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Importance of large, deep-burrowing and anecic earthworms in forested and cultivated areas (vineyards) of northeastern Italy

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ABSTRACT

Through their activities earthworms and especially the long, deep-burrowing and anecic species, may modify the chemical-physical, biological and mechanical properties of the soil. They build mull soils. Their presence has a key role in soil ecology relating to its quality in agricultural ecosystems and especially in orchards, on vineyards and in woodlands. For comparison, this study shows the distribution of earthworm species on 29 vineyards and 32 deciduous forests in northeastern and central Italy and in parts of Croatia and Slovenia. The study located 3215 specimens: 1193 from woodlands and 2022 from cultivated areas collected between 2010 and 2017. Twelve anecic and 19 endogeic/epigeic species were identified. Rarely more than two or three anecic species live together. Some species of *Octodrilus* Omodeo, 1956 and some other species from the collecting sites were subject to barcoding analysis. The phylogenetic tree based on COI is rather consistent with current taxonomy. Of the few genera studied, the key genus *Octodrilus* is most abundant with *Oc. complanatus* having wide circum-mediterranean range and another three endemic species having restricted ranges: *Oc. mimus*, *Oc. tergestinus* and *Oc. istrianus*. They cover the prime wine producing area in the Friuli Venezia Giulia region, including the Collio with a wine of the same name. Another couple of species *Octodrilus* nov. sp. and *Eophila crodabepis* are distributed throughout the classical prosecco wine production area. Although natural deciduous forests are anecic earthworm's selective habitat, in certain circumstances however, rural areas can receive anecic recruits from nearby forests or hedgerows. Reducing tillage, trampling, and use of pesticides along with introduction of mulching are a means to make the rural environment and specially vineyards more attractive to these anecic deep-burrowing and beneficial species.

1. Introduction

Through their activity, earthworms can modify both the chemical-physical, mechanical and biological soil properties (Edwards and Bohlen, 1996; Brown, 1995). They have a key role in the ecology of soil in many regions of the world being classified as *soil ecosystem engineers*. Earthworms species are divided by their size, coloration, and their

behavior into different ecological categories namely epigeics (litter species), endogeics (topsoil and subsoil species) and anecics (Darwin, 1881; Bouché, 1972; Sims and Gerard, 1985; Lee, 1985; Edwards, 1998; Paoletti, 1999; Paoletti et al., 2013). Anecic earthworms (deep-burrowing species that feed on the surface litter) in particular have positive influences on soil biotic and abiotic properties (Bagni et al., 2006; Dauber et al., 2005; Edwards and Bohlen, 1996; Edwards, 2004; Lee

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Fig. 1. Typical mull soil in which *Eophila crodabepis* and *Octodrilus* sp. nov. live near Valdobbadiene.

1985; Paoletti, 1988, 1999; Paoletti et al., 1998; Scullion and Malik, 2000; Van Straalen, 1998) and they are very important in promoting organic matter decomposition and mixing in the soil profile, nutrient cycling and soil formation, thereby accelerating the pedological process (Kiyasudeen et al., 2015).

Figs. 1 and 2 show a typical mull soil habitat of anecic earthworms *Eophila crodabepis* and *Octodrilus* n. sp. near Valdobbadiene and *Octodrilus tergestinus* and *Oc. complanatus* in the Motovun forest, Croatia. These forested stands of Motovun with mull soils produced by the activity of *Octodrilus tergestinus* support collection of truffles (*Tuber magnatum*) (in particular St 1 and St 5) (Bragato et al., 2004). *Hormogaster redii* and *Octodrilus complanatus* dominate the landscape in Rome. *H. redii* and *Scherotheca targionii* dominate the rural landscape in Tuscany (ex. San Carlo farm).

Information on deep-burrowing (anecic) earthworm' taxonomy, ecology and relationship with other earthworm species in different environments and agriculture is fragmentary in Italy, except for some data from the northeast (Paoletti and Omodeo, 1981; Paoletti, 1988; Paoletti et al., 2010, 2016). Our research in the northeast of Italy and its bordering countries is concentrated on the larger anecic species that have a consistent role in building fertile soil mediated by bacteria and fungi (Thakuria et al., 2010; Scullion and Malik, 2000).

Anecic species tend to large size, in general more than 20 cm length, and are thus exposed in cultivated areas to soil management practices such as tillage, trampling, soil erosion, crop inputs such as fertilizers and pesticides. They are also affected by loss of heterogeneity in the landscape such as due to removal of hedgerows, fallows and woodland. Grey-brown pigmentation is present especially dorsally, however a color banding in each segment could be seen on *Octodrilus mimus* and

Oc. tergestinus, a puce violet pigmentation banding on *Eophila tellinii* and *Eo. crodabepis* (Paoletti et al., 2016). In general they produce a system of vertical galleries (Pelosi et al., 2009).

Agricultural areas, if compared with forested areas and permanent grasslands, have earthworm community declines due to several inappropriate management factors that result in a very fragmented distribution and a very low biomass especially for large burrowing species. In a recent survey in 12 European countries, based on 205 farms and 1470 fields in which 30,000 adults belonging to 49 species were identified, and only 10 species with a few specimens, less than of 5%, belonged to the anecic category (Lüscher et al., 2014; Paoletti et al., 2012).

Anecic earthworms such as *Lumbricus terrestris*, come to the soil surface usually during the night or when it is more humid and draw leaf litter down into the lower strata. Other species (e.g., *Eophila tellinii* and *Eo. crodabepis*) consume seasoned litter of the deciduous vegetation close to the soil surfaces where they migrate at night or when it is very wet and have been documented mating (Fig. 3). Furthermore, deep-burrowing earthworms can live in a system of semi-permanent burrows, often going vertically to a few meters depth (Lamparski et al., 1987; Paoletti et al., 2013; Pelosi et al., 2009).

Casts are different for typology: in some anecic species such as *Octodrilus mimus* and *Octodrilus tergestinus* characteristic tall vertical tube-shaped casts that look like towers are produced (Fig. 4). Other species like *Eophila tellinii* or *Eophila crodabepis*, instead produce castings scattered and spread irregularly when they are at the soil surface. Most earthworms feed on decomposed litter, however, *L. terrestris* and many other anecic (*L. polyphaemus* and *F. platyura platyura*, *F. platyura montana*, *F. platyura depressa*) pull leaves into a vertical gallery producing a sort of midden. Similar behavior has been documented in areas newly colonized by *Octodrilus mimus* (Fig. 5).

In southern Europe some species included in genera *Allolobophora*, *Aporrectodea*, *Eophila*, *Hormogaster*, *Lumbricus*, *Octodrilus*, *Scherotheca* and from the families Lumbricidae and Hormogastridae due to their behavior are considered anecic (Braido et al., 1997; Csuzdi et al., 2003, 2011; Hackenberger and Hackenberger, 2013; Mršić, 1992; Paoletti, 1988; Paoletti et al., 2013).

The native populations of these deep-burrowing species live in deciduous forests and some grasslands, and some like *L. terrestris* have been adapted to wet meadows and gardens (Paoletti, 1988).

Following is the current state of art about the deep-burrowing species for these genera:

Aporrectodea includes 43 valid species according to Blakemore (2008), but 50 species listed in <http://taxo.drilobase.org/>. The anecic species reported are *A. longa*, with distribution similar to *L. terrestris* (Atlavinytė, 1989; Blakemore, 2006, 2007a, 2007b; Carpenter et al., 2012; Christian and Zicsi, 1999; Csuzdi and Zicsi, 2003; Csuzdi and Szlávecz, 2003; Garbar and Vlasenko, 2007; Gudleifsson et al., 2003; Haraldsen et al., 1994; Ivask et al., 2006; Kasprzak, 1989; Makin et al., 2014; Omodeo et al., 2003; Perel, 1979; Reynolds, 1995, 2012; Stojanović et al., 2013; Szederjesi, 2013; Terhivuo, 1988; Valchovski, 2012; Ventinš, 2011; Zangerlé, 2011; Zicsi, 1986) and *A. carpathica* endemic species of Carpatian region (Cherevatov, 1994; Kasprzak, 1989; Pop et al., 2012; Zicsi and Csuzdi, 1986).

Allolobophora include 66 species listed in <http://taxo.drilobase.org/>, but 49 valid species according to Blakemore (2008). The anecic species in Italy are *A. januaeargenti* and *A. asconensis* (Diaz Cozín et al., 1992; Paoletti et al., 2013).

Eophila has two Italian species: *Eo. tellinii* and *Eo. crodabepis*. *E. tellinii* is endemic to the Eastern Friuli Venezia-Giulia area along the peaks of Ciaurlec mount and the hills of Ragonna and Cravest (Braido et al., 1997; Paoletti et al., 2016); *E. crodabepis* is located in the

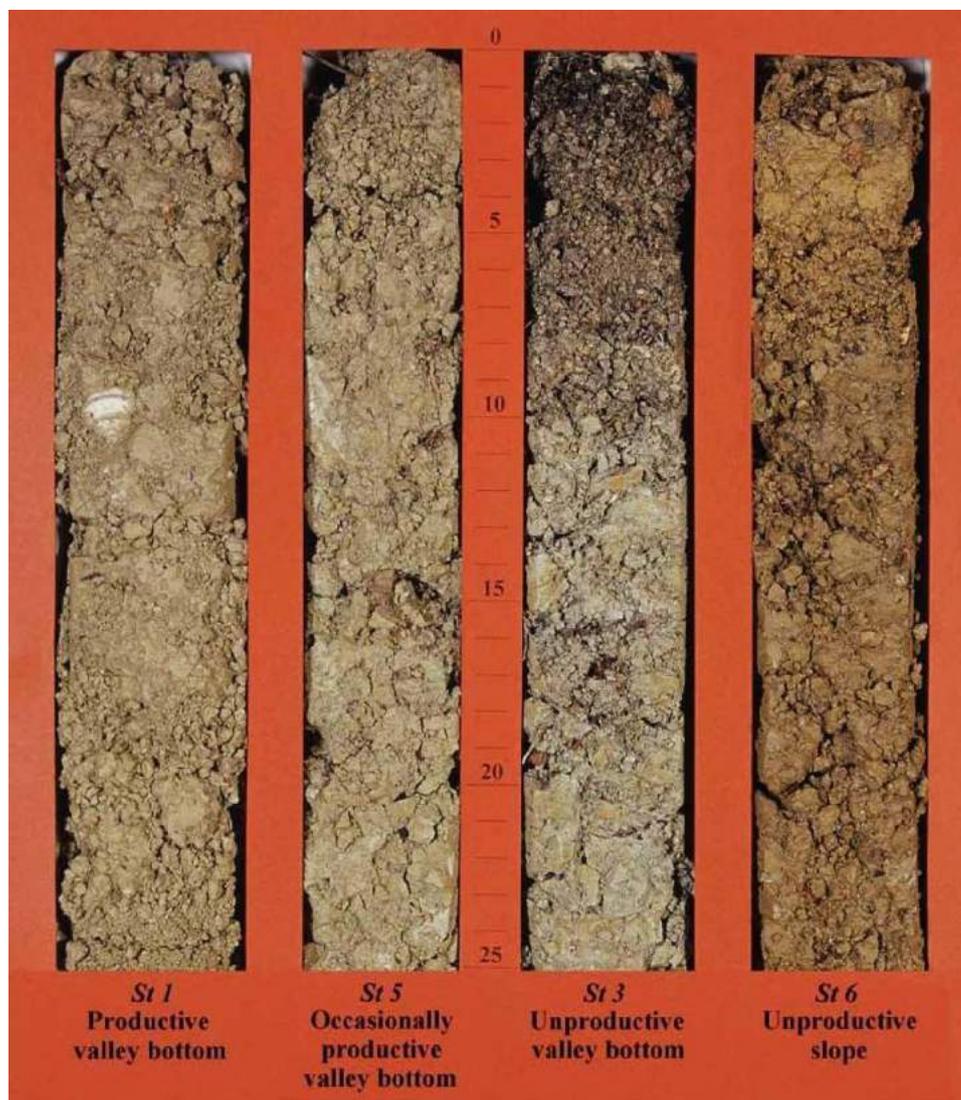


Fig. 2. Typical mull soil (St 1 and St 5) in which the anecic *Octodrilus tergestinus* and *Oc. complanatus* live in Motovun woodland, Croatia. In these mull stands truffles are collected (*Tuber magnatum*).

Venetian Prealps between hills of Conegliano, Montello, Asolo and the Asiago plateau (Paoletti et al., 2016). Its distribution range cover the classic prosecco wine production territory (Figs. 7 and 8).

Hormogaster is a west Mediterranean genus with two species living in the central Italy: *H. redii* (Fig. 6) and *H. samnitica*.

Lumbricus includes 13 species according to Blakemore (2008), and 14 species listed in <http://taxo.drilobase.org/> (including *L. badensis*) of which 5 species overall are anecic (<http://taxo.drilobase.org/>). *L. terrestris* has a palearctic distribution, but due to human introductions it is frequent in North America and it is found also in New Zealand, Tasmania, Chile and Mexico (Lee, 1952, 1985; Reynolds, 1995, 2012; Richter, 2010; Zicsi, 2007; Blakemore, 2016). *L. polyphemus* is present in East Europe (Csuzdi et al., 2003; Hackenberger and Hackenberger, 2013; Blakemore, 2007a, 2007b; Christian and Zicsi, 1999; Fedorovich, 1991; Kasprzak, 1989; Lamparski et al., 1987; Lehmitz et al., 2014; Milutinović et al., 2010; Pižl, 2001; Pop and Pop, 2004; Szederjesi, 2013; Zicsi and Csuzdi, 1986; Zicsi, 1983; Valchovski, 2012). It has not yet been collected for sure in Italy, even though a specimen at the Specola Museum is labeled “Belluno”.

L. badiensis the largest species in this genus is endemic to the Black

Forest (Germany) (Lamparski et al., 1987), and the newly refound *L. herculeus* in the Western Countries (James et al., 2010). *L. friendi* in France, Ireland and UK but introduced in the USA as well (Csuzdi and Szlavecz, 2003; Thakuria et al., 2010).

Octodrilus counts 46 species in Europe (<http://taxo.drilobase.org/>) and 14 of them in Italy some of which are anecic. *Oc. complanatus* has the most extensive distribution not only in Italy but in all Circum-Mediterranean lands and in the North of Alps (Switzerland and Austria) (<http://www.fauna-eu.org>).

Perelia includes 31 valid species (Blakemore, 2008; <http://taxo.drilobase.org/>) and the deep-burrowing earthworm *Perelia gestroi*, distributed in North Italy (Csuzdi, 2012; Paoletti et al., 2013) is most diffuse in the vineyards (Fig. 7). This species is endogeic but it is usually unpigmented and did not come to the surface for feeding.

Scherotheca counts 24 species according to Blakemore (2011) but 36 species listed in <http://taxo.drilobase.org/>. In fact Blakemore consider 12 of them as subspecies. The Italian anecic species are: *S. targionii* (Fig. 7), *S. andreinii* and *S. dugesi*, the first two present just in Italy, the third in Italy, Spain and France (Bouche, 1972; Díaz Cosín et al., 1992; Paoletti et al., 2013).



Fig. 3. *Eophila*, the largest Italian anecic earthworm. A) *Eo. tellinii* mating at the soil surface (photographed at Ragogna, Udine, by Donato Franculli, November 24, 2016); B) *Eo. crodabepis*, immatures specimens collected in Roncavezzai, Follina, Treviso, May 14, 2017.

This work is focused on the study of the ecology and distribution of the deep-burrowing anecic species, especially the *Octodrilus* genus in North-East of Italy in the Veneto and Friuli Venezia-Giulia Regions and in the nearby areas of Slovenia and Croatia, in order to estimate their abundance and their role in natural and rural environments. In addition, barcoding analysis is used for a better clarification about the taxonomical differences between the species. A few central Italian stations are also considered for comparison.

2. Material and methods

2.1. Area of study

The specimens for this study were collected between the 2010 and 2017. The study area is focused on 29 deciduous woodlands sites and 32 cultivated sites (vineyards). This area extends into the northeast of Italy, through Friuli Venezia Giulia, Veneto regions (Italy). Some additional stations are in Tuscany and Emilia-Romagna, Lazio Regions and outside of Italy: Switzerland, Slovenia, Croatia. The exact locations where the specimens were found are summarized in Table 1.

2.2. Earthworms sampling

The sampling was made after two or three days of rainfall events in order to increase the probability of capture, since anecic species tend to stay near the soil surface when it is wet. The collecting method was hand-sorting with irritant water solution on a 1 m² soil surface. After cleaning from the litter, the solution (3–5 L) was poured three times within intervals of 5–7 min. The irritant solution used was in alternative, formaldehyde (0.2%), Allyl isothiocyanate (1 mL AITC into 10 L of water) or mustard powder (25 g/l); when possible, because the soil had not hard root system or too many stones, a spade fork was used to dig 40 × 40 cm holes and to hand-sort specimens (Paoletti et al., 2013).

The specimens were preserved in ethanol 80% and stored at +4 °C at the Biology Department of the University of Padova, Via Ugo Bassi 58b, 35121 Padova, Italy (Paoletti et al., 2016), at Natural History Museum of Verona and at the Hungarian Natural History Museum, Baross str. 13, H-1088, Budapest, Hungary.

2.3. Specimens identification

Morphological and anatomical identification of the specimens were carried out by using LOMBRI software (Paoletti and Gradenigo, 1996) and especially Mršić (1991). The anatomical features analyzed were: length, body pigmentation, type of prostomium, distance of setae, position of clitellum and number of spermathecae. We upgraded species names following Blakemore (2008) and Csuzdi (2012) and the website <http://taxo.drilobase.org>. Some specimens have been subjected to morph-anatomical dissection in order to optimize the identification and one of us (Csaba Csuzdi) did an additional supervision of our specimens and made an additional accurate control of the species identification.

2.4. Barcoding

Some specimens especially of the genus *Octodrilus* and some other genera *Allolobophora*, *Eophila*, *Hormogaster*, *Perelia* and *Scherotheca* from the collecting sites were subject to barcoding analysis in particular the taxonomically problematic species. Total genomic DNA was extracted from ventral integument tissue samples using the DNeasy Tissue Kit (QIAGEN) with two consecutive steps of elution (70 µL of buffer each). The mitochondrial molecular marker COI (subunit 1 of cytochrome c oxidase) was amplified using the same primer sequences, polymerase chain reactions (PCR) and sequencing reactions as in Pérez-Losada et al. (2009).

Reference sequences from Pérez-Losada et al. (2015), Novo et al. (2015) and Paoletti et al. (2016) were included into the dataset.



Fig. 4. Different typologies of tall vertical tube-shaped casts. A) *Octodrilus tergestinus* from Grimalda and B) its cast near Motovun; C) *Octodrilus mimus* and D) its casts in Romagno woodland.

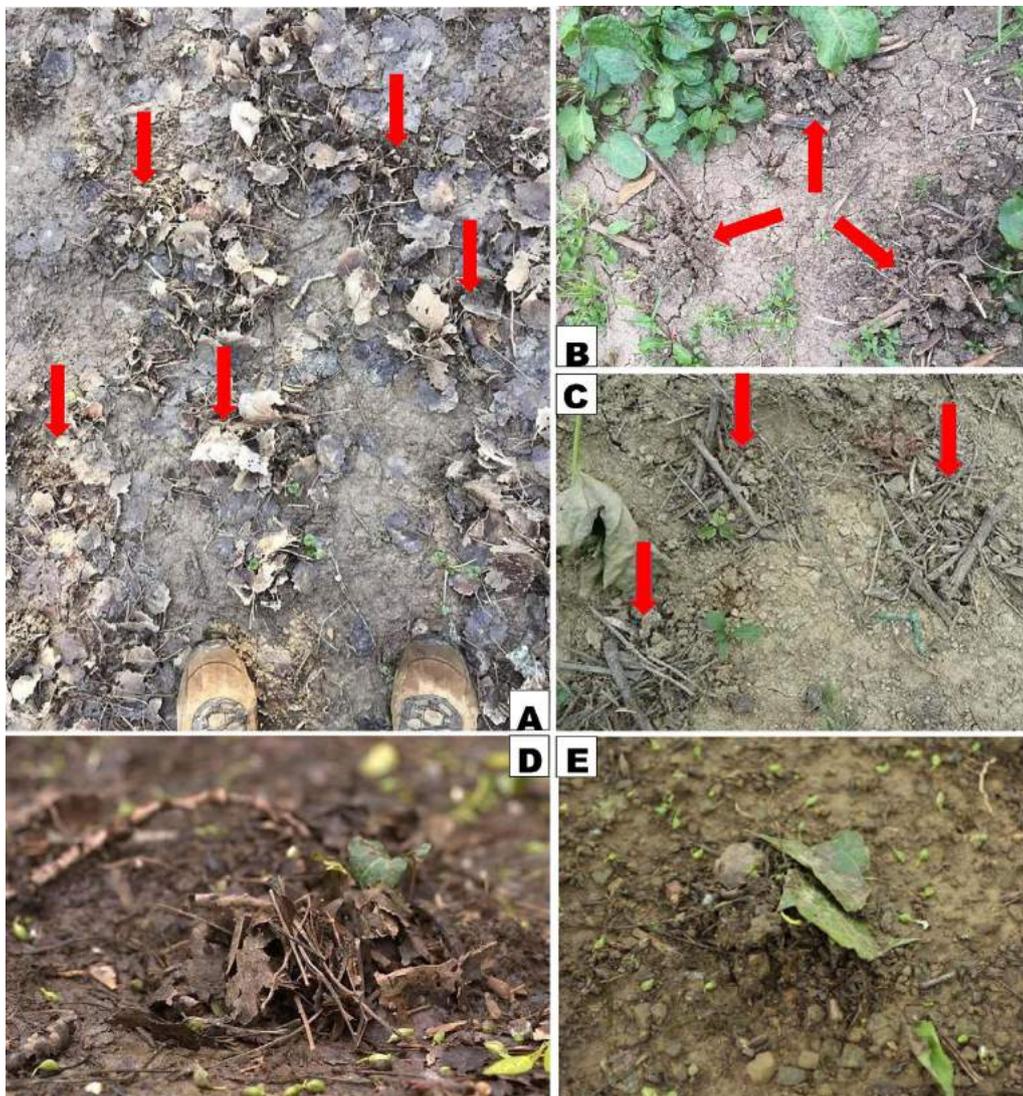


Fig. 5. *Lumbricus terrestris* middens. At A) S. Agostino lowland forest under *Populus nigra* stand, Ferrara; at B) Cartizze, Valdobbiadene, Treviso in a vineyard of prosecco wine (March 2017) and C) in a vineyard of Vo'3, Euganean hills, Padova. This anecic species can efficiently incorporate in the soil grape branches if cutted in small pieces. D and E) example of middens made by *O. mimus*. This species make middens very similar to *L. terrestris* but less complexed in newly colonized places.

Sequences were aligned in MAFFT 7 (Kato and Standley, 2013) resulting in a matrix of 629 base pairs. jModelTest v. 2.1.3 (Darriba et al., 2012) was used to select the best-fit evolutionary model using the Akaike information criterion (AIC; Akaike, 1973), and Bayesian information criterion (BIC; Schwarz, 1978). GTR + I + G was selected as best-fitting evolutionary model.

Bayesian Inference of the phylogeny was estimated with MRBAYES v.3.2.6 (Ronquist and Huelsenbeck, 2003) as implemented in the CIPRES Science Gateway V. 3.3 (Miller et al., 2010 <http://www.phylo.org/index.php/portal/>). Parameters were set to ten million generations and 10,000 trees were sampled for every 1000th generation, initiating the analysis from a random tree. Two independent analyses were performed and 20% of the trees were discarded as burn-in. The remaining trees were combined to find the maximum *a posteriori* probability estimate of phylogeny.

3. Results

We collected a total of 3215 specimens, 1193 in woodlands and 2022 specimens in cultivated areas. Final abundance for the collecting sites shown in Table 2 and 3 is the total mean of the different sampling replicates for each collecting sites. 12 were anecic species and 19 epigeic and endogeic species (Table 2 and 3). The anecic species tended to be in a higher than expected ratio to epigeic and endogeic species (Paoletti et al., 2012). As expected the number of species collected is proportional at the specimens collected (Fig. 9). Fig. 8 offers the distribution of the key anecic species.

L. terrestris is rather abundant in places with deciduous vegetation and sometimes in the cultivated areas with limited impacts like tillage, trampling and pesticide residues. In forested areas it is sometimes present only along the margins and cannot move in the mature stands of woodlands where *Oc. mimus* and *Oc. tergestinus* are dominant. The transect in the Coda Manin lowland woodland is one example (Fig. 10) where the stations 4 and 5 had no *L. terrestris* but instead had *Oc.*



Fig. 6. *Hormogaster redii* in olive grove on natural grassland vegetation at S. Carlo, Grosseto. A) a specimen at the soil surface; B) a specimen with the lighter regenerated tail.



Fig. 7. The other four main species found in the study. *Eophila tellinii* and *E. crodabepis*, *Perelia gestroi* and *Scherotheca* cfr *andreinii*.

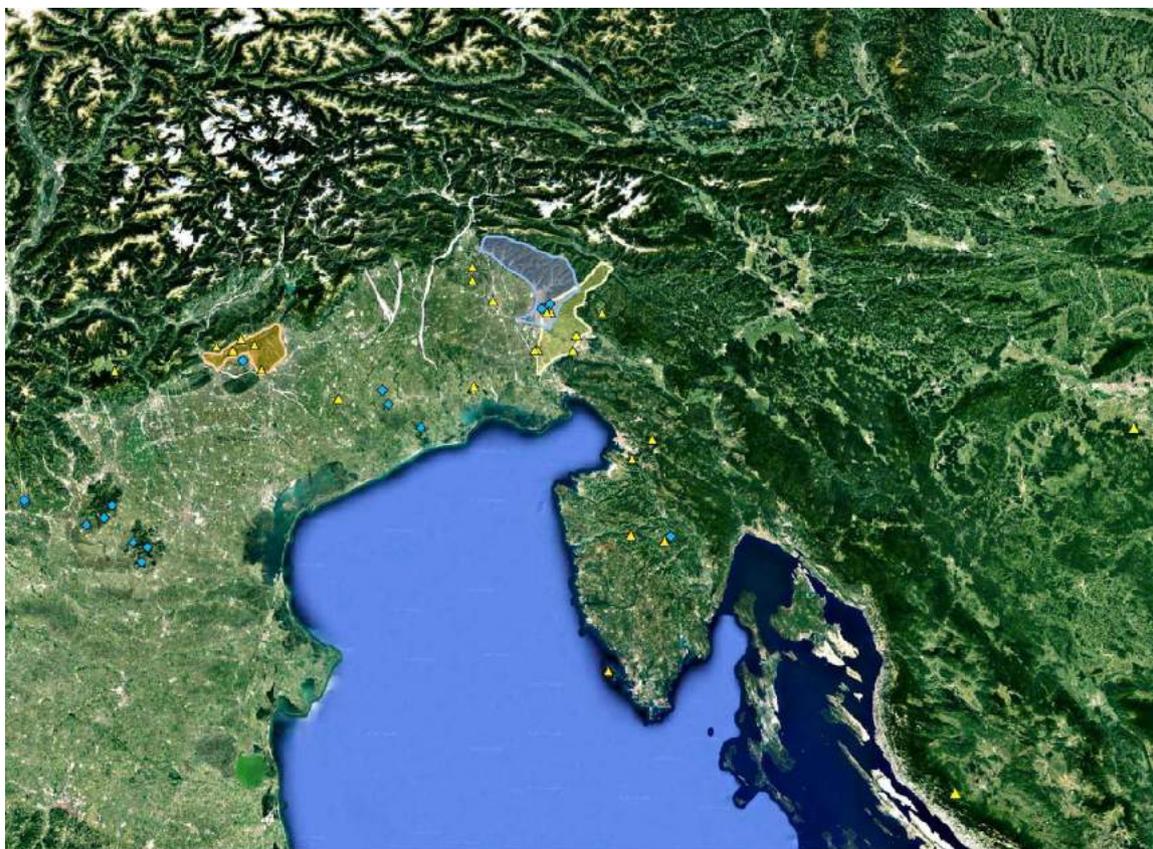


Fig. 8. Geographical distribution of the collecting sites. Blue signs are the vineyards, yellow signs the woodlands. The three colour areas describe the main wine production areas: Orange the Valdobbiadene, Blue the Oriental hills, Yellow the Collio region. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

tergestinus.

Oc. complanatus, as expected, is the most abundant species in the deciduous woodlands and in some vineyards.

It is present in grasslands, deciduous forests and vineyards but it suffers of management practices including soil compaction, tillage and herbicides application. In fact glyphosate herbicide severely damages population of this anecic species even more than *L. terrestris* (Stellin et al., 2017; in press).

Perelia gestroi is the species present in multiple sites and with a considerable density of individuals per m², among our deep burrowing species is the less mobile and possibly more geophagous. Its galleries have, in most cases, horizontal development.

Oc. mimus, *Oc. tergestinus* and *Octodrilus* sp. nov. with *Eo. tellinii* and *Eo. crodabepis*, have apparently a more restricted range and are endemic species.

It was difficult to find many different anecic species living together. Only in Medea and Cerreto few meters apart we found specimens of *Oc. mimus* and *Oc. tergestinus*, and sometimes *Oc. complanatus*.

Among the deep burrowing species *L. terrestris* has the higher population density with respectively 10 and 20.9 individuals per m² found at the margin of the Coda Manin lowland woodlands and in S. Agostino woodland (Fig. 5). This invasive species, however, seems to suffer from the presence of *Oc. mimus* and *Oc. tergestinus*, as it was sampled together with them in very limited number of cases.

The tube shaped casts produced by *Oc. mimus* and *Oc. tergestinus* endemic species and their secretions can produce a volatile compound that performs like the alarm pheromone that we have under study (Nicolussi, 2016) that could discourages the invasive *L. terrestris* to enter and colonize their forest territories.

3.1. Barcoding

The phylogenetic tree based on COI provided good phylogenetic resolution at supra-specific level, as it was able to recover monophyletic genera with high support.

After revision of conflictive identifications the main clades obtained in the phylogenetic tree (Fig. 11) were mainly consistent with described species of the genus *Octodrilus*. However, there were some unexpected results.

Oc. pseudocomplanatus although well recovered (1 PP) contained an advanced clade. This clade differs from *pseudocomplanatus* in the number of spermathecae (7 vs. 5), the length of the clitellum (29–37, 38 vs. ½28, 29–40) but the tubercles are on the same position ½29–39, 40. Also both groups have aligned nephridiopores in b. Therefore these specimens can represent an advanced population of *pseudocomplanatus* or a new species if analysing additional genes will results in recovering monophyletic *pseudocomplanatus* proper and clade B specimens (Table 4).

Table 1
List of the geographical location of sampling. In the list is noted: habitat, exact position, altitude and codification used for the analysis. Localities mark with the V are vineyard, WL for woodlands.

Nation	Province	Site	Code	Map	Habitat	Coordinates	Altitude (MSL)
Italy	Padova	Arquà Petrarca	Cinto2	1	<i>Vitis vinifera</i>	45°15'44.63"N 11°41'59.32"E	80
	Padova	Baone	Baone	2	<i>Vitis vinifera</i>	45°14'39.34"N 11°42'22.32"E	24
	Padova	Faedo	Cinto1	3	<i>Vitis vinifera</i>	45°18'16.23"N 11°41'52.85"E	62
	Padova	Galzignano 258			Galzignano	4	V
<i>Vitis vinifera</i>	45°18'18.85"N 11°44'10.08"E						
	Padova	Teolo	Teolo	5	<i>Vitis vinifera</i>	45°20'43.40"N 11°40'46.88"E	2
	Padova	Vo'	Vo'1	6	<i>Vitis vinifera</i>	45°19'52.65"N 11°39'52.67"E	85
	Padova	Vo'	Vò2	7	<i>Vitis vinifera</i>	45°19'30.22"N 11°39'2.18"E	40
<i>Vitis vinifera</i>	Padova	Vo'	Vò3	8	<i>Vitis vinifera</i>	45°19'52.65"N 11°39'52.67"E	170
	Padova	Vo'	Vò4	9	<i>Vitis vinifera</i>	45°18'47.91"N 11°38'35.59"E	18
	Treviso	Valdobbiadene – Cartizze 245			Cartizze	10	V
	45°53'36.29"N 12°26'81"E						
Udine	Casa Rossa	Casa Rossa		11	<i>Vitis vinifera</i>	46° 0'10.58"N 13°27'4.89"E	100
	Gramogliano, Como di Rosazzo	Gramogliano, Como di Rosazzo			Gramogliano		12
V	<i>Vitis vinifera</i>	45°59'00"N 13°27'00"E	24	13	<i>Vitis vinifera</i>	45°57'18.01"N 13°29'23.80"E	120
	Udine	Scrìo Annone Veneto 2	Scrìo		Annone	14	V
<i>Vitis vinifera</i>	45°45'58.52"N 12°40'59.61"E	Blessaglia	Bless- aglia	15	<i>Vitis vinifera</i>	45°48'29.34"N 12°43'18.46"E	5
	Venezia	Comugna di Pramaggiore 2	Comugna di Pramaggiore 2		Comugnaga	16	V
<i>Vitis vinifera</i>	45°46'31.53"N 12°42'26.26"E	Loncon di Annone Veneto	Lonc- on1	17	<i>Vitis vinifera</i>	45°46'22.95"N 12°43'24.58"E	2
	Venezia	Loncon di Annone Veneto	Lonc- on2	18	<i>Vitis vinifera</i>	45°46'10.05"N 12°43'3.23"E	2
<i>Vitis vinifera</i>	45°26'59.20"N 11°10'25.93"E	Portogruaro	Lison	19	<i>Vitis vinifera</i>	45°44'44.62"N 12°45'47.69"E	3
	Verona	Brogoligo di Monteforte d'Alpone	Brog- noligo	20	<i>Vitis vinifera</i>	45°24'2.79"N 11°31'32.77"E	190
<i>Vitis vinifera</i>	45°26'59.20"N 11°10'25.93"E	Colognola ai Colli 113	Colognola ai Colli 113		Colognola	21	V
	Verona	Fittà di Soave	Fittà	22	<i>Vitis vinifera</i>	45°27'11.35"N 11°15'16.41"E	160
<i>Vitis vinifera</i>	45°22'17.53"N 11°26'41.40"E	San Zeno di Colognola ai Colli	San Zeno	23	<i>Vitis vinifera</i>	45°26'39.31"N 11°10'29.96"E	50
	Vicenza	Alonte 170	Alonte		Alonte1	24	V
<i>Vitis vinifera</i>	45°23'2.10"N 11°25'35.01"E	Barbarano Vicentino	Barba- rano	25	<i>Vitis vinifera</i>	45°24'42.29"N 11°32'15.51"E	160
	Vicenza	Lonigo 94	Lonigo		Alonte2	26	V
<i>Vitis vinifera</i>	45°22'33.99"N 11°25'16.53"E	Lonigo 135	Lonigo		Alonte3	27	V
	Vicenza	Toara di Villaga Villaga	Toara	28	<i>Vitis vinifera</i>	45°22'34.70"N 11°31'0.56"E	77
<i>Vitis vinifera</i>	45°22'33.99"N 11°25'16.53"E			29	<i>Vitis vinifera</i>	45°23'25.09"N 11°31'5.85"E	50
	Vicenza						

(continued on next page)

Table 1 (continued)

Nation	Province	Site	Code	Map	Habitat	Coordinates	Altitude (MSL)
132	Gorizia	Colle di Medea	Villag- a Medea	30	WL	8, 13, 15	45°55'17.15"N 13°25'15.71"E
114	Gorizia	Dolegna del Collio	Doleg- na	31	WL	1, 15, 17	46° 1'55.12"N 13°29'4.96"E
–	Gorizia 45°57'6.31"N 13°35'44.38"E	Groinizza 72	Groinizza		Groinizza	32	WL
50	Gorizia	Judrio	Judrio	33	WL	5, 13	45°55'1.80"N 13°24'45.97"E
49	Treviso	Savogna d'Isonzo	Savo- gna	34	WL	–	45°55'00"N 13°35'00"E
1400	Treviso	Cesen Mount	Cesen	35	WL	–	45°55'38.82"N 12°0'56.98"E
–	Treviso 45°51'59.15"N 12°14'24.83"E	Crevada 95	Crevada		Crevada	36	WL
187	Treviso	Follina-Roncavezzai	Ronc- avezz- ai	37	WL	1, 2, 3, 4, 10, 16	45°57'1.62"N 12° 7'32.54"E
1, 15, 17	Treviso 45°45'48.00"N 12°33'2.10"E	Gorgo al Monticano – Bosco Cavalier 7			Cavalier	38	WL
170	Treviso	Ruio Vall'Alta	Ruio	39	WL	–	45°56'27.48"N 12°8'22.97"E
77	Treviso	Susegana-Villa Jacur	Villa Jacur	40	WL	1, 15, 17	45°51'27.57"N 12°12'43.12"E
140	Treviso	Val Posan	Val Posan	41	WL	–	45°51'18.05"N 12°8'55.04"E
458	Trieste	Muggia-Osapska Reka	Ospo	42	WL	5, 15	45°34'45.89"N 13°50'15.96"E
133	Udine	Cividaie del Friuli – Bosco di Romagno	Roma- gno	43	WL	1, 2, 3, 4, 10, 14, 16	46° 2'2.89"N 13°27'50.95"E
113	Udine	Colloredo di Montalbano – Laibacco	Laiba- cco	44	WL	16, 18	46°10'23.052"N 13°8'24.32"E
267	Udine	Moruzzo – Modotto	Morru- zzo	45	WL	1, 14	46° 7'53.47"N 13° 8'24.50"E
–	Udine	Muzzana del Turgnano – Bosco Coda Manin	Coda Manin	46	WL	1, 2, 5, 7, 16	45°48'7.04"N 13°8'51.09"E

(continued on next page)

Table 1 (continued)

Nation	Province	Site	Code	Map	Habitat	Coordinates	Altitude (MSL)
260	Udine	Ragogna	Ragogna	47	WL	-	46°11'11.23"N 12°58'9.10"E
-	Udine 46°11'18.37"N 12°51'39.45"E	Travesio 270			Travesio	48	WL
1300	Vicenza	Ekar Mount	Ekar	49	WL	2, 7	45°50'56.58"N 11°34'16.72"E
14	Ferrara 44°46'49.19"N 11°23'22.63"E	Sant'Agostino 15	S.		Agostino	50	WL
2	Grosseto	San Carlo	S. Carlo	51	WL	9	42°42'4.59"N 11°1'27.27"E
Croatia 1, 5, 12, 16	Cerreto 45°18'58.55"N 13°58'48.53"E	Grimalda 287			Cerreto	52	WL
275	Ziepcari	Ziepcari	Ziepcari	53	WL	-	45°21'10.02"N 13°51'49.83"E
270	Montona	Montona	Montona	54	WL	7, 15	45°20'13.07"N 13°50'1.88"E
0	Brioni	Brioni Isle – Vanga	Vanga	55	WL	-	44°54'28.64"N 13°43'51.94"E
565	Velebit Planina	Trnovac-Velebit	Velebit	56	WL	-	44°31'48.22"N 15°14'53.15"E
75	Velika Gorica	Velika	Velika	57	WL	-	45°40'38.55"N 16°25.81"E
Slovenia	Nova Gorica	Banjšće-Podlaka	Podlaka	58	WL	2, 6, 11, 18	46°1'27.15"N 13°42'45.84"E
589	Sezana-Obcina	Kokos Mount – Sezana	Sezana	59	WL	-	45°38'47.96"N 13°55'0.98"E
384	Engleberg	Engleberg	Engleberg	60	WL	-	46°48'55.57"N 8°24'35.84"E
1050–>							

Habitat species numbers: 1 *Carpinus betulus*; 2 *Corylus avellana*; 3 *Acer campestre*; 4 *Acer pseudoplatanus*; 5 *Acer sp.*; 6 *Fagus sp.*; 7 *Fagus sylvatica*; 8 *Laurus nobilis*; 9 *Olea europaea*; 10 *Ostrya carpinifolia*; 11 *Pinus nigra*; 12 *Pinus sp.*; 13 *Populus alba*; 14 *Populus nigra*; 15 *Quercus petraea*; 16 *Quercus robur*; 17 *Robinia pseudoacacia*; 18 *Tilia platyphyllos*.

Table 2
List of the species found in the woodlands. The list with their abundances divided for ecological categories in number and species.

Species	Deciduous Forests/Woodland															
	Cavalier	Cesen	Carreto	Coda Manin	Crevada	Dolegna	Ekar	Engleberg	Groinizza	Judrio	Medea	Motvun	Moruzzo	Ospo	Podlaka	
EPIGEIC and ENDOGEIC																
<i>Allobophora chlorotica</i>				1,5							2,2	1,3			9,9	
<i>Allobophoridaella eiseni</i>				2,2							2,2					
<i>Aporrectodea caliginosa</i>				0,2		6,3										
<i>Aporrectodea jassyensis</i>																
<i>Aporrectodea rosea</i>																
<i>Aporrectodea sp.</i>																
<i>Eisenia foetida</i>																
<i>Eiseniona singaporensis</i>						6,3										
<i>Lumbricus castaneus</i>						6,3									2,2	
<i>Lumbricus rubellus</i>																
<i>Lumbricus sp.</i>						12,5										
<i>Microcolex dubius</i>																
<i>Octodriloides eubenthami</i>																
<i>Octodrilus lissaensis</i>																
<i>Octodrilus transpandanoides</i>																
<i>Octodrilus transpandanus</i>																
<i>Octolasion lacteum</i>																
<i>Perelia nematogena</i>																
<i>Proctodrilus antipae</i>																
Epigeic and Endogeic	0	0	0	3,8	0	31,3	0	0	0	0	4,4	1,3	0	0	12,1	
Density																
Epigeic and Endogeic	0	0	0	3	0	4	0	0	0	0	2	1	0	0	2	
Species Number																
ANECIC																
<i>Eophila crodabepis</i>		2			4		3									
<i>Eophila tellinii</i>																
<i>Hormogaster redii</i>																
<i>Lumbricus terrestris</i>				10		1,3			7	10					6,6	
<i>Octodrilus mimus</i>			1,6		8					1	6,6					
<i>Octodrilus pseudocomplanatus</i>																
<i>Octodrilus tergestinus</i>			4	2,8						8	11	7,9	1	2	8,8	
<i>Octodrilus complanatus</i>			4								2,2	1,3		1		
<i>Octodrilus istrjanus</i>																
<i>Octodrilus nov. sp.</i>	1	1,2														
<i>Perelia gestroi</i>																
<i>Scherotheca targionii</i>																
Anecic Density	1	3,2	9,5	12,8	4	9,3	3	0	7	19	24,2	9,2	1	5	15,4	
Anecic Species Number	1	2	3	2	1	2	1	0	1	3	4	2	1	3	2	
Total Density	1	3,2	9,5	16,7	4	40,5	3	0	7	19	28,6	10,4	1	5	27,5	
Total Species Number	1	2	3	5	1	6	1	0	1	3	6	3	1	3	4	
Deciduous Forests/Woodland																
Species	Posan	Praderad- ego	Ragogna	Romango	Roncavez- zai	Ruio	S. Agostino	S. Carlo	Savogna	Sesana	Travesio	Vanga	Velebit	Velika	Villa Jacur	Ziepcari
EPIGEIC and ENDOGEIC																
<i>Allobophora chlorotica</i>				0,8			1,5									
<i>Allobophoridaella eiseni</i>																
<i>Aporrectodea caliginosa</i>					6,6		3	5							5,2	

Table 2 (continued)

Deciduous Forests/Woodland																
Species	Posan	Praderad- ego	Ragogna	Romango	Roncavez- zai	Ruio	S. Agostino	S. Carlo	Savogna	Sesana	Travesio	Vanga	Velebit	Velika	Villa Jacur	Ziepcari
<i>Aporrectodea jassyensis</i>					1,9	4,286									13,5	
<i>Aporrectodea rosea</i>						4,25	7									
<i>Aporrectodea</i> sp.						1,5										
<i>Eisenia foetida</i>					1,9											
<i>Eisenia sineporis</i>			3,6		5,6											
<i>Lumbricus castaneus</i>			0,5			1										
<i>Lumbricus rubellus</i>			3,1		7,5										13,5	
<i>Lumbricus</i> sp.						19,6										
<i>Microcotex dubius</i>			6,6		3,8		9									
<i>Octodriloides eubenihami</i>			4,4		6,2											
<i>Octodrilus lissaeensis</i>			6,8		8,8		9,875								1,3	
<i>Octodrilus transpadanoides</i>															1,0	
<i>Octodrilus transpadanus</i>					1,3											
<i>Octolasion lacteum</i>			2,3		4,4											
<i>Perelia nematogena</i>			0,8													
<i>Proctodrilus anipae</i>							5									
Epigeic and Endogeic Density	0	0	0	29	47,8	0	35,2	0	34,6	0						
Epigeic and Endogeic Species Number	0	0	0	9	10	0	7	5	0	0	0	0	0	0	5	0
ANECIC																
<i>Eophila erodabepis</i>	6	2			2	1					3				3	
<i>Eophila tellinii</i>																
<i>Hormogaster redii</i>		5														
<i>Lumbricus terrestris</i>				10,9											1,3	
<i>Octodrilus nitidus</i>			4,8				20,9									
<i>Octodrilus pseudocomplanatus</i>			3,1		4,2				1	1			1			4,082
<i>Octodrilus tergestinus</i>																
<i>Octodrilus istrizianus</i>			7,6										1			8,163
<i>Octodrilus nov. sp.</i>													1			
<i>Perelia gestroi</i>			1,4		3,1											
<i>Scherotheca largionii</i>																
Anecic Density	6	2	5	27,7	9,3	1	20,9	1	1	1	3	2	2	1	4,3	12,2
Anecic Species Number	1	1	1	5	3	1	1	2	1	1	1	2	2	1	2	2
Total Density	6	2	5	56,7	57,1	1	56,1	1	1	1	3	2	2	1	38,9	12,2
Total Species Number	1	1	1	14	13	1	8	7	1	1	1	2	2	1	7	2

Table 3
List of the species found in the vineyards. The list with their abundances divided for ecological categories in number and species.

Species	Vineyards													
	Alonte1	Alonte2	Alonte3	Annone	Loncon 2	Baone	Barbarano	Brognofigo	Cartizze	Casa Rossa	Cinto2	Colognola	Fittà	Galzignano
EPIGEIC and ENDOGEIC														
<i>Allophora chlorotica</i>	1,5	4,4	0,1		18,8	1,4	18,2	28,1		1,5	2,4	1,9		0,3
<i>Allophoridella antipae</i>	1,5	3,2		71,9	7,8			21,9			1,6			1,4
<i>Aporetodea caliginosa</i>	1,5	3,2		25	12,5			35,9						
<i>Aporetodea jassyensis</i>				6,3	50			12,5						
<i>Aporetodea rosea</i>														
<i>Dendrobaena byblica</i>														
<i>Dendrobaena octaedra</i>														
<i>Eisenona sineporis</i>				12,5	3,1			6,3						
<i>Lumbricus castaneus</i>					6,3		5,7	12,5					6,3	
<i>Lumbricus rubellus</i>									137,5					
<i>Lumbricus sp.</i>														
<i>Microcolex dubius</i>				9,4	3,1							6,3		0,6
<i>Octodriloides eubenthami</i>										3				
<i>Octodrilus lissaensis</i>				3,1										
<i>Octodrilus transpadanus</i>							0,3							
<i>Octolasion lacteum</i>				6,3	68,8									0,3
Epigeic and Endogeic	4,5	10,7	0,1	134,4	170,3	1,4	24,1	117,2	137,5	4,5	4,2	8,2	6,3	2,6
Density	3	3	1	7	8	1	3	6	1	2	3	2	1	4
Species Number														
ANECIC														
<i>Lumbricus terrestris</i>				9,4				12,5		4,3				
<i>Octodrilus minus</i>														
<i>Octodrilus pseudo-complanatus</i>			1,8				1,4							
<i>Octodrilus complanatus</i>		2,8		3,1	12,5									
<i>Perelia gestroi</i>		0,9					5,6							
Anecic Density	0	3,7	1,8	12,5	12,5	0	7	12,5	37,5	6,5	0,3	0	3,1	0
Anecic Species Number	0	2	1	2	1	0	2	1	1	2	1	0	1	0
Total Density	4,5	14,4	2	146,9	182,8	1,4	31,1	129,7	175	11	4,5	8,2	9,4	2,6
Total Species Number	3	5	2	9	9	1	5	7	2	4	4	2	2	4

Species	Vineyards													
	Lison	Loncon 1	Monteforte	Pramaggiore1	Pramaggiore2	San Zano	Sgribò	Teolo	Toara	Villaga	Vò1	Vò2	Vò3	Vò4
EPIGEIC and ENDOGEIC														
<i>Allophora chlorotica</i>	3,1	9,4		4,7		13,5		6,7	3,3	1,9	0,4	1,3	4,9	31,3
<i>Allophoridella antipae</i>			3,8	15,6		18,8		0,3			0,1			
<i>Aporetodea caliginosa</i>	6,3		1,4	42,9	3,1	7,9		1,6		0,1		5	7,9	21,9
<i>Aporetodea jassyensis</i>		9,4	7,3	4,7				0,6				3,1		
<i>Aporetodea rosea</i>	3,1	3,1			3,1		0,5	0,6				2,5	2,6	
<i>Dendrobaena byblica</i>														
<i>Dendrobaena octaedra</i>														
<i>Eisenona sineporis</i>							8,6							
<i>Lumbricus castaneus</i>		81,3	5,3	12,5		2,8	2,4					13,8	25,5	
<i>Lumbricus rubellus</i>				3,1		14,6		0,6	3,7				17,6	
<i>Lumbricus sp.</i>														
<i>Microcolex dubius</i>				21,9								1,3	1	
<i>Octodriloides eubenthami</i>							3,8							

Table 3 (continued)

Species	Vineyards													
	Lison	Loncon 1	Monteforte	Pramaggiore1	Pramaggiore2	San Zano	Sgriò	Teolo	Toara	Villaga	Vò1	Vò2	Vò3	Vò4
<i>Octodrilus lissaensis</i>			13,5		4,3									
<i>Octodrilus transpadanus</i>											0,1			
<i>Octolasion lacteum</i>	15,6	9,4	1,4	34,4	15,6		1,9			2,2	11,3			15,6
Epigeic and Endogeic Density	28,1	112,5	32,7	139,7	21,9	57,6	21,6	10,4	7	2,2	38,1	59,6	68,8	
Epigeic and Endogeic Species Number	4	5	6	8	3	5	6	6	2	3	7	6	3	
ANECIC														
<i>Lumbricus terrestris</i>	3,1	15,6		13,3		2,8	0,5							
<i>Octodrilus nitimus</i>														
<i>Octodrilus pseudocomplanatus</i>			1,4					0,1						
<i>Perelita gestroi</i>				6,3	12,5	2,8	0,5	2,7			7,5			6,3
Anecic Density	6,3	28,1	1,4	19,5	12,5	9,8	3,4	3,7	0	0,3	7,5	6,5	6,3	
Anecic Species Number	2	2	1	2	1	3	4	3	0	1	1	1	1	
Total Density	34,4	140,6	34,1	159,2	34,4	67,4	25	14,1	7	2,5	45,6	66,1	75	
Total Species Number	6	7	7	10	4	8	10	9	2	4	8	7	4	

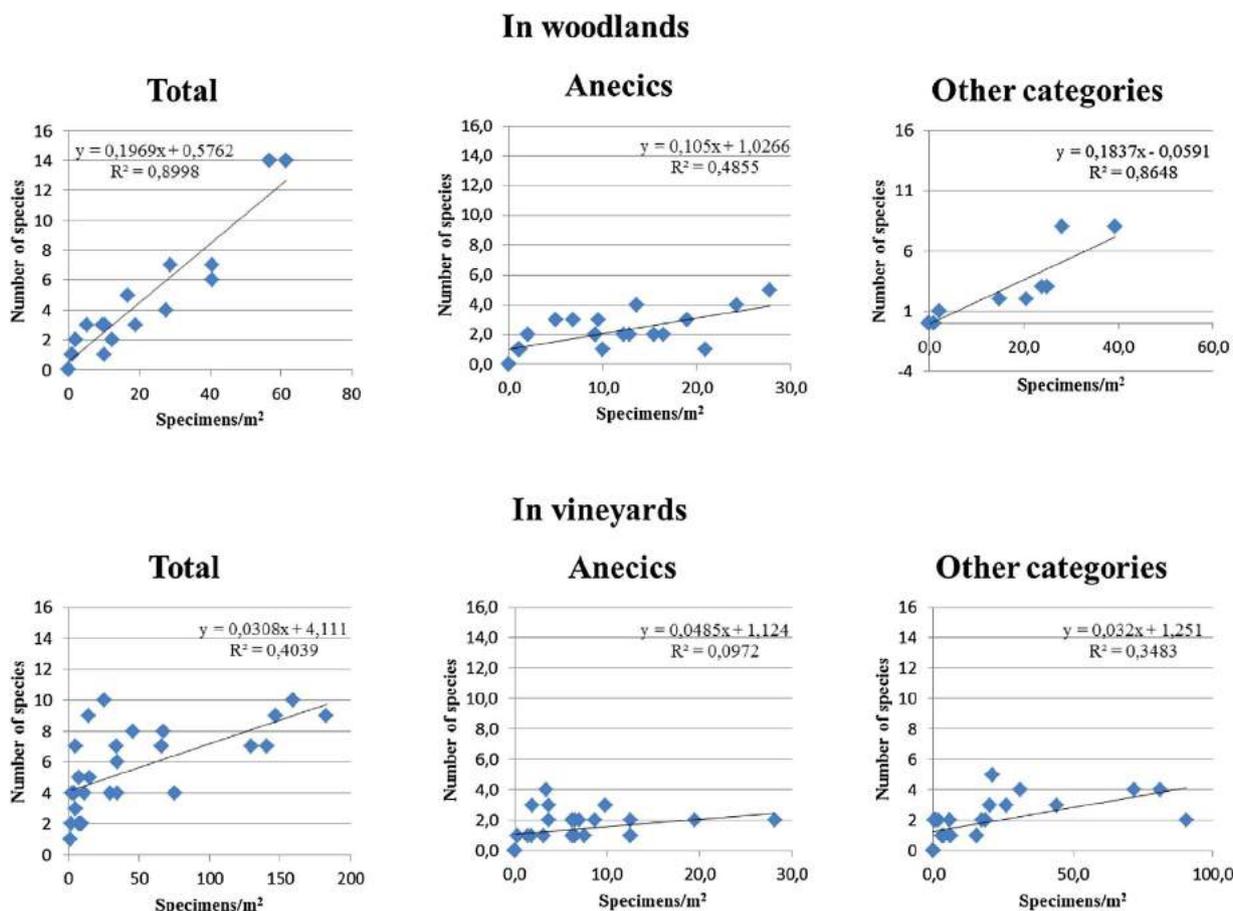


Fig. 9. Regression trends for number of species and abundance of specimens versus specimens collected in the two environment studied. The figures describe the regression for the total of earthworms found in the environment and for the anecic and the sum of the other ecological categories.

The clade containing the large burrowing *Oc. istrianus* is rather complex. The Torrente 1, 5 and 6 specimens shows identical characters with “*istrianus*” (according to morphology) but Roncavezzai 6 and 7 and Villadose 1 are typical *Oc. complanatus*. However, it should be noted that from the above mentioned three specimen we have much shorter sequences (somewhat less than 500 bp instead of the 629 full length) therefore this placement can be an artefact as well.

What is interesting, the “*istrianus*” specimens differ from *complanatus* not only in the position of the clitellum ($\frac{1}{2}29-42,43$ vs. $\frac{1}{2}29-37$) but also in the form of testis sacs. In *complanatus* they are suboesophageal in *istrianus* perioesophageal. To clarify the position of this compound clade re-sequencing of the problematic specimens, wider specimen sampling and more genes are needed (Table 4).

The three clades in the *Oc. tergestinus sensu lato* group are united perhaps unintentionally (with 0.51 PP). The second and third clades of them clearly represent *Oc. tergestinus* (but not forming a monophylum). One eastern clade covers Istria and Croatia, while the western clade covers the Italian station in Friuli Venezia-Giulia. The first clade shows the same clitellum, tubercles and testis sacs’ data as the typical *Oc. mimus*. It seems that their phylogenetic position cannot be determined using a single gene (COI).

Octodrilus mimus, *Oc. sp. nov.* and *Oc. complanatus* together form a well supported clade (1 PP) and COI barcoding highly supported all the

three species too. It is worth to note, that *Oc. complanatus* consists of three highly divergent clades which show not only a genetically division from the hills (Romagno, Euganean and Berici hills sites) and lowlands sites (Vo’1, Alonte sites) but there is difference also from vineyards surrounded by the woodland like Cinto 1 from Cinto 2, with less woodland contact.

Summing up the barcoding results, we can conclude that it successfully recovered most of the morphospecies however, some unexpected branches also appeared. These patterns are not easily explained and could be caused by a high rate of morphological evolution in the genus which results in ongoing speciation and intraspecific polymorphism which process reminiscent of Pop (1994) observations on the accelerated speciation of the large anecic *Octodrilus* species of the Apuseni Mts. Romania (Pop, 1989).

3.2. Presence in woodlands and in cultivated areas

The analyzed woodlands are predominantly deciduous forests and the key vegetation is reported in Table 1. It emerges a constant presence of *Oc. complanatus* (3–12 specimens per m^2), in particular, it is present in most sites like *L. terrestris* (up to 20.9 specimens per m^2 at S. Agostino woodland) (Fig. 5).

The two *Eophila* species are endemic having a restricted range of

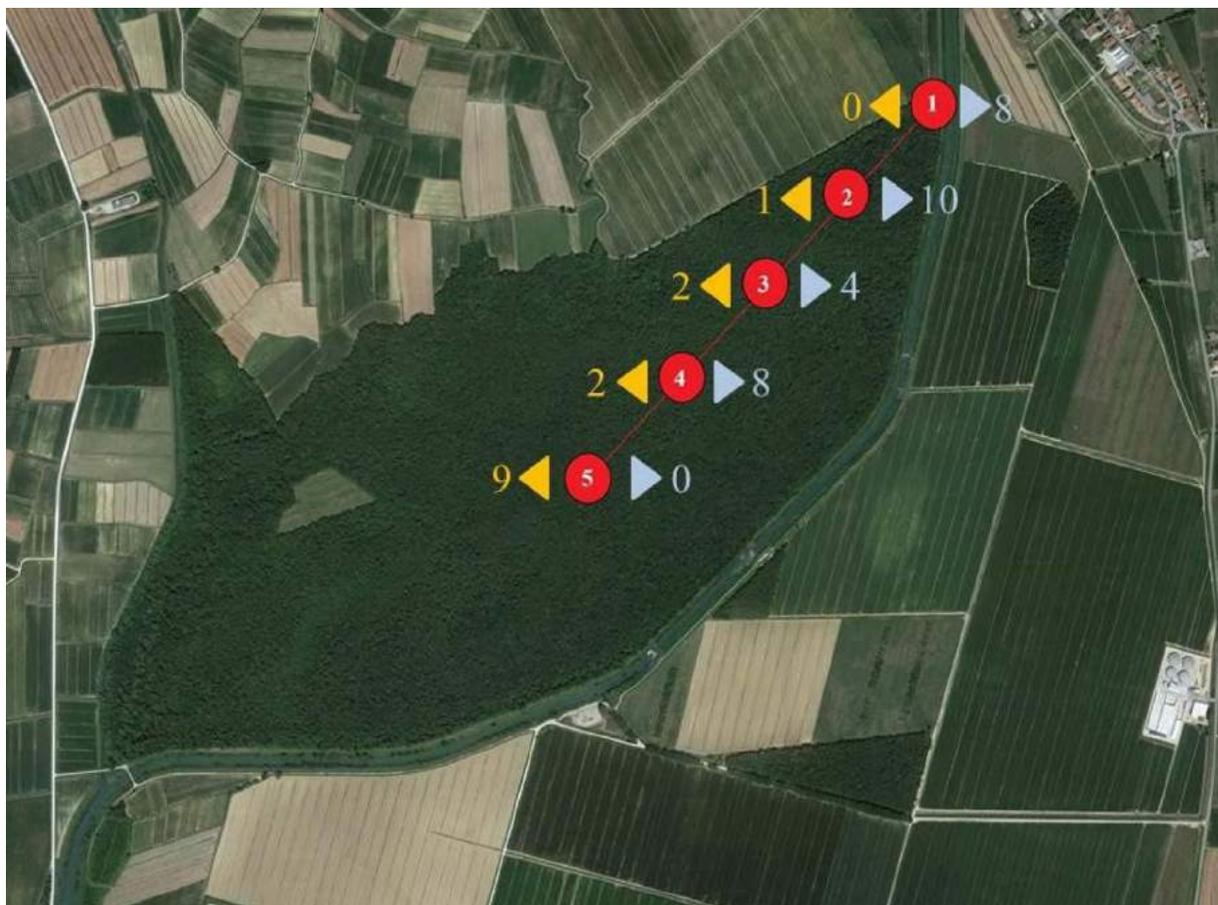


Fig. 10. Transect of five stations at Coda Manin lowland forest. For each station, red spot, the decreasing abundance of *O. tergestinus* from the woodland to the margins, in yellow, and *L. terrestris* from the margins to the woodlands, in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

distribution (Fig. 7). *Eo. crodabepis* covers in particular the classic prosecco wine production area, however, it is found close to vineyards but not inside it except in Roncavezzai where it can be found on the margin of one removed vineyard. In any case this colourful earthworm is together with *Octodrilus* species (*Oc. sp. nov.*, *Oc. complanatus* and *Oc. pseudocomplanatus*) the key soil engineer of these areas producing mull soils (Fig. 1) (Lazzarini, 2015; Paoletti et al., 2016).

Oc. mimus and *Oc. tergestinus* are characterized by banded color produce in general tube shaped casts above soil. *Oc. mimus* produces in most cases tube shaped casts, a sort pretty regular tube of 5–7 cm diameter and 8–16 cm height (Fig. 4). *Oc. tergestinus*, however, produces slightly irregular tube casts, of larger diameter: 8–9 cm and 8–14 cm height (Fig. 4). In the lowland forest of Coda Manin, despite the presence of *Oc. tergestinus* tower casts were not observed. In Podlaka, Croatia no tube shaped casts were found on the *Oc. tergestinus* stations. At Ziepcari *Octodrilus mimus* and *Oc. tergestinus* casts were found.

At Cerreto station in Croatia up to eight tube shaped casts per m² were observed, while in the other sites a maximum of 3–4 tube shaped casts per m² were found. Up to now the production of tower casts was not observed for the species *Oc. complanatus*, *Oc. pseudocomplanatus*, *Octodrilus nov. sp.*, *Eo. tellinii*, *Eo. crodabepis* and *L. terrestris*. When present their casts are distributed irregularly at the soil surface.

A sampling transect made at Medea was characterized by a first station with Mediterranean vegetation, with *Laurus nobilis* and *Asparagus sp.* in which *Oc. tergestinus* was found. In the second station, a few meters apart and characterized by the presence of poplar (*Populus nigra*), was caught *Oc. mimus*. In this site and then, for the first time, not exactly in the same station, but at a short distance, a simultaneous presence of these two anecic endemic species was documented. The *Oc. rucneri* (Plisko and Zicsi, 1970) was not found in Medea as cited by Zicsi (1981). However, examining the praeadult specimens in the Hungarian Natural History Museum, Budapest it is most probably a misidentification for *Oc. tergestinus*.

L. terrestris has the behavior of dragging litter collected at the entrance of its vertical gallery to produce what is termed middens. At Medea (site with *Populus nigra*) and at Romagno (in a picnic areas station) the same behavior was also observed for the species *Oc. mimus* but in these two cases were new colonized places sites (Fig. 5).

A collection transect of five stations was made at Coda Manin lowland forest: from the outer edge epigeic and endogeic species and the deep-burrower *L. terrestris* (Station 1) was found. Along the transect (2–3–4 stations), the species found at the margins decrease in number while increased the number of specimens of the *Oc. tergestinus*, and reaching the exclusive presence at the inner and mature forest stand (Station 5) (Fig. 10).

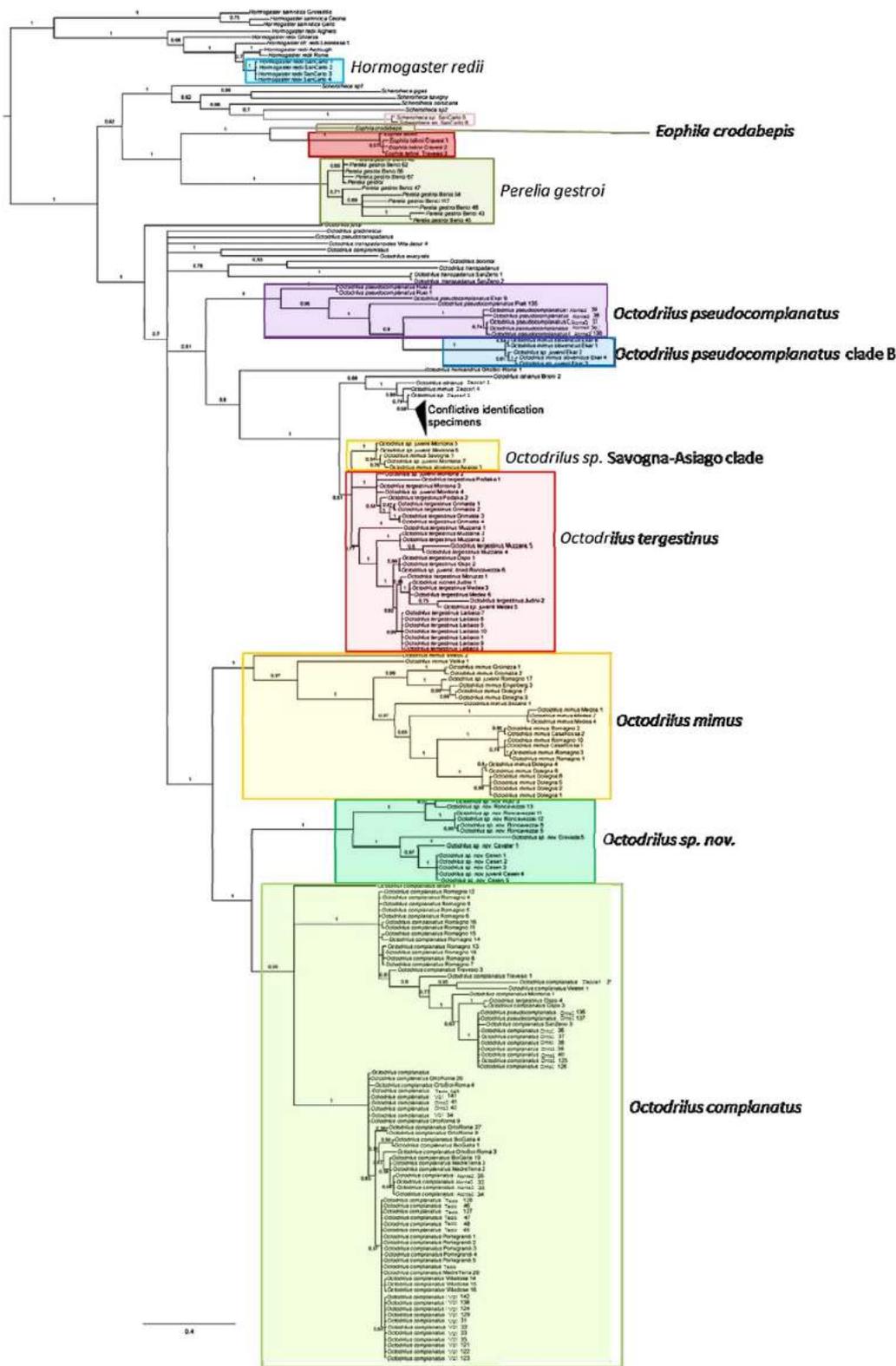


Fig. 11. Barcoding Cluster from left to right: *Hormogaster redii*, *Scherotheca* cfr *targionii*, *Eophila crodabepis*, *E. tellinii*, *Perelia gestroi*, *Octodrilus pseudocomplanatus*, *Oc. tergestinus*, *Oc. mimus*, *Oc. sp. nov.*, *Oc. complanatus*.

Table 4
The list of *Octodrilus* genus with their taxonomical key features.

Species	Clitellum	Tubercles	Spermathecae	Vesicles	Testis sacs	Nephridial Pores	Setae	Color	Size (mm)
<i>Octodrilus complanatus</i> (Dugés, 1828)	1/n 28, 29–37, 1/n 38	1/n 28, 29–39, 40	6/7–12/13, 7	9–12	10, 11 suboes	irregular	3.5:1.8:1.5:1:7:8	grey	80–270/6–10
<i>Octodrilus hemiandrus</i> (Cognetti, 1901)	29–37	29–39	6/7–12/13, 7	10, 12	11 suboes	irregular	2.2:1.5:1.3:1:4	reddish-grey	60–100/6
<i>Octodrilus istrianus</i> Mišić, 1991	1/2 29, 29–42	30–41, 1/2 42	6/7–12/13, 7	9–12	10, 11 perioes	irregular	4.6:1.1:1.2:1:19	reddish-brown	240–280/10–12
<i>Octodrilus lissaensis</i> (Michaelsen, 1891)	1/n 28, 29–36	29–36	5/6–10/11	9–12	10, 11 perioes	aligned in b	2.8:1.4:1.1:1:5:8	reddish-brown	30–150/4–5
<i>Octodrilus minimus</i> (Rosa, 1889)	1/228–1/240	1/228–40	6/7–12/13, 7	9–12	10, 11 perioes	irregular	5:1.6:1.5:1:12	chestnut-brown	180–260/10–12
<i>Octodrilus pseudocomplanatus</i> (Omodeo, 1962)	1/2 29, 30–37, 1/2 38	1/2 29, 30–39, 40	6/7–12/13, 7	9–12	10, 11 perioes	aligned in b	2.1:1.5:1.1:1:3:6	striped reddish-brown	60–180/6–10
<i>Octodrilus pseudocomplanatus</i> clade B.	1/2 28, 29–39, 1/n 40	29–39	6/7–10/11, 5	9–12	10, 11 perioes	aligned in b	4.7:2.5:2:1:10.7	grey brown?	220–250/12–15
<i>Octodrilus</i> Savogna-Asiago clade	1/2 28, 28–1/n 40, 40	29–39, 40	6/7–10/11, 12/13, 5, 7	9–12	10, 11 perioes	irregular	3.4:1.8:1.2:1:8.2	brown	150–180/12–15
<i>Octodrilus</i> sp. nov.	1/2 28, 29–39, 1/n 40	1/n 28, 29–39, 1/n 40	6/7–12/13, 7	9, 10, 12	10, 11 suboes	irregular	4.3:1.7:2.1:1:12	striped?	120–200/10–15
<i>Octodrilus tergestinus</i> (Michaelsen, 1910)	1/2 29, 30–44	30–44	(5/6) 6/7–12/13, 7–8	9–12	10, 11 perioes	irregular	6.2:2.1:3:1:16.2	dark-brown	180–420/15–20
<i>Octodrilus transpadanoides</i> Zicsi, 1981	30–37	30–37	6/7–10/11, 5	9, 10, 11, 12	10 perioes, 11 suboes	aligned in b	2.1:1.5:1.3:1:3:3	striped reddish	4.5–8/4–6
<i>Octodrilus transpadanus</i> (Rosa, 1884)	1/n 29 30–37, 1/n 38	30–37	6/7–10/11, 5	9–12	10, 11 perioes	irregular	2.2:1.2:1.4:1:4.6	grey	40–200/4–8



Fig. 12. Transect in the vineyards located at Casa Rossa in contact to the Romagna woodland. In the spots, the progressive decreasing of the abundance for *O. mimus* from the woodland to the vineyard.

The vineyards located at Casa Rossa is settled nearby the Romagna woodland. A transect made from the center of the vineyard to the margin of the semi-natural, deciduous woodland shows an increasing number of individuals/m² collected (anecic earthworms) in particular *Oc. mimus* (Fig. 12). Similar situation exists in the organic vineyard at Scriò surrounded by woodlots that hosts both *Oc. mimus* and *P. gestroi*.

It is quite evident that anecic earthworms can live inside vineyards, and having wooded deciduous infrastructures such as hedgerows or forest fragments, greatly improve chances to have a rich amount of anecic species.

3.3. Color banding

The presence of bands of pigmentation is observed in some anecic species. This kind of color pattern was found in some deep-burrower species such as *Eo. tellinii*, *Eo. crodabepis*, *Oc. mimus* and *Oc. tergestinus* (Paoletti, 1988; Paoletti et al., 2016; <http://www.youtube.com/watch?v=hSyX8g1V3bc>), but this feature tends to be variable in the very same species and less visible for the specimens preserved in alcohol.

In particular in *Oc. mimus* each segment has a band that occupies almost the entire width of the same segment, extending from the dorsal to the ventral bristles (Figs. 4 and 13).

Oc. tergestinus has a pigmentation pattern similar to *Oc. mimus*. Such pigmentation, however, is more evident in the dorsal portion of the tail (Fig. 4) and in locations such as Podlaka and Grimalda a different

pigmentation darker and homogeneous of *Oc. tergestinus* specimens was recorded (Fig. 13).

Eo. tellinii, instead, exhibits a rosy ventral coloring interspersed on each segment from dorsal bands of darker pigment in the front of the body, and violet colored bands along the rear portion of the body. *Eo. crodabepis* has purplish-brown bands in the middle of each segment with puce intersegments; bands are less visible or absent caudally in some specimens, or pigment is lacking from posterior half of ventral side or from ventral and lateral sides or entirely lacking ventrally (Paoletti et al., 2016) (Fig. 13).

Other anecic species such as *Oc. complanatus*, *Oc. pseudocomplanatus*, *Oc. istrianus*, *Octodrilus* n. sp. and *L. terrestris*, although are pigmented, do not show the banded pattern. The banding pattern is not exclusive of anecic earthworms. In fact the specimens of *Dendrobaena veneta* and *Eisenia foetida* that we collected show pigmentation similar to *Oc. mimus*.

Pigmentation could be a possible defense mechanism to deter predation for the anecic species that come to the surface during the rainfall season to gain new territories and to mate (Fig. 3).

4. Discussion

In Northeastern Italy a set of anecic earthworms are very active in the few wooded deciduous forested areas and others persist under agriculture. Extending viticulture over larger areas without



Fig. 13. The anecic color banding. A) *Oc. mimus*, extending from the dorsal to the ventral bristles. *Oc. tergestinus* pigmentation pattern more evident in the dorsal portion of the tail in B) Zipcari/Torrente and C) Podlaka. D) *E. tellinii* with a bottom rosy coloring interspersed on each segment from dorsal bands of darker pigment in the front of the body, and violet bands the rear portion of the body. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



Fig. 14. Distribution of the two main species collected during the study in the northeastern of Italy. The square for *Oc. tergestinus*, the round for *Oc. mimus*. Each number correspond to the collecting sites listed in Table 1.

interruptions is detrimental to the environment and sustainable farming. Anecic earthworms produce soils of good quality and areas such as the Classic Prosecco or the Collio-Oriental hills vineyards bear several endemic species. Here we demonstrated that the key anecic species are important for building mull soils and are associated with truffle production.

Oc. mimus and *Oc. tergestinus* are present in the North-East of Italy: in Friuli Venezia-Giulia region mainly. These two species occur mainly in hills areas within an average annual rainfall higher than 1100 mm and require conditions of high humidity and are more thermophilic than *L. terrestris* or *P. gestroi*.

L. terrestris, *Oc. complanatus* and *Oc. pseudocomplanatus* have a wider geographic range and the two *Eophila* species, *Oc. mimus*, *Oc. tergestinus* and *Oc. sp.nov.* have a smaller range (Figs. 14 and 15). Some species are poorly studied in central Italy and the *Hormogaster* we collected, *H. redii* comes from Rome's botanical garden and San Carlo at Grosseto (Fig. 6) and *Scherotheca targionii* from grassland with some young olive trees at San Carlo near the coast (Fig. 7).

The *Octodrilus* species we collected in disturbed environments such as the vineyards were able to survive because of mosaic landscape with refuge areas of woodlands, hedgerows, or shelterbelts. The transect Bosco Romagno-Casa Rossa vineyard (Fig. 12) demonstrated that *Oc.*

mimus could colonize the vineyards from an adjacent woodland.

In the mature woodland portion of Romagno and Coda Manin, *Oc. mimus* and *Oc. tergestinus* were rather abundant. We hypothesize that the tower-cast could help earthworms to diffuse alarm pheromones that dissuade lowland forested areas to be colonized by the invading *L. terrestris*. In fact, in Romania the presence of *Oc. frivaldskyi* and its casts (4–6 cm in high and 2–4 cm in diameter) (Fig. 4) would exclude the presence of *L. polyphemus*, another anecic *Lumbricus* (Pop, 2004; Nicolussi, 2016; Zicsi, 1983).

It is known that the earthworms are very sensitive to the stress of the soil management, such as tillage, plowing, use of agricultural chemicals or chemical fertilizers and soil compaction. This sensitivity is more developed in the deep burrowing earthworms, (as earthworms with reproductive rates lower than those of other ecological categories) (Paoletti, 1999; Curry and Schmidt, 2007; Lavelle and Spain, 2007; Pelosi et al., 2016). Efforts have to be made to improve their abundance in rural areas to ameliorate soil quality conditions and their presence has been scored high in the QBS-e that measures soil sustainability (Paoletti et al., 2013).

Reducing the use of chemicals, introducing of living or dead mulches; reducing soil compaction could improve the stability of the soil in which these organisms live as a paradigm of sustainability.

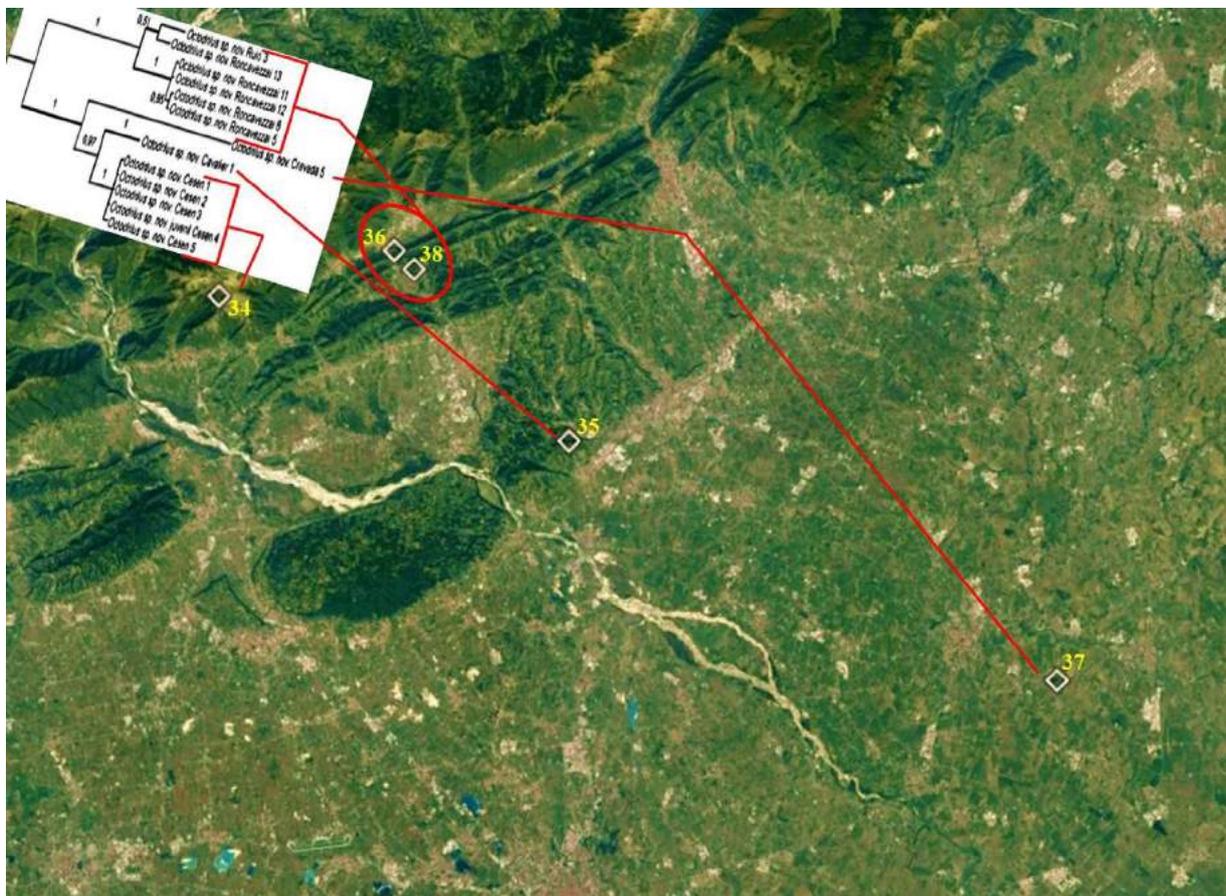


Fig. 15. The barcoding tree of *Octodrilus sp. nov.* in relationship with its geographical collection sites distributions.

This study confirmed that *Oc. mimus*, *Oc. tergestinus*, *Oc. complanatus* and *Oc. pseudocomplanatus* are not only morphologically and genetically different, but their ecological patterns differ as well.

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