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Outdoor surviving experiment with three green house enchytraeid species (Oligochaeta: Enchytraeidae)

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Abstract. Some enchytraeid species of tropical, subtropical or Mediterranean origin can appear in artificial environments, e.g. green houses due to the worldwide commercial network. Since the used soil from green houses is often disposed outdoors, a question raised that these exotic enchytraeid species could survive under continental climate conditions. In this experiment two of the resettled green house species survived outdoors the winter frost and the arid summer season in Hungary.

Keywords. Pot-worms, exotic species, introduction, survival.

INTRODUCTION

Development of human civilization entailed the development and growth of worldwide commercial network. Due to this process, several animal species were propagated accidentally which have not been able to spread under natural circumstances. The expansion of certain oligochaete species is an expressive example of this phenomenon. Importation of foreign earthworm or enchytraeid species is hardly perceptible. Apart from their hidden lifestyle, as saprophagous animals, occasional species exchange is not necessarily observable in the effect produced on environment, since native and introduced species can decompose similarly.

In spite of this fact, frequent monitoring detected invasive earthworm species in a number of cases: Csuzdi and Szlávecz (2003) have recently recorded the exotic species *Lumbricus friendi* Cognetti, 1904 in North America, previously known only from the Atlantic region of Europe (Bouché, 1972). *Dichogaster bolaui* (Michaelsen, 1891), which is of Eastern African origin, was found not only in green houses but also in bathrooms in Hungary and Israel (Csuzdi *et al,*. 2008). The same species have repeatedly been found in the sewage systems of buildings in Sweden and Finland as well (Terhivuo, 1990). Such species were defined as a 'domicole' which are able (or adapted) to live in urban dwellings. Earlier, Michaelsen (1903), Lee (1987) and others used the expression 'peregrine' for earthworm species which are anthropochorous and are usually confined to disturbed, human-modified habitats, such as gardens, lawns or green houses. The greenhouse invertebrate fauna of Hungary have previously been investigated by Korsós *et al*. (2002) reporting on Isopods and Diplopods and Hídvégi (1994) who examined earthworms. Both investigations demonstrated the presence of introduced peregrine species.

Despite of the broad range of the aforementioned literature, enchytraeids have not yet been the subject of similar studies, albeit their presence is more evident in consequence of their size. In 2006 a comprehensive study was launched to investigate the Enchytraeid fauna of green houses in Hungary. The aim was to describe the enchytraeid fauna in a sample of 10 green houses across Hungary and to prove the presence of exotic species. The hypothesis of the research was that (sub)tropical and Mediterranean enchytraeid species were imported in earth-balls left on the roots of the plants, or even more among the roots themselves, and the species settled down due to the human-made optimal conditions found in green houses. As a result, some species detected earlier in subtropical or Mediterranean climate were found in the samples, e.g. *Fridericia pretoriana* Stephenson, 1930, *Enchytraeus dudichi* Dózsa-Farkas, 1995, moreover, 2 unidentified species

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belonging to the genus *Hemienchytraeus* were described (Boros and Dózsa-Farkas, 2007). A new species to science, *Marionina scintillans* Boros and Dózsa-Farkas, 2008 was also discovered (Boros and Dózsa-Farkas, 2008). The abundance of this species was extremely high: *ca*. 342.000 ind./m^2. This high number can be explained with the artificial circumstances, since the highest abundance of enchytraeids in the nature was 289.000 ind./m^2 (Peachey, 1963). Since the used soil from green houses is often disposed outdoors, the question raised by this paper is whether introduced exotic enchytraeid species could survive under continental climate conditions, especially with that high abundance. In the course of outdoor sampling in botanical gardens, has not been found an evidence of surviving exotic populations, therefore an experiment was carried out to answer this question.

This experiment was carried out in the Botanical Garden of the Eötvös Loránd University. Three species were chosen: *Enchytraeus bigeminus* Nielsen & Christensen, 1963; the above mentioned *Marionina scintillans,* and a fragmenting *Marionina* species found in the botanical garden of Szeged University in 2009 and uniquely in the genus able to multiply by architomy. Although stable breeding cultures are sustained, no sexually matured specimens of this species are known yet. Even so, the species was considered as suited to this study due to the special asexual reproduction like that of *E. bigeminus*.

MATERIAL AND METHODS

The research were carried out in the Botanical Garden of the Eötvös Loránd University. A 0.25 m^2 (0.5x0.5 m) undisturbed lawn area was enclosed where lack of any gardening intervention was guaranteed. Before resettlement, qualitative samples were taken from this area to establish the present Enchytraeid species.

Individuals of the 3 species were placed in the following numbers: 100 specimens each of *Enchytraeus bigeminus* and *Marionina scintillans,* and 40 specimens of the fragmenting *Marionina* species. Breeding cultures of the fragmenting *Marionina* sp. increase slowly, so only a small number of worms were to be expended.

The reasons why these species were chosen are 1) these species were missing from the research area so the presence of the newly introduced species were traceable 2) due to reliable breeding cultures relatively high number of enchytraeids were available.

Breeding cultures were sustained in Petri-dishes on 1% agar-agar medium with soil on top, earlier sterilized by heat-treatment. Foods were rolled oats and dried leaves of nettle *(Urtica dioica)*. Breeding cultures were kept between 10–12 °C in refrigerator.)

Quantitative samples were taken 5 times in the year of 2011: 2 times both in spring (09. 04. and 28. 04.) and in summer (24. 06. and 11. 07.) and once in autumn (06. 09.). Every time 5 samples were taken with a 20 cm^2 surface split soil corer. Samples were 10 cm deep which means 200 cm^3 volumes each.

Enchytraeids were extracted from the soil by the wet funnel method (O'Connor, 1962) and were identified alive with a Zeiss Axioskop Imager.A2 microscope, using DIC (differential interference contrast) illumination. Voucher specimens were preserved in 70% ethanol and stored in 600 µl. Eppendorf tubes.

RESULTS

Just before the resettlement (05. 04. 2010.) six species of five genera were found in the research field:

Achaeta eiseni Vejdovský, 1878
Buchholzia appendiculata (Buchholz, 1862)
Enchytraeus buchholzi Vejdovský, 1879
Enchytraeus sp. juv.
Fridericia bulboides Nielsen & Christensen, 1959
Henlea ventriculosa (d'Udekem, 1854)

It was impossible to identify specimens of *Enchytraeus* sp. juv. due to the lack of sexual organs. However, based on setal complements and the number of setal follicles, it was obvious that this species is identical neither with *E. bigeminus* nor *E. buchholzi*.

The following species were found in the spring samples (recollected species are represented in **bold**):

Buchholzia appendiculata
Enchytraeus bigeminus
Enchytraeus buchholzi
Enchytraeus sp. juv.
Fridericia bulboides
Henlea ventriculosa
Marionina scintillans

Two of the resettled species were present which justify their survival of the winter frost. All specimens of *Marionina scintillans* were adults, in some of them matured egg was observable. At the same time, individuals of the other *Marionina* species completely disappeared.

Species found during the summer sampling were:

Enchytraeus bigeminus
Fridericia bulboides
Enchytraeus buchholzi

Low numbers of individuals were present from all the three species. In some samples there were absolutely no enchytraeids.

Species found during the autumn sampling were:

Buchholzia appendiculata
Enchytraeus bigeminus
Enchytraeus buchholzi
Fridericia bulboides
Marionina scintillans

Moisture demanding *Buchholzia appendiculata* and *M. scintillans* reappeared in the samples, but only in low numbers.

From the original fauna of the research field, *Achaeta eiseni* were not found in any samples after introduction of the 3 new species.

DISCUSSION

The number of species of the research area was relatively low compared to other lawns in the region, e.g. 5–15 species in the meadows near Tisza river, Hungary (Dózsa-Farkas *et al.,* 2003), 7–12 species in lawn parks of Brno, Czech Republic (Schlaghamerský and Pižl, 2009) or 6–10 species in the grasslands of Sas-hegy Nature Conservation Area, Budapest, Hungary (Boros, 2007). This low number of species was especially prominent in the dry summer season when specimen numbers were also small. The periodical lack of *Buchholzia appendiculata* also confirms the importance of aridity in the seasonal development of species' abundances which is also in line with earlier findings.

In the course of the experiment two displaced species were found outdoors. *E. bigeminus* is well known from compost fields, and is considered as a common species in Southern Europe (Schmelz and Collado, 2010). Now, it seems we can find it in colder continental climate as well. Expressly, an advantage is that this species can multiply asexually by fragmentation (architomy) so increases their populations quickly. Earlier experiences showed that this species can contaminate other enchytraeid breeding cultures so it can be an invasive species.

M. scintillans was so far known only from green houses. However, this experiment proved its ability to survive outdoors as well. This species was missing from the samples in the summer but reappeared during the moist autumn. A presumable explanation for this behaviour is that only cocoons survived the arid period and all the worms died. Another possibility is that these worms go deeper than the sampling depth of 10 cm during the arid season.

The fragmenting *Marionina* species was not found outdoors in the course of the study which possibly means the extinction of these enchytraeids during the harsh winter conditions. Another

explanation could be the lower initial number of the test worms.

As it was already mentioned, *Achaeta eiseni* was also missing in every sample, in spite of the fact it was present in the study area before. This phenomenon should be placed under more thorough scrutiny in the future.

The preliminary results of the experiment herewith presented show that several green house species are able to survive outdoors under continental climate. For the more reliable explanation of their behaviour and survival further experiments are needed with higher number of enchytraeid species and individuals.

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