PROJECT NUMBER 289706

COLLABORATIVE PROJECT

Assessing and Monitoring The Impacts of Genetically Modified Plants on Agro-ecosystems

Deliverable 4.7 - Report on potential earthworm species as appropriate focal species for cropping systems with maize and potato from different biogeographical regions

Organisation name of lead contractor for this deliverable: Stefan Schrader, Thünen Institute of Biodiversity, 38116 Braunschweig, Germany

Report compiled by: Christine van Capelle Christine.vancapelle@ti.bund.de)

Project funded by the European Commission within the Seventh Framework Programme (2007-2013)		
Dissemination Level		
PU	Public	**x**
PP	Restricted to other programme participants (including the Commission	
RE	Restricted to a group specified by the consortium (including the Commission	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Summary

By means of a literature survey, earthworm species of significant functional relevance in Irish, Swedish and Spanish AMIGA sites were identified. These focal earthworm species, defined here according to the EFSA ERA guidance document (EFSA, 2010), are representative for crop rotations with maize and/or potatoes within the respective biogeographical regions (Atlantic, Boreal, Mediterranean). Species should be used as non-target decomposers in a standardized laboratory test system for GM crop risk assessment based on life-history traits.
In total, 40 literature sources were collected to provide information on species diversity, individual density and specific functional relevance. By means of condensed literature data, species which (1) play an important part in respective soil systems, (2) are well adapted to biogeographical regions, (3) are expected to occur in high abundances under cultivation of maize (Sweden and Spain) or potato (Ireland) and (4) fulfill the requirements along with the development of the test system based on performance traits (availability, suitability to testing under laboratory conditions, sensitivity against GM crop residues) were identified. This way, 8 anecic and endogeic earthworm species (5 for each biogeographical region) belonging to two families (Lumbricidae, Acanthodrilidae) were identified as potential focal species: *Aporrectodea caliginosa, Aporrectodea rosea, Aporrectodea longa, Allolobophora chlorotica, Lumbricus terrestris, Lumbricus friendi, Octodrilus complanatus* and *Microscolex phosphoreus.* The species *Aporrectodea caliginosa* (endogeic, secondary decomposer) and *Lumbricus terrestris* (anecic, primary decomposer) were finally selected to be included in the laboratory test system.

Introduction

Earthworms as important detritivores are often considered to represent the “keystone” group of soil invertebrates due to their role in decomposing plant litter (Lavelle & Spain, 2005; Wall et al., 2012). They contribute to important soil processes, like bioturbation, the formation of organo-mineral complexes during the gut passage, the regulation of nutrient cycling processes and are highly involved in increasing soil fertility (Edwards et al., 1995; Parmelee et al., 1998). Due to their burrowing activity, consumption of leaf litter and promotion of microbial activity they play an important role in soil formation (Tomlin et al.,

1995; Wall et al., 2012). Moreover, earthworms represent an important part of the diet of many vertebrates and other invertebrates (Edwards & Bohlen, 1996).

Due to these functional properties, there is a reasonable doubt that earthworms may be affected by genetically modified (GM) crops. Impacts might occur via GM-induced expression of specific proteins, like the Cry1Ab protein in Bt maize, whose degradation from litter material is accelerated by earthworm activity (Schrader et al., 2008; Emmerling et al., 2011). Furthermore, GM crops differ from the near-isoline in the amount of major plant components such as cellulose, lignin, fructose or soluble carbohydrates (Escher et al., 2000; Flores et al., 2005; Poerschmann et al., 2005; Saxena & Stotzky, 2001). These modifications affect nutritional parameters of plant material (Clark & Coats, 2006) and the decomposability of plant residues in soil (Flores et al., 2005; Hönemann et al., 2008; Zwahlen et al., 2007).

Thus, earthworms are, on the one hand, closely associated to GM crops and their compounds and, on the other hand, they contribute to numerous important ecosystem functions and services. According to the combination of these both issues earthworms represent appropriate non-target organisms in the context of GM crop risk assessment (EFSA, 2010).

Against this background, Task 4.4 of Work Package 4 aims at developing a laboratory test system that serves for standard testing of the impact of GM crops (here: maize and potatoes) of selected European biogeographical regions on earthworms.

Previous risk assessment studies on earthworms mainly focus on common laboratory species, usually *Eisenia fetida* (e.g. Ahl Goy et al., 1995; Clark & Coats, 2006). As these species are often irrelevant in arable field soils and therefore may only be of limited value for risk analyses, the present test system should base on focal earthworm species. These species are, according to the EFSA ERA guidance document (EFSA, 2010), defined as species with a high potential exposure linked to a significant functional importance in soils of a specific biogeographical region under cultivation of a respective crop. This focal species approach avoids that laboratory tests might be classified as “not ecologically realistic” due to the selection of non-target species which may not represent the most sensitive examples

from the functional group in the regional food web, an often critical point in previous risk assessment studies (Lövei & Arpaia, 2005).

Moreover, tests designed to assess acute toxicity over short-term exposure may not predict effects of chronic exposure, like sublethal direct or indirect effects on non-target species over several generations (Birch et al., 2007). To meet this need for risk assessment under chronic exposure conditions, the test system should include growth, reproduction and survival of focal earthworm species as main components of their fitness and relevant performance traits to conclude on potential long-term effects and changes in ecological functions (Pey et al., 2014; Violle et al., 2007). GM crop risk assessment should, therefore, base on biomass, cocoon production, cocoon size, percentage of cocoon hatching, as well as survival, biomass, growth and development of offspring as measurable endpoints. According to this approach, selected focal earthworm species should be culturable and suitable to testing under laboratory conditions.

A literature survey was conducted to identify potential focal earthworm species which (1) are of high functional relevance in soil systems at different European AMIGA sites (Ireland, Sweden and Spain), belonging to three biogeographical regions (Atlantic, Boreal and Mediterranean) and (2) fulfill the requirements along with the development of the test system based on performance traits (availability of species, suitability to testing under laboratory conditions, sensitivity against GM crop residues).

Procedure of data assessment

In order to identify appropriate focal species for development of the standardized laboratory test system peer-reviewed papers in relevant scientific journals, proceedings and reports of projects, and online-available species lists were screened. The literature sources were examined for data on earthworm communities in Irish, Swedish and Spanish arable soils. Finally, 40 literature sources (10 sources for Irish soils, 10 sources for Swedish soils, and 20 sources for Spanish soils) were collected to provide appropriate information, allowing the selection of focal earthworm species (see Appendix for the complete list of sources).

Data on species diversity, individual density and specific functional relevance were analyzed and condensed. By means of these integrated analyses, those species were identified, which (1) play an important part in respective soil systems, (2) are well adapted to biogeographical regions, and (3) are expected to occur in high abundances under cultivation of maize (Sweden and Spain) or potato (Ireland).

If not stated, the dominance distribution within species communities was calculated by means of total or relative abundances of species in relation to total individual densities. The calculation of the dominance structure of the species assemblage was assessed following the classification system of Engelmann (1978). According to this system, species represented by greater than 10 % of the total density are classified as dominant; those comprising 3.2–9.9 % of the total density are subdominant, 1.0–3.1 % recedent, 0.32–0.99 % subrecedent and <0.32 % sporadic. For reasons of clarity, dominant and subdominant as well as recedent and subrecedent species were pooled.

Results of data evaluation and discussion

The literature sources indicate the occurrence of earthworm species in Ireland, Sweden, and Spain either by presenting whole species lists (7 literature sources), records of certain species (6 literature sources) or earthworm community compositions (27 literature sources). Studies on field-derived data, moreover, partially indicate species numbers or abundances and/or biomasses of whole communities or species as relevant parameters for selecting appropriate focal species (Tab. 1).

Information from total species lists of countries were combined with field-derived data on species records to compile region-specific lists of species including their ecological classification (Tab. 2, 3, 4).

Tab. 1: Number of literature sources [n] on earthworm parameters relevant to the selection of focal earthworm species in the three biogeographical regions represented by Ireland (IR), Sweden (S) and Spain (ES).

	Number of literature sources [n]		
	Atlantic [IR]	**Boreal [S]**	**Mediterranean [ES]**
Total [n]	10	10	20
Species lists	3	2	2
Records of communities	7	6	14
Records of species	0	2	4
Crop (potato or maize)	1	0	2
Species number [n]	10	7	9
Total earthworm abundance [ind. m^{-2}]	6	3	1
Total earthworm biomass [g m^{-2}]	5	3	0
Species abundance [ind. m^{-2}]	5	3	1
Species biomass [g m^{-2}]	0	3	0
Relative abundance of species [%]	6	4	1

The species list for Irish soils comprises 30 species belonging to 3 families (Acanthodrilidae, Lumbricidae and Sparganohilidae), whereof 12 belong to the endogeic, 3 to the anecic, and 14 to the epigeic ecological group. One species was classified as aquatic. In total 6 of these species (4 endogeic and 2 anecic ones) were recorded under cultivation of potato (Tab. 2).

The species list for Swedish soils includes 22 species, all belonging to the family Lumbricidae, whereof 8 are endogeic, 2 anecic, 11 epigeic and 1 epiendogeic (Tab. 3).

The list of earthworm species for Spanish soils comprises 75 species belonging to 7 families (Acanthodrilidae, Criodrilidae, Haplotaxidae, Hormogastridae, Lumbricidae, Megascolecidae and Ocnerodrilidae). Of these species, 27 are classified as endogeic, 6 as anecic and 19 as epigeic. Moreover, 3 semiaquatic and 2 aquatic species are included. For 18 species no records on respective ecological groups exist. In total, 8 of these species (4 endogeic and 4 epigeic) are described to occur under cultivation of maize (Tab. 4).

Tab. 2: Earthworm species from Ireland, their ecological group and the respective number of literature sources [n], indicating their occurrence.

FAMILY resp. species	**Ecological group**	**Literature sources [n]**
ACANTHODRILIDAE		
Microscolex phosphoreus (Dugès, 1837)	endogeic	3
LUMBRICIDAE		
Allolobophora chlorotica (Savigny, 1826)*	endogeic	10
Allolobophora cupulifera (Tétry, 1937)	endogeic	2
Allolobophoridella eiseni (Levinsen, 1884)	endogeic	2
Aporrectodea caliginosa (Savigny, 1826)*	endogeic	10
Aporrectodea icterica (Savigny, 1826)	endogeic	2
Aporrectodea limicola (Michaelsen, 1890)	endogeic	4
Aporrectodea longa (Ude, 1885)*	anecic	8
Aporrectodea rosea (Savigny, 1826)*	endogeic	10
Dendrobaena attemsi (Michaelsen, 1902)	epigeic	2
Dendrobaena octaedra (Savigny, 1826)	epigeic	5
Dendrobaena pygmaea (Savigny, 1826)	epigeic	1
Dendrodrilus rubidus (Savigny, 1826)	epigeic	5
Eisenia andrei (Bouché, 1972)	epigeic	1
Eisenia fetida (Savigny, 1826)	epigeic	5
Eisenia hortensis (Michaelsen, 1890)	epigeic	4
Eiseniella tetraedra (Savigny, 1826)	epigeic	4
Helodrilus oculatus (Hoffmeister, 1845)	epigeic	3
Lumbricus castaneus (Savigny, 1826)	epigeic	7
Lumbricus eiseni (Levinsen, 1884)	epigeic	2
Lumbricus festivus (Savigny, 1826)	epigeic	7
Lumbricus friendi (Cognetti, 1904)	anecic	5
Lumbricus rubellus (Hoffmeister, 1843)	epigeic	8
Lumbricus terrestris (Linnaeus, 1758)*	anecic	9
Murchieona minuscula (Rosa, 1906)*	endogeic	5
Octolasion cyaneum (Savigny, 1826)	endogeic	5
Octolasion tyrtaeum (Savigny, 1826)	endogeic	5
Prosellodrilus amplisetosus (Bouché, 1972)	endogeic	1
Satchellius mammalis (Savigny, 1826)	epigeic	9
SPARGANOPHILIDAE		
Sparganophilus tamesis (Benham, 1892)	aquatic	1

*recorded under cultivation of potato

Tab. 3: Earthworm species from Sweden, their ecological group and the respective number of literature sources [n], indicating their occurrence.

FAMILY resp. species	**Ecological group**	**Literature sources [n]**
LUMBRICIDAE		
Allolobophora chlorotica (Savigny, 1826)	endogeic	4
Allolobophora cupulifera (Tétry, 1937)	endogeic	1
Allolobophoridella eiseni (Levinsen, 1884)	endogeic	1
Aporrectodea caliginosa (Savigny, 1826)	endogeic	7
Aporrectodea limicola (Michaelsen, 1890)	endogeic	2
Aporrectodea longa (Ude, 1885)	anecic	6
Aporrectodea rosea (Savigny, 1826)	endogeic	10
Dendrobaena attemsi (Michaelsen, 1902)	epigeic	2
Dendrobaena hortensis (Michaelsen, 1890)	epigeic	2
Dendrobaena octaedra (Savigny, 1826)	epigeic	6
Dendrodrilus rubidus (Savigny, 1826)	epigeic	3
Eisenia fetida (Savigny, 1826)	epigeic	2
Eisenia nordenskioldi (Eisen, 1879)	epiendogeic	1
Eiseniella tetraedra (Savigny, 1826)	epigeic	6
Lumbricus castaneus (Savigny, 1826)	epigeic	4
Lumbricus eiseni (Levinsen, 1884)	epigeic	1
Lumbricus festivus (Savigny, 1826)	epigeic	2
Lumbricus meliboeus (Rosa, 1884)	epigeic	2
Lumbricus rubellus (Hoffmeister, 1843)	epigeic	5
Lumbricus terrestris (Linnaeus, 1758)	anecic	6
Octolasion cyaneum (Savigny, 1826)	endogeic	5
Octolasion tyrtaeum (Savigny, 1826)	endogeic	4

Tab. 4: Earthworm species from Spain, their ecological group and the respective number of literature sources [n], indicating their occurrence.

FAMILY resp. species	**Ecological group**	**Literature sources [n]**
ACANTHODRILIDAE		
Microscolex dubius (Fletcher, 1887)	endogeic	6
Microscolex phosphoreus (Dugès, 1837)	endogeic	6
CRIODRILIDAE		
Criodrilus lacuum (Hoffmeister, 1845)	aquatic	3
HAPLOTAXIDAE		
Delaya corbarensis (Delay, 1972)	aquatic	1
HORMOGASTRIDAE		
Hormogaster elisae (Álvarez, 1977)	endogeic	6
Hormogaster pretiosa (Michaelsen, 1889)	unknown	1
Xana omodeoi (Díaz Cosín et al., 1989)	unknown	2
LUMBRICIDAE		
Allolobophora s.l. asconensis (Bretscher, 1900)	unknown	1
Allolobophora chlorotica (Savigny, 1826)	endogeic	6
Allolobophora morenoe (Díaz Cosín et al., 1985)	unknown	1
Allolobophora oculata (Hoffmeister, 1845)	endogeic	3
Aporrectodea caliginosa (Savigny, 1826)*	endogeic	10
Aporrectodea chitae (Díaz Cosín et al., 1988)	unknown	4
Aporrectodea dubiosa monchicana (Trigo et al., 1990)	unknown	1
Aporrectodea georgii (Michaelsen, 1890)	endogeic	4
Aporrectodea iberica (Trigo et al., 1988)	unknown	4
Aporrectodea icterica (Savigny, 1826)	endogeic	2
Aporrectodea molleri (Rosa, 1889)	endogeic	6
Aporrectodea oliveirae (Rosa, 1894)	epigeic	5
Aporrectodea opisthosellata (Graff, 1961)	endogeic	1
Aporrectodea rosea (Savigny, 1826)*	endogeic	9
Aporrectodea terrestris (Savigny, 1826)	anecic	1
Dendrobaena alpina (Rosa, 1884)	epigeic	1
Dendrobaena alvaradoi (Moreno et al., 1982)	unknown	4
Dendrobaena attemsi (Michaelsen, 1902)	epigeic	2
Dendrobaena byblica (Rosa, 1893)	epigeic	4
Dendrobaena franzi (Zicsi, 1965)	unknown	2
Dendrobaena hortensis (Michaelsen, 1889)	epigeic	2
Dendrobaena lacustris (Stephenson, 1913)	unknown	1
Dendrobaena lusitana (Graff, 1957)	epigeic	2
Dendrobaena madeirensis (Michaelsen, 1891)*	endogeic	7
Dendrobaena octaedra (Savigny, 1826)*	epigeic	6
Dendrobaena pantaleonis (Chinaglia, 1913)	unknown	3
Dendrobaena pygmaea (Savigny, 1826)	epigeic	5
Dendrodrilus rubidus (Savigny, 1826)*	epigeic	8

Eisenia andrei (Bouché, 1972)	epigeic	4
Eisenia fetida (Savigny, 1826)	epigeic	3
Eisenia hortensis (Michaelsen, 1890)	epigeic	3
Eisenia lucens (Waga, 1857)	epigeic	1
Eiseniella tetraedra (Savigny, 1826)*	epigeic	7
Iberoscolex albolienatus (Díaz Cosín et al., 1989)	semiaquatic	3
Iberoscolex carpetanus (Alvarez, 1970)	semiaquatic	4
Iberoscolex pseudorroseus (Moreno et al., 1982)	unknown	4
Lumbricus castaneus (Savigny, 1826)	epigeic	10
Lumbricus centralis (Bouché, 1972)	anecic	1
Lumbricus eiseni (Levinsen, 1884)	epigeic	6
Lumbricus festivus (Savigny, 1826)*	epigeic	3
Lumbricus friendi (Cognetti, 1904)	anecic	13
Lumbricus rubellus (Hoffmeister, 1843)	epigeic	12
Lumbricus terrestris (Linnaeus, 1758)	anecic	4
Murchieona minuscula (Rosa, 1906)	endogeic	4
Octodrilus complanatus (Dugès, 1828)	anecic	3
Octodrilus lissaensis (Michaelsen, 1891)	unknown	1
Octolasion cyaneum (Savigny, 1826)*	endogeic	8
Octolasion tyrtaeum (Savigny, 1826)	endogeic	7
Orodrilus paradoxus (Cognetti, 1904)	unknown	1
Postandrilus bertae (Díaz Cosín et al., 1985)	endogeic	1
Prosellodrilus amplisetosus (Bouché, 1972)	endogeic	1
Prosellodrilus fragilis (Bouché, 1972)	endogeic	1
Prosellodrilus idealis (Bouché, 1972)	endogeic	1
Prosellodrilus praticola (Bouché, 1972)	endogeic	1
Prosellodrilus pyrenaicus (Cognetti, 1904)	endogeic	1
Satchellius mammalis (Savigny, 1826)	epigeic	5
Scherotheca campoii (Lainez & Jordana, 1983)	endogeic	1
Scherotheca gigas aquitania (Bouché, 1972)	anecic	1
Scherotheca guipuzcoana (Bouché, 1972)	unknown	1
Scherotheca navarrensis (Lainez & Jordana, 1983)	unknown	1
Scherotheca occidentalis (Michaelsen, 1922)	endogeic	1
Scherotheca savignyi (Guerne & Horst, 1893)	unknown	1
MEGASCOLECIDAE		
Amynthas corticis (Kinberg, 1867)	endogeic	3
Amynthas morrisi (Beddard, 1892a)	endogeic	1
Metaphire californica (Kinberg, 1867)	unknown	1
Pontodrilus litoralis (Grube, 1855)	semiaquatic	1
OCNERODRILIDAE		
Eukerria saltensis (Beddard, 1895)	endogeic	2
Ocnerodrilus occidentalis (Eisen, 1878)	endogeic	2

*recorded under cultivation of maize

As a first step to identify potential focal earthworm species among entire species on the initial lists, aquatic and semiaquatic species, as well as species of unknown ecological group, which are ineligible as focal species, were excluded.

Epigeic species are characterized by feeding on plant residues on the soil surface and the creation of non-permanent horizontal burrows. Deep-burrowing anecic species feed on decaying plant residues from the soil surface and create permanent vertical burrows, allowing the incorporation of organic matter deeper in the soil profile. Endogeic species form network-like semi-permanent burrows and feed on topsoil and associated strongly decomposed organic matter. Hence, earthworm species belonging to the remaining ecological groups differ strongly concerning their vertical distribution, burrowing activity and used food sources. According to these behavioural and functional differences, it has to be expected that impacts of GM crops on these soil organisms differ between endogeic, anecic and epigeic species. As individual densities of surface-active epigeic species are usually comparatively low in arable soils (Kladivko, 2001) they were not considered in this context and as well excluded from the lists. Thus, to ensure a high sensitivity and to provide an optimal validity of the test system, endogeic and anecic earthworm species were selected as potential focal species which might be appropriate to be included in laboratory studies.

For further condensation of the lists of remaining anecic and endogeic species, literature data were surveyed for information on the dominance distribution of these species in arable soils and their occurrence under cultivation of maize and potato.

However, in total only three literature sources indicate the earthworm community structure under cultivation of potato in Ireland (1 literature source) and maize in Spain (2 literature sources). With regard to Swedish soils, no literature data on the species composition in maize fields were available (Tab. 1). For that reason, data on the dominance distribution of species under cultivation of other crops (e.g. wheat, barley, oats and rye) were considered as well.

In total 11 literature sources on field-derived data (Ireland: 6; Sweden: 4; Spain: 1) mention the dominance of detected species or indicate abundances [ind. m^{-2}] or relative abundances [%] of species in combination with total individual densities [ind. m^{-2}], allowing to determine the dominance distribution of species according to Engelmann (1978) (Tab. 5, 6 and 7).

Tab. 5: Data sources on total records, records under cultivation of potato, and dominance distribution of anecic and endogeic earthworm species occurring in Ireland. Sources are identified by their consecutive number given in the Appendix.

		Total sources	Sources on dominance			Sources on potato
FAMILY resp. species	Ecological group		dominant	recedent	sporadic	
ACANTHODRILIDAE						
Microscolex phosphoreus (Dugès, 1837)	endogeic	2, 3, 8	-	-	-	-
LUMBRICIDAE						
Allolobophora chlorotica (Savigny, 1826)	endogeic	1, 2, 3, 4, 5, 6, 7, 8, 9, 10	1, 5, 6, 7, 8, 9	6		6
Allolobophora cupulifera (Tétry, 1937)	endogeic	2, 3	-	-	-	-
Allolobophoridella eiseni (Levinsen, 1884)	endogeic	2, 3	-	-	-	-
Aporrectodea caliginosa (Savigny, 1826)	endogeic	1, 2, 3, 4, 5, 6, 7, 8, 9, 10	5, 6, 7, 8, 9	-	-	6
Aporrectodea icterica (Savigny, 1826)	endogeic	2, 3	-	-	-	-
Aporrectodea limicola (Michaelsen, 1890)	endogeic	2, 3, 4, 7	7	-	-	-
Aporrectodea longa (Ude, 1885)	anecic	2, 3, 5, 6, 7, 8, 9, 10	7	5, 6, 7, 9	-	6
Aporrectodea rosea (Savigny, 1826)	endogeic	1, 2, 3, 4, 5, 6, 7, 8, 9, 10	1, 7, 8	5, 6, 9	-	6
Lumbricus friendi (Cognetti, 1904)	anecic	2, 3, 4, 7, 8	7	7	-	-
Lumbricus terrestris (Linnaeus, 1758)	anecic	2, 3, 4, 5, 6, 7, 8, 9, 10	-	5, 6, 7	6, 9	6
Murchieona minuscula (Rosa, 1906)	endogeic	2, 3, 6, 8, 9	6, 9	6	-	6
Octolasion cyaneum (Savigny, 1826)	endogeic	2, 3, 4, 7, 8	-	7	7	-
Octolasion tyrtaeum (Savigny, 1826)	endogeic	1, 2, 3, 4, 7	1	-	7	-
Prosellodrilus amplisetosus (Bouché, 1972)	endogeic	8	8	-	-	-

Tab. 6: Data sources on total records, records under cultivation of maize, and dominance distribution of anecic and endogeic earthworm species occurring in Sweden. Sources are identified by their consecutive number given in the Appendix.

		Total sources	Sources on dominance			Sources on maize
FAMILY resp. species	Ecological group		dominant	recedent	sporadic	
LUMBRICIDAE						
Allolobophora chlorotica (Savigny, 1826)	endogeic	11, 13, 14, 20	14	13	-	-
Allolobophora cupulifera (Tétry, 1937)	endogeic	11	-	-	-	-
Allolobophoridella eiseni (Levinsen, 1884)	endogeic	11	-	-	-	-
Aporrectodea caliginosa (Savigny, 1826)	endogeic	11, 12, 13, 14, 15, 16, 20	12, 13, 14, 15	-	-	-
Aporrectodea limicola (Michaelsen, 1890)	endogeic	11, 20	-	-	-	-
Aporrectodea longa (Ude, 1885)	anecic	11, 12, 13, 14, 16, 20	12, 14	13	-	-
Aporrectodea rosea (Savigny, 1826)	endogeic	11, 12, 13, 14, 15, 16, 17, 18, 19, 20	14	13	12	-
Lumbricus terrestris (Linnaeus, 1758)	anecic	11, 12, 13, 14, 15, 20	12	12, 13, 14	-	-
Octolasion cyaneum (Savigny, 1826)	endogeic	11, 17, 18, 19, 20	-	-	-	-
Octolasion tyrtaeum (Savigny, 1826)	endogeic	11, 18, 19, 20	-	-	-	-

Tab. 7: Data sources on total records, records under cultivation of maize, and dominance distribution of anecic and endogeic earthworm species occurring in Spain. Sources are identified by their consecutive number given in the Appendix.

		Total sources	Sources on dominance			Sources on maize
FAMILY resp. species	Ecological group		dominant	recedent	sporadic	
ACANTHODRILIDAE						
Microscolex dubius (Fletcher, 1887)	endogeic	26, 27, 31, 32, 34, 38	-	32	-	-
Microscolex phosphoreus (Dugès, 1837)	endogeic	26, 27, 29, 31, 32, 38	-	32	-	-
HORMOGASTRIDAE						
Hormogaster elisae (Álvarez, 1977)	endogeic	31, 32, 35, 36, 37, 40	32	-	-	-
LUMBRICIDAE						
Allolobophora chlorotica (Savigny, 1826)	endogeic	26, 27, 28, 31, 34, 38	-	-	-	-
Allolobophora oculata (Hoffmeister, 1845)	endogeic	26, 31, 38	-	-	-	-
Aporrectodea caliginosa (Savigny, 1826)	endogeic	23, 24, 26, 27, 28, 29, 31, 32, 34, 38	32	-	-	23, 24
Aporrectodea georgii (Michaelsen, 1890)	endogeic	26, 31, 34, 38	-	-	-	-
Aporrectodea icterica (Savigny, 1826)	endogeic	31, 38	-	-	-	-
Aporrectodea molleri (Rosa, 1889)	endogeic	26, 27, 29, 31, 34, 38	-	-	-	-
Aporrectodea opisthosellata (Graff, 1961)	endogeic	38	-	-	-	-
Aporrectodea rosea (Savigny, 1826)	endogeic	23, 24, 26, 27, 28, 31, 32, 34, 38	32	-	-	23, 24
Aporrectodea terrestris (Savigny, 1826)	anecic	31	-	-	-	-
Dendrobaena madeirensis (Michaelsen, 1891)	endogeic	24, 26, 27, 28, 29, 31, 34	-	-	-	24
Lumbricus centralis (Bouché, 1972)	anecic	31	-	-	-	-
Lumbricus friendi (Cognetti, 1904)	anecic	21, 22, 25, 26, 27, 28, 29, 30, 31, 33, 34, 38, 39	-	-	-	-
Lumbricus terrestris (Linnaeus, 1758)	anecic	26, 31, 34, 38	-	-	-	-
Murchieona minuscula (Rosa, 1906)	endogeic	26, 27, 31, 38	-	-	-	-
Octodrilus complanatus (Dugès, 1828)	anecic	31, 32, 38	32	32	-	-
Octolasion cyaneum (Savigny, 1826)	endogeic	24, 26, 27, 28, 29, 31, 34, 38	-	-	-	24
Octolasion tyrtaeum (Savigny, 1826)	endogeic	26, 27, 28, 29, 31, 34, 38	-	-	-	-
Postandrilus bertae (Díaz Cosín et al., 1985)	endogeic	31	-	-	-	-
Prosellodrilus amplisetosus (Bouché, 1972)	endogeic	31	-	-	-	-
Prosellodrilus fragilis (Bouché, 1972)	endogeic	31	-	-	-	-
Prosellodrilus idealis (Bouché, 1972)	endogeic	31	-	-	-	-
Prosellodrilus praticola (Bouché, 1972)	endogeic	29	-	-	-	-
Prosellodrilus pyrenaicus (Cognetti, 1904)	endogeic	31	-	-	-	-
Scherotheca campoii (Lainez & Jordana, 1983)	endogeic	31	-	-	-	-
Scherotheca gigas aquitania (Bouché, 1972)	anecic	29	-	-	-	-
Scherotheca occidentalis (Michaelsen, 1922)	endogeic	31	-	-	-	-
MEGASCOLECIDAE						
Amynthas corticis (Kinberg, 1867)	endogeic	26, 31, 38	-	-	-	-
Amynthas morrisi (Beddard, 1892a)	endogeic	31	-	-	-	-
OCNERODRILIDAE						
Eukerria saltensis (Beddard, 1895)	endogeic	31, 38	-	-	-	-
Ocnerodrilus occidentalis (Eisen, 1878)	endogeic	31	-	-	-	-

Species which are assumed to generally occur in high abundances in respective arable field soils were identified according to (1) total species records, (2) occurrence under cultivation of either maize (Sweden, Spain) or potato (Ireland), and (3) information on their dominance distribution as presented in Tabs. 5-7. For each biogeographical region, three endogeic and two anecic species were selected, to allow consideration of specific impacts of GM crop residues on species of different ecological groups and functions.

With regard to Irish soils, those species which occurred under cultivation of potato (6 species) and, additionally, are recorded in at least 80 % of total literature sources (5 species: *Al. chlorotica*, *Ap. caliginosa*, *Ap. longa*, *Ap. rosea* and *L. terrestris*) are selected as focal species for the Atlantic region. Of these five species, four are described as dominant species in at least one literature source (Tab. 5, Tab. 8).

For Swedish soils no information on the species distribution under cultivation of maize was available. Therefore, for this region those endogeic and anecic earthworm species which are described as dominant in literature sources on other arable crops (barley, wheat, oats and rape), and which are, moreover, recorded in at least 40 % of total literature sources on Swedish soils (*Ap. rosea*: 100 %; *Ap. caliginosa*: 70 %; *Ap. longa* and *L. terrestris*: 60 %; *Al. chlorotica*: 40 %) were selected as focal species for the Boreal region (Tab. 6, Tab. 8).

With regard to anecic species in Spanish soils, the circummediterranean species *Octodrilus complanatus* (recorded in three, and described as dominant in one literature source) and *Lumbricus friendi* (recorded in 13 literature sources) (Díaz Cosín et al., 1992; Rodriguez et al., 1997) were selected as focal species. The species *Lumbricus terrestris* was excluded as it was described only to occur in very low densities in Spanish soils (Briones et al., 2009). Concerning the endogeic species, *Aporrectodea caliginosa* and *Aporrectodea rosea* were selected as focal species, as both species are recorded under the cultivation of maize and, furthermore, are described as dominant species (Tab. 7). As third potential focal species, *Microscolex phosphoreus* as indicative species of agricultural activity (Talavera & Pérez, 2009), which was described in 6 literature sources, was selected (Tab. 7, Tab. 8)

Based on condensed literature data, 8 earthworm species (5 for each AMIGA site; Tab. 8), typically occurring in Irish, Swedish or Spanish arable soils, were finally selected as appropriate focal species for the respective biogeographical region.

The focal species belong to the following oligochaete families: Lumbricidae (*Aporrectodea caliginosa, Aporrectodea rosea, Aporrectodea longa, Allolobophora chlorotica, Lumbricus terrestris, Lumbricus friendi, and Octodrilus complanatus*) and Acanthodrilidae (*Microscolex phosphoreus*).

Tab. 8: Selected focal earthworm species for the three biogeographical regions Atlantic (Ireland), Boreal (Sweden) and Mediterranean (Spain).

Biogeographical region **AMIGA site**	**Atlantic** **Ireland**	**Boreal** **Sweden**	**Mediterranean** **Spain**
Endogeic	*Ap. caliginosa* *Al. chlorotica* *Ap. rosea*	*Ap. caliginosa* *Al. chlorotica* *Ap. rosea*	*Ap. caliginosa* *Ap. rosea* *M. phosphoreus*
Anecic	*Ap. longa* *L. terrestris*	*Ap. longa* *L. terrestris*	*O. complanatus* *L. friendi*

According to the availability of species, their suitability to testing under laboratory conditions and the sensitivity of different life-history traits against GM plant residues, the species *Aporrectodea caliginosa* (secondary decomposer) and *Lumbricus terrestris* (primary decomposer) were finally selected to be included in the standardized laboratory test system. The results of this objective were submitted as Milestone (MS 11) to the members' area of the AMIGA website in November 2012.

General discussion

Earthworms are well documented from many parts of the world, particularly Europe. However, only less information on earthworm communities in Irish, Swedish or Spanish arable systems, where specifically maize or potatoes were cultivated, is available. Therefore, literature data on earthworm abundances and species diversity from different types of agroecosystems were considered as well during the literature survey and included into analyses.

Several studies, mainly older ones from before the nineties of the last century, were published only in the respective native language (especially Spanish studies). Results of these studies were, nonetheless, considered on the basis of literature citations, like, for instance, given in Briones et al., 2009.

Endemic species, such as *Hormogaster elisae*, *Dendrobaena madeirensis*, *Prosellodrilus pyrenaicus*, *Scherotheca campoii* or *Xana omodeoi* which occur in high abundances in arable soils in central Spain (Briones et al., 1994; Díaz Cosín et al., 1992; Novo et al., 2009), were excluded during selection, to ensure the applicability of the test system in different countries or sites of one biogeographical region. For this reason, only species of global (*Al. chlorotica, Ap. caliginosa, Ap. longa, Ap. rosea, L. terrestris, M. phosphoreus and O. complanatus*) (Blakemore, 2006a, 2006b; de Jong, 2011; Klinkenberg, 2012) or Mediterranean *(L. friendi*) (Csuzdi & Szlavecz, 2003) distribution were identified as focal species.

References

Ahl Goy, P.; Warren, G.; White, J.; Privalle, L.; Fearing, P. (1995). Interaction of an insect tolerant maize with organisms in the ecosystem. Proceedings of the key biosafety aspects of genetically modified organisms, 10-11 April 1995. Braunschweig, Germany.

Birch, A.N.E.; Griffiths, B.S.; Caul, S.; Thompson, J.; Heckmann, L.H.; Krogh, P.H.; Cortet, J. (2007). The role of laboratory, glasshouse and field scale experiments in understanding the interactions between genetically modified crops and soil ecosystems: a review of the ECOGEN project. Pedobiologia 51, 251–260.

Blakemore, R.J. (2006a). Cosmopolitan Earthworms - An Eco-Taxonomic Guide to the Peregrine Species of the World, 2nd Edition, VermEcology, Japan. 600 pp.

Blakemore, R.J. (2006b). http://www.annelida.net/earthworm/Mexico species.pdf, accessed November 2012.

Briones, M.J.I.; Mascato, R.; Mato, S. (1994). Biogeographical considerations in Asturias, Leon, Zamora, and Salamanca (Spain) in relation to their earthworm fauna. European Journal of Soil Biology 30, 149-155.

Briones, M.J.I.; Moran, P.; Posada, D. (2009). Are the sexual, somatic and genetic characters enough to solve nomenclatural problems in lumbricid taxonomy? Soil Biology & Biochemistry 41, 2257-2271.

Clark, B.W.; Coats, J.R. (2006). Subacute effects of Cry1Ab Bt corn litter on the earthworm *Eisenia fetida* and the springtail *Folsomia candida*. Environmental Entomology 35 (4), 1121-1129.

Csuzdi, C.; Szlavecz, K. (2003). *Lumbricus friendi* Cognetti, 1904 a new exotic earthworm in north America, Norteastern Naturalist 10, 77-82.

de Jong, Y.S.D.M. (Ed.) (2011). Fauna Europaea version 2.4. Web Service available online at http://www.faunaeur.org, accessed November 2012.

Díaz Cosín, D.J.; Trigo, D.; Mascato, R. (1992). Earthworms of the Iberian Peninsula. Species list and some biogeographical considerations. Soil Biology & Biochemistry 24, 1351-1356.

Edwards, C. A.; Bohlen, P.J. (eds.) (1996). Biology and ecology of earthworms. Chapman & Hall, London, UK

Edwards, C. A.; Bohlen, P. J.; Linden, D.R.; Subler, S. (1995). Earthworms in agroecosystems, pp. 185-214. In: P.F. Hendrix (ed.), Earthworm ecology and biogeography in North America. CRC, Boca Raton, FL.

EFSA (European Food Safety Authority) Panel on Genetically Modified Organisms (GMO) (2010). Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal 8 (11): 1879, 111p.

Emmerling, C.; Strunk, H.; Schobinger, U.; Schrader, S. (2011). Fragmentation of Cry1Ab protein from Bt-maize (MON810) through the gut of the earthworm species *Lumbricus terrestris L*. European Journal of Soil Biology 47, 160-164.

Engelmann, H.D. (1978). Zur Dominanzklassifizierung von Bodenarthropoden. Pedobiologia 18, 378–380.

Escher, N.; Käch, B.; Nentwig, W. (2000). Decomposition of transgenic Bacillus thuringiensis maize by microorganisms and woodlice Porcellio scaber (Crustacea: Isopoda). Basic and Applied Ecology 1, 161–169.

Flores, S.; Saxena, D.; Stotzky, G. (2005). Transgenic Bt plants decompose less in soil than non-Bt plants. Soil Biology & Biochemistry 37, 1073–1082.

Hönemann, L.; Zurbrügg, C.; Nentwig, W. (2008). Effects of Bt-corn decomposition on the composition of the soil meso- and macrofauna. Applied Soil Ecology 40, 203-209.

Kladivko, E.J. (2001). Tillage systems and soil ecology. Soil & Tillage Research 61, 61-76.

Klinkenberg, B. (Ed.) (2012). E-Fauna BC: Electronic Atlas of the Fauna of British Columbia [www.efauna.bc.ca]. Lab for Advanced Spatial Analysis, Department of Geography, University of British Columbia, Vancouver. accessed November 2012.

Lavelle, P.; Spain, A.V. (2005). Soil Ecology. Springer, Dordrecht.

Lövei, G.L.; Arpaia, S. (2005). The impact of transgenic plants on natural enemies: a critical review of laboratory studies. Entomologia Experimentalis et Applicata 114, 1-14.

Novo, M.; Almodóvar, A.; Díaz Cosín, D.J. (2009). High genetic divergence of hormogastrid earthworms (Annelida, Oligochaeta) in the central Iberian Peninsula: evolutionary and demographic implications. Zoologica Scripta 38, 537-552.

Parmelee, R. W.; Bohlen, P.J.; Blair, J.M. (1998). Earthworms and nutrient cycling processes: integrating across the ecological hierarchy, pp. 123-146. In: C. A. Edwards (ed.), Earthworm ecology. CRC, Boca Raton, FL.

Pey, B.; Nahmani, J.; Auclerc, A.; Capowiez, Y.; Cluzeau, D.; Cortet, J.; Decaëns, T.; Deharveng, L.; Dubs, F.; Joimel, S.; Briard, C.; Grumiaux, F.; Laporte, M.A.; Pasquet, A.; Pelosi, C.; Pernin, C.; Ponge, J.F.; Salmon, S.; Santorufo, L.; Hedde, M. (2014). Current use of future needs for soil invertebrate functional traits in community ecology. Basic and Applied Ecology 15, 194-206.

Poerschmann, J.; Gathmann, A.; Augustin, J.; Langer, U.; Górecki, T. (2005). Molecular composition of leaves and stems of genetically modified Bt and near-isogenic non-Bt maize-characterisation of lignin patterns. Journal of Environmental Quality 34, 1508–1518.

Rodriguez, T.; Trigo, D.; Díaz Cosín, D.J. (1997). Biogeographical zonation of the western Iberian peninsula on the basis of the distribution of earthworm species. Journal of Biogeography 24, 893-901.

Saxena, D.; Stotzky, G. (2001). Bacillus thuringiensis (Bt) toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi in soil. Soil Biology & Biochemistry 33, 1225–1230.

Schrader, S.; Münchenberg, T.; Baumgarte, S.; Tebbe, C.C. (2008). Earthworms of different functional groups affect the fate of the Bt-toxin Cry1Ab from transgenic maize in soil. European Journal of Soil Biology 44, 283-289.

Talavera, J.A.; Pérez, D.I. (2009). Occurrence of the genus Microscolex (Oligochaeta, Acanthodrilidae) at western Canary Islands. Bonner Zoologische Beiträge 56 (1/2), 37-41.

Tomlin, A.D.; Shipitalo, M.J.; Edwards, W.M.; Protz, R. (1995). Earthworms and their influence on soil structure and infiltration, pp. 159-184. In: P.F. Hendrix (ed.), Earthworm ecology and biogeography in North America. CRC, Boca Raton, FL.

Violle, C.; navas, M.L.; Vile, D.; Kazakou, E.; Fortunel, C.; Hummel, I.; Garnier, E. (2007). Let the concept of trait be functional! Oikos 116, 882-892.

Wall, D.H.; Bardgett, R.D.; Behan-Pelletier, J.E.; Herrick, J.E.; Jones, T.H.; Ritz, K.; Six, J.; Strong, D.R.; van der Putten, W.H. (2012). Soil Ecology and Ecosystem Services. Oxford University Press, Oxford.

Zwahlen, C.; Hilbeck, A.; Nentwig, W. (2007). Field decomposition of transgenic Bt maize residue and the impact on non-target soil invertebrates. Plant and Soil 300, 245–257.

Appendix: Literature sources selected for identifying focal earthworm species

Ireland:

[1] Baker, G.H. (1983). Distribution, abundance and species association of earthworms (Lumbricidae) in a reclaimed peat soil in Ireland. Ecograpgy 6, 74-80.

[2] Blakemore, R.J. (2008). British and Irish earthworms – a checklist of species updated from Sims & Gerard (1999). http://www.annelida.net/earthworm/Britain & Ireland.pdf, accessed November 2012.

[3] Carpenter, D.; Sherlock, E.; Jones, D.T.; Chiminoides, J.; Writer, T.; Neilson, R.; Boag, B.; Keith, A.M.; Eggleton, P. (2012). Mapping of earthworm distribution for the British Isles and Eire highlights the under-recording of an ecologically important group. Biodiversity and Conservation 21, 475-485.

[4] Cotton, D.C.F. (1978). A revision of the Irish earthworms (Oligochaeta: Lumbricidae) with the addition of two species. The Irish Naturalists' Journal 19, 257-260.

[5] Curry, J.P.; Byrne, D. (1992). The role of earthworms in straw decomposition and nitrogen turnover in arable land in Ireland. Soil Biology and Biochemistry 24, 1409-1412.

[6] Curry, J.P.; Byrne, D.; Schmidt, O. (2002). Intensive cultivation can drastically reduce earthworm populations in arable land. European Journal of Soil Biology 38, 127-130.

[7] Curry, J.P.; Doherty, P.; Purvis, G.; Schmidt, O. (2008). Relationships between earthworm populations and management intensity in cattle-grazed pastures in Ireland. Applied Soil Ecology 39, 58-64.

[8] Melody, C.; Schmidt, O. (2012). Northward range extension of an endemic soil decomposer with a distinct trophic position. Biology Letters, http://rsbl.royalsocietypublishing.org/ content/early/2012/07/20/rsbl.2012.0537.full, accessed November 2012.

[9] Schmidt, O.; Curry, J.P. (2001). Population dynamics of earthworms (Lumbricidae) and their role in nitrogen turnover in wheat and wheat-clover cropping systems. Pedobiologia 45, 174-187.

[10] Schmidt, O.; Keith, A.M.; Arroyo, J.; Bolger, T.; Boots, B.; Breen, J.; Clipson, N.; Doohan, F.M.; Griffin, C.T.; Hazard, C.; Niechoj, R. (2005). The CréBeo Soil Biodiversity Project

CréBeo – Baseline Data, Response to Pressures, Functions and Conservation of Keystone Micro- and Macro-Organisms in Irish Soils, STRIVE Report No. 67, 43p.

Sweden:

[11] Blakemore, R.J. (2007). Scandinavian earthworms - a checklist of species. http://www.annelida.net/earthworm/Scandinavian.pdf, accessed November 2012, 7p.

[12] Boström, U. (1995). Earthworm populations (Lumbricidae) in ploughed and undisturbed leys. Soil & Tillage Research 35, 125-133.

[13] Lagerlöf, J.; Goffre, B.; Vincent, C. (2002). The importance of field boundaries for earthworms (Lumbricidae) in the Swedish agricultural landscape. Agriculture, Ecosystems & Environment 89, 91-103.

[14] Lagerlöf, J.; Pålsson, O.; Arvidsson, J. (2012). Earthworms influenced by reduced tillage, conventional tillage and energy forest in Swedish agricultural field experiments. Acta Agriculturae Scandinavica, Section B – Soil & Plant Science 62, 235-244.

[15] Lofs-Holmin, A. (1982). The influence of routine pesticide spraying on earthworms (Lumbricidae) in field experiments with winter wheat. Swedish Journal Agricultural Research 12, 121-123.

[16] Lofs-Holmin, A. (1983). Influemce of agricultural practices on earthworms (Lumbricidae). Acta Agriculturae Scandinavica 33, 169-225.

[17] Terhivuo, J.; Saura, A. (1996). Clone pool structure and morphometric variation in endogeic and epigeic North-European parthenogenetic earthworms (Oligochaeta: Lumbricidae). Pedobiologia 40, 226–239.

[18] Terhivuo, J.; Saura, A. (1997). Island biogeography of North European parthenogenetic Lumbricidae: I. Clone pool affinities and morphometric differentiation of Åland populations. Ecography 20, 185-196.

[19] Terhivuo, J.; Saura, A. (2006). Dispersal and clonal diversity of North-European parthenogenetic earthworms. Biological Invasions Belowground: Earthworms as Invasive Species 8, 1205-1218.

[20] Timm, T.; Erséus, C.; Gustavsson, L. (1994). Check-list of Clitellata recorded from Sweden.http://www.nrm.se/download/18.b7068e512065b473cb80002908/oligochaet+checklist. pdf, accessed November 2012, 6p.

Spain:

[21] Alvarez, J. (1966). Oligoquetos terrícolas de España I. Las lombrices de tierra de la región central, Boletín de la Real Sociedad Española de Historia Natural (Sección Biología) 64, 133–144. Cit. ex Briones et al., 2009.

[22] Alvarez, J. (1973). Oligoquetos terrícolas ibéricos 2. Lumbrícidos (2ª parte). Boletín de la Real Sociedad Española de Historia Natural (Sección Biología) 71, 209–222. Cit. ex Briones et al., 2009.

[23] Briones, M.J.I.; Bol, R.; Sleep, D.; Allen, D.; Sampedro, L. (2001). Spatio-temporal variation of stable isotope ratios in earthworms under grassland and maize cropping systems. Soil Biology & Biochemistry 33, 1673-1682.

[24] Briones, M.J.I.; Bol, R.; Sleep, Sampedro, L. D.; Allen, D. (1999). A dynamic study of earthworm feeding ecology using stable isotopes. Rapid Communications in Mass Spectrometry 13, 1300-1304.

[25] Briones, M.J.I.; Mariño, F.; Trigo, D.; Díaz-Cosín, D.J. (1992). Lombrices de tierra de Asturias, León, Zamora y Salamanca. II. Familia Megascolecidae, Acanthodrilidae y Hormogastridae y otros Lumbricidae. Boletín de la Real Sociedad Española de Historia Natural (Sección Biología) 88, 19–29. Cit. ex Briones et al., 2009.

[26] Briones, M.J.I.; Mascato, R.; Mato, S. (1992). Relationships of earthworms with environmental factors studied by means of detrended canonical correnspondence analysis. Acta Oecologica 13 (5), 617-626.

[27] Briones, M.J.I.; Mascato, R.; Mato, S. (1994). Biogeographical considerations in Asturias, Leon, Zamora, and Salamanca (Spain) in relation to their earthworm fauna. European Journal of Soil Biology 30, 149-155.

[28] Briones, M.J.I.; Mascato, R.; Mato, S. (1995). Autecological study of some earthworm species (Oligochaeta) by means of ecological profiles. Pedobiologia 39, 97-106.

[29] Briones, M.J.I.; Moran, P.; Posada, D. (2009). Are the sexual, somatic and genetic characters enough to solve nomenclatural problems in lumbricid taxonomy? Soil Biology & Biochemistry 41, 2257-2271.

[30] Butt, K.R.; Briones, M.J.I. (2011). Life cycle studies of the earthworm *Lumbricus friendi* (Cognetti, 1904). Pedobiologia 54S, S27-S29.

[31] Díaz Cosín, D.J.; Trigo, D.; Mascato, R. (1992). Earthworms of the Iberian Peninsula. Species list and some biogeographical considerations. Soil Biology & Biochemistry 24, 1351-1356.

[32] Hernandez, P.; Fernandez, R.; Novo, M.; Trigo, D.; Diaz Cosin, D. (2007). Geostatistical and multivariate analysis of the horizontal distribution of an earthworm community in El Molar (Madrid, Spain)**.** Pedobiologia 51, 13—21.

[33] Mariño, F.; Mascato, R.; Díaz-Cosín, D.J. (1986). Lombrices de tierra de Galicia. Mapas provisionales de distribución. Trabajos Compostelanos de Biología 13, 89–100. Cit. ex Briones et al., 2009.

[34] Monroy, F.; Aira, M.; Domínguez, J.; Mariño, F. (2003). Distribution of earthworms in the north-west of the Iberian Peninsula. European Journal of Soil Biology 39, 13-18.

[35] Novo, M.; Almodóvar, A.; Díaz Cosín, D.J. (2009). High genetic divergence of hormogastrid earthworms (Annelida, Oligochaeta) in the central Iberian Peninsula: evolutionary and demographic implications. Zoologica Scripta 38, 537-552.

[36] Novo, M.; Almodóvar, A.; Fernández, R.; Trigo, D.; Díaz Cosín, D.J. (2010). Cryptic speciation of hormogastrid earthworms revealed by mitochondrial and nuclear data. Molecular Phylogenetics and Evolution 56, 507-512.

[37] Novo*,* M.; Almodóvar, A.; Fernández, R.; Giribet, G.; Díaz Cosín, D.J. (2011). Understanding the biogeography of a group of earthworms in the Mediterranean basin - the phylogenetic puzzle of Hormogastridae (Clitellata: Oligochaeta). Molecular Phylogenetics and Evolution 61, 125-135.

[38] Rodriguez, T.; Trigo, D.; Díaz Cosín, D.J. (1997). Biogeographical zonation of the western Iberian peninsula on the basis of the distribution of earthworm species. Journal of Biogeography 24, 893-901.

[39] Souto, B.F. (1992). Faunística y Ecología de las lombrices de tierra de las provincias deLa Coruña y Pontevedra. Microchip PhD Thesis. Servicio de Publicacións e Intercambio Científico. Cit. ex Briones et al., 2009.

[40] Valle, J.V.; Moro, R.P.; Garvin, M.H.; Trigo, D.; Díaz Cosín, D.J. (1997). Annual Dynamics of the earthworms *Hormogaster elisae* (Oligochaeta, Hormogastridae) in Central Spain. Soil Biology & Biochemistry 29, 309-312.