainfall of 660 mm.
55 The soil is a deep Luvisol (FAO soil classification) developed on loess. The homogeneity of the
56 physicochemical characteristics in the experimental site was verified by random sampling a soil core
57 (0-20 cm-depth) at seven different locations in the site (Table 1).

58

59 2.2. Sampling and identification

60

61 Ninety-six soil cores were sampled both in April and November 2016, following a regular grid
62 of 60 m x 40 m and using a split soil corer (diameter of 5 cm) at 0-5 cm and 5-10 cm depths. Each
63 sample was transferred separately into a plastic bag and stored at 4°C. Enchytraeids were extracted
64 using wet funnel extractors under a light from incandescent light bulbs. Soil samples were heated up
65 from 17°C to 43°C on their upper surface for 3 hours (O'Connor, 1959; Kobetičová and
66 Schlaghamerský, 2003). All the individuals were kept in Petri dishes in tap water and counted. Adult
67 and sub-adult individuals were identified at the species level under a light microscope (up to 400x
68 magnification) according to the key of Schmelz and Collado (2010).

69

70 Results

71

72 In total, twenty four species of enchytraeids were identified, belonging to six genera, i.e.,
73 eleven *Fridericia* species, four *Enchytraeus* species, four *Achaeta* species, two *Buchholzia* species,
74 two *Marionina* species and one *Enchytronia* species. Species are listed below, with available
75 information on their presence in Europe, habitat and life strategy (based on Graefe and Schmelz, 1999
76 and Schmelz and Collado, 2000):

77

- 78 - *Achaeta bohemica* (Vejdovský, 1879): widespread, fresh soils, absent in wet habitats,
79 slightly acid to slightly alkaline conditions, never in strongly acid soils, *K*-strategist, soil
80 dweller.

- 81 - *Achaeta iberica* Graefe, 1989: found in Spain, Italy (Schmelz and Collado, 2000), and
82 Switzerland (Amossé et al., 2016), only known from the original description.
83 - *Achaeta pannonica* Graefe, 1989: widespread, fresh soils, absent in wet habitats, slightly
84 acid to slightly alkaline conditions, never in strongly acid soils, *K*-strategist, soil dweller.
85 - *Achaeta unibulba* Graefe, Christensen & Dózsa-Farkas, 2005: widespread.
86 - *Buchholzia appendiculata* (Buchholz, 1862): common and widespread in neutral to
87 slightly acidic soils, *r/F* (fragmenting)-strategist, litter dweller.
88 - *Buchholzia fallax* Michaelsen, 1887: widespread.
89 - *Enchytraeus buchholzi* Vejdovský, 1879: widespread, very common, species complex,
90 not in strongly acidic soils, *r*-strategist, OF and soil dweller.
91 - *Enchytraeus bulbosus* Nielsen & Christensen, 1963: widespread, not in strongly acidic
92 soils, *r*-strategist, OF and soil dweller.
93 - *Enchytraeus christenseni* Dózsa-Farkas, 1992: widespread but not common, wet habitats,
94 not in strongly acidic soils, *r*-strategist, OF and soil dweller.
95 - *Enchytraeus lacteus* Nielsen & Christensen, 1961: widespread, not in strongly acidic
96 soils, *r*-strategist, OF and soil dweller.
97 - *Enchytronia parva* Nielsen & Christensen, 1959: common and widespread in neutral to
98 moderately acidic soils, probably a species complex, soil dweller, stress tolerant species
99 adapted to acidity stress.
100 - *Fridericia bulboides* Nielsen & Christensen, 1959: widespread and common, slightly acid
101 to slightly alkaline conditions, never in strongly acid soils, *K*-strategist, soil dweller
102 - *Fridericia christeri* Rota & Healy, 1999: Northern half of Europe, very common in
103 Germany.
104 - *Fridericia galba* (Hoffmeister, 1843): widespread and very common in Central Europe,
105 slightly acid to slightly alkaline conditions, never in strongly acid soils, *K*-strategist, soil
106 dweller.
107 - *Fridericia isseli* Rota, 1994: widespread and common.
108 - *Fridericia maculata* Issel, 1905: widespread, probably a species complex, slightly acid to
109 slightly alkaline conditions, never in strongly acid soils, *K*-strategist, soil dweller.
110 - *Fridericia nix* Rota, 1995: Italy, Germany.
111 - *Fridericia paroniana* Issel, 1904: widespread and common, slightly acid to slightly
112 alkaline conditions, never in strongly acid soils, *K*-strategist, soil dweller.
113 - *Fridericia tuberosa* Rota, 1995: moist soil, widespread.
114 - *Fridericia ulrikae* Rota & Healy, 1999: Northern and Central Europe.
115 - *Fridericia viridula* Issel, 1904: found in Ireland, The Netherlands, and Italy.
116 - *Fridericia glandifera* Friend, 1911: few records in England, Denmark, Germany and
117 France.
118 - *Marionina argentea* (Michaelsen, 1889): very common and widespread, wet sites also
119 aquatic, slightly salt-tolerant, slightly acid to slightly alkaline conditions, never in strongly
120 acid soils, *K*-strategist, soil dweller.
121 - *Marionina communis* Nielsen & Christensen, 1959: widespread, fresh soils but not wet,
122 slightly acid to slightly alkaline conditions, never in strongly acid soils, *K*-strategist, OF
123 dweller.

124
125 A total of 5 633 and 3 390 individuals were identified in Spring 2016 and in Autumn 2016,
126 respectively. The number of individuals and the percentage of enchytraeid species are presented in
127 Table 2. In Spring, the three most abundant species were *E. buchholzi* (23,6 %), *F. galba* (14,4 %),
128 and *F. isseli* (13,2 %). In autumn, the three most abundant species were *En. parva* (12,7%), *F.*
129 *christeri* (12,2 %), and *E. buchholzi* (11,8 %).

131 Discussion

132
133 This study, in which twenty four species were found, represents the first record of
134 enchytraeid's community in France. Schmelz and Collado (2010) reported only four to five species
135 identified in France: *Guaranidrilus europaeus* (Healy, 1979), *Enchytraeus varithecatius* (Bouguenec &

136 Giani, 1987), *Enchytraeus doerjesi* (Westheide & Graefe, 1992), *Fridericia glandifera*, and *Fridericia*
137 *stephensoni* (Moszyński, 1933, need to be confirmed). A bit more than 200 species are listed in the key
138 for terrestrial enchytraeids of Europe (Schmelz and Collado, 2010). Among them, 126 have been
139 sampled in Germany (Römbke et al. 2013), and 50 of them can be classified as common for Central
140 Europe (Didden et al., 1997).

141

142 When looking at the published literature on enchytraeid communities in grasslands, meadows
143 and pastures, only forty publications could be found. Among them, some were performed under
144 tropical climates, in Brazil (e.g., Schmelz et al., 2008). Schlaghamerský and Kobetičová (2005)
145 studied *Enchytraeidae*, *Tubificidae*, *Aeolosomatidae* in a field experiment in the Czech Republic
146 during meadow restoration on arable land and in a well-preserved meadow nearby. They found 22
147 species of *Enchytraeidae* in the meadow and considered it as a relatively high diversity. The same
148 authors also studied two sites in the White Carpathians (Czechia) at four dates during one year
149 (Schlaghamerský and Kobetičová, 2006). Each sites consisted of one meadow and one cattle pasture.
150 They found respectively 16 and 17 enchytraeid species in the two meadows. The high percentages of
151 *Fridericia* spp. in all plots were considered to correspond with their rather low soil acidity. In the
152 present study, despite the neutral pH, we also found many *Fridericia* individuals and eleven out of
153 twenty four species belonged to this genus. To our knowledge, the highest diversity reported in a
154 meadow was 23 species (Schlagamerský et al., 2007) in White Carpathians (Czechia), with twelve
155 *Fridericia* species. The diversity found in our study was close to these results and thirteen species
156 were in common. Some species such as *F. galba*, *E. buchholzi* or *M. argentea* appeared to be
157 relatively well represented in the different meadows of the published studies. Contrarily, some others,
158 such as *F. christeri* are more rarely found.

159

160 Regarding enchytraeid species distribution in European countries and soil characteristics (pH,
161 soil water and C_{org} contents), the species found in our study are in accordance with the observations of
162 Schmelz and Collado (2010) and Graefe and Schmelz (1999). We found horizontal heterogeneity in
163 the meadow concerning the occurrence of the different species of enchytraeids. This was probably due
164 to the variations in soil properties in the plot (e.g. soil moisture, temperature, vegetation). For
165 example, numerous *M. argentea* were found in few soil cores and absent in others. This could be
166 mainly explained by wetter soil conditions in one part of the plot. By contrast, species such as *F.*
167 *galba*, *F. isseli*, *F. christeri*, *E. buchholzi* or *En. parva* were observed in most of soil samples. Few
168 species were mainly found in the soil layer at 5-10 cm depth such as *Achaeta* spp. or *F. nix*. Those
169 observations confirm Graefe and Schmelz study (1999) as they indicated that *A. bohémica* and *A.*
170 *pannonica* are known to be soil dwellers. However, no information was found about *F. nix* habitat
171 requirements.

172

173 We found a total of five *r*-strategists (*Enchytraeus* spp. and *B. appendiculata*) including one
174 fragmenting species (*B. appendiculata*) among the sampled species. We also observed numerous
175 specimens of *Buchholzia* sp. with two chaeta, which is unusual except for very young specimens just
176 hatched from cocoons and not “born” by fragmentation (Schmelz, pers. observ.). DNA test will
177 confirm if a new species of *Buchholzia* has been found.

178

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180

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Table 1. Physicochemical characteristics of the soil at the experimental site (n = 7, ± Standard Deviation)

Parameters	Meadow soil
Texture (USDA, 1975)	Loamy soil
Clay (%)	22.9 ± 0.7
Silt (%)	48.0 ± 1.3
Sand (%)	29.1 ± 1.2
pH _{H2O}	7.5 ± 0.2
C _{org} (g kg ⁻¹)	18.9 ± 1.0
N _{tot} (g kg ⁻¹)	1.5 ± 0.1
C _{org} /N _{tot}	12.7 ± 0.3
P ₂ O ₅ (g kg ⁻¹)	0.08 ± 0.01
CaCO ₃ (g kg ⁻¹)	23.3 ± 8.1
Cu _{tot} (mg.kg ⁻¹)	25.2 ± 1.6

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Table 2. Enchytraeid density (ind.m⁻²) per species and per season (Spring and Autumn 2016). Nomenclature follows Schmelz and Collado (2010).

Genus	Species	Spring 2016		Autumn 2016	
		number of individuals	% abundance	number of individuals	% abundance
<i>Achaeta</i>	<i>bohemica</i>	0	0,00	20	0,59
<i>Achaeta</i>	<i>iberica</i>	311	5,52	186	5,49
<i>Achaeta</i>	<i>pannonica</i>	12	0,21	34	1,00
<i>Achaeta</i>	<i>unibulba</i>	13	0,23	28	0,83
<i>Buchholzia</i>	<i>appendiculata</i>	238	4,23	120	3,54
<i>Buchholzia</i>	<i>fallax</i>	27	0,48	9	0,27
Enchytraeus	<i>buchholzi</i>	1330	23,61	399	11,77
<i>Enchytraeus</i>	<i>bulbosus</i>	97	1,72	29	0,86
<i>Enchytraeus</i>	<i>christenseni</i>	32	0,57	0	0,00
<i>Enchytraeus</i>	<i>lacteus</i>	2	0,04	0	0,00
Enchytronia	<i>parva</i>	441	7,83	432	12,74
<i>Fridericia</i>	<i>bulboides</i>	0	0,00	31	0,91
Fridericia	<i>christeri</i>	426	7,56	412	12,15
Fridericia	<i>galba</i>	813	14,43	397	11,71
<i>Fridericia</i>	<i>glandifera</i>	0	0,00	2	0,06
Fridericia	<i>isseli</i>	745	13,23	386	11,39
<i>Fridericia</i>	<i>maculata</i>	10	0,18	55	1,62
<i>Fridericia</i>	<i>nix</i>	17	0,30	30	0,88
<i>Fridericia</i>	<i>paroniana</i>	319	5,66	320	9,44
<i>Fridericia</i>	<i>tuberosa</i>	254	4,51	270	7,96
<i>Fridericia</i>	<i>ulrikae</i>	152	2,70	102	3,01
<i>Fridericia</i>	<i>viridula</i>	62	1,10	23	0,68
<i>Marionina</i>	<i>argentea</i>	244	4,33	37	1,09
<i>Marionina</i>	<i>communis</i>	3	0,05	4	0,12

NI	85	1,51	64	1,89
Total	5633	100 %	3390	100 %

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Figure 1. Meadow located in the park of Versailles palace

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