Report on the research 'Investigation of the Hungarian enchytraeid (Oligochaeta: Enchytraeidae) fauna: taxonomical and zoogeographical synthesis IV. Enchytraeids of Őrség, Kőszegi Mountains, Keszthelyi Mountains and remnant hills of Balaton. Synthesis of the Hungarian data and publishing the general work ("Enchytraeids of Hungary")'conducted between 01.01.2014 and 31.12.2019 (NRDIO 108582)

The investigation was going on according to plan, but unfortunately, I fall behind my research schedule in 2017, since after an accident (my arm was broken in several points) I had a series of operations which did not allow working for a relatively long period. Therefore I asked for a permission to delay the finishing of the project ((NRDIO 108582) with one year, and finally I got this permission. So I give account of the work of five years (01.01.2014–31.12.2018).

Results

Some result of the former investigation of the Hungarian enchytraeid fauna (project III) supported by the previous HSRF-project was published only in 2015. The results of the analyses of the **fauna of Dráva plain is as follows:** a total of 14 enchytreid genera were detected, including 49 species and 2 other annelid worms (*Hrabeiella periglandulata* and *Rhyacodrilus falciformis*), moreover 4 species (*Fridericia connatiformis* sp. n., *Fridericia phaeostriata* sp. n., *Fridericia longiducta* sp. n. and *Cernosvitoviella buekkhati* sp. n.) which were new to science were described (Dózsa-Farkas et al 2015)

According to the research schedule, it started with fauna investigations of the western mountains of Hungary in **Örség National Park** from different ecosystems (mixed forests and mountain hayfields of Szentgyörgy valley, specially protected alder marsh areas and hayfields of Gödörházi meadows, old oak-woods of Kercaszomor, and *Sphagnum* mires of Szőce and Farkasfa). 14 enchytraeid genera including 47 species and one other annelid worm (*Hrabeiella periglandulata*) were identified and one enchytraeid species new to science (*Fridericia zicsii* sp. nov) was described. One new species for the Hungarian fauna (*Cognettia chalupskyi*) was found. This enchytraeid fauna is quite diverse, and consists mostly of species typical to the Hungarian or wider Central European fauna (Schmelz & Collado 2010). In terms of species number of each investigated sites, the hay meadow of Gödörházi rétek showed with 32 species the highest value. The species composition of this site considerably reflects the mountain or subalpine character of the area.

From all other studied Hungarian mountain ranges, the fauna of Őrség NP shows the most similarities with the fauna of Zemplén Mts (Dózsa-Farkas 2007). *Mesenchytraeus armatus, Mesenchytraeus glandulosus* and *Marionina simillima* are North European or subalpine fauna elements. Comparing the two *Sphagnum* mires studied here with the four other *Sphagnum* mires in the north-eastern part of Hungary investigated earlier (Dózsa-Farkas 1990, 1991), it is worth noting that the enchytraeid fauna of the mire in Farkasfa was poor in species (4 species) while the mire in Szőce with its 20 recorded species widely differed from these. Interestingly, this species-rich sampling site was located in the middle of the mire, while at its edge only seven species were recorded. This was quite interesting, as we expected an opposite trend. The reason for this could be the higher pH values measured (6.8–6.9) in the mire at Szőce, the site was not as nutrient-poor as in case of the other mires (Pócs *et al.* 1958) and furthermore, besides *Sphagnum* spp. other moss species were also present (Dózsa-Farkas & Felföldi 2016).

From the results of the investigation of the **Kőszeg Mountains** and comparative studies with the investigated fauna of Rax Mountains have been arised **5 publications**

(Dózsa-Farkas & Felföldi 2017a, b, 2018, Dózsa-Farkas et al 2017, Nagy et al 2018) and in total **6 species new to sciences** (*Fridericia szoevenyii sp.n*, *F. ventrochaetosa sp. n.*, *F. alpica sp. n.*, *F. raxiensis sp. n.*, *Cernosvitoviella farkasi sp. n.*, *Achaeta tothi* sp.n.) were described.

Besides the description of the new *Cernosvitoviella* species which was detected only in one habitat of Kőszeg Mts., we made a **comparison with other five** *Cernosvitoviella* **species** living in Hungary, [founded newly in Örség N.P. and Kőszeg Mts. and two additional habitats from Eastern Hungary (Vámosatya, Istvánkút) were also included in our analysis]. The results of the comparison of five *Cernosvitoviella* species based on morphological and molecular taxonomical analysis and new traits are presented, together with a detailed photodocumentation of all studied species (Dózsa-Farkas et al 2017).

A new species (*Achaeta tothi* sp. n.) and five other *Achaeta* species (*A. affinis*, *A. bohemica sensu stricto*, *A. camerani*, *A. cf. danica*, *A. unibulba*) were found during the investigation of Örség National Park and Kőszeg Mts. We give some additional morphological data and photos about these *Achaeta* species. Such information can have importance in subsequent taxonomic studies and will aid the discrimination among the members of the genus. These new information were supplemented with molecular taxonomic analyses targeting the nuclear ribosomal ITS region, the mitochondrial cytochrome c oxidase subunit I (CO1) gene and the nuclear histone 3 (H3) gen and DNA-based taxon identification (Dózsa-Farkas & Felföldi 2017a).

During the investigation of the fauna of Kőszeg Mountains (Hungary) and Rax Mts. (Austria) a large *Fridericia* species was detected, and its spermatheca was very similar to the spermatheca of *Fridericia galba* (Hoffmeister, 1843) in having 6–8 diverticula. Therefore we presented **comparative taxonomic information for this new species and** *F. galba*. Therefore, it seemed to be interesting to examine if these species have any other different morphological traits, and if the shape of spermatheca or the position of chaetae or any other morphological traits are much more important in the distinction between species. Molecular taxonomic methods were also involved in our study to solve this problem incorporating other *Fridericia* species which have more diverticula, so a new species was described (*F. ventrochaetosa sp. n.*). These results also pointed out that in the determination of enchytraeid species, although the shape of spermatheca can be determine their taxonomic identity, it is still very important to consider several morphological characters of the worms. Apart form the new species, Gen-Bank reference sequences are provided for the first time for *F. hegemon* and *F. regularis*, too (Nagy et al. 2018).

Our investigation focusing on the enchytraeid fauna of the Kőszeg Mountains (Günser Mountains) of Hungary and Austria. To compare these subalpine areas and the **Rax mountain** range soil samples were taken from five different habitats from the upper terminal of the Rax cable car and from the western side of the mountain. 41 enchytraeid species belonging to 12 genera were found and additionally the terrestrial polychaeta *Hrabeiella periglandulata*. Two new species were found (*Fridericia alpica* sp. n. *and F. raxiensis* sp. n.) and were described based on both morphological and molecular taxonomic methods. Both species were found in the Kőszeg Mts (Hungary), too (Dózsa-Farkas & Felföldi 2018).

From the enchytraeid fauna of **Kőszeg Mountains** (Western Hungary and Eastern Austria), hitherto unknown 59 species were recorded which belonged to 15 enchytraeid genera, moreover, two terrestrial polychaete species, *Hrabeiella periglandulata* Pižl & Chalupský, 1984 and *Parergodrilus heideri* Reisinger, 1925 were also detected. *Parergodrilus heideri* was a new record for the fauna of Hungary. The fauna of Kőszeg Mts. is comparable to the fauna of Rax Mts. in Austria, as both have subalpine or alpine climate. Comparing these two regions, similarities could be found regarding the enchytraeid fauna,

which includes some characteristic species of the Alps. For example, *F. alpica* described from Rax was also found in Kőszeg Mts., moreover, at an interest site (alder carr at creekside) with its very cool and humid subclimate, a habitat is provided for such species as *F. discifera*, *F. raxiensis*, *Euenchytraeus clarae* and *Fridericia ventrochetosa* sp. *n*. (which was later in 2018 described). Additionally, these four species were recorded only from the Kőszeg Mts (in one site) and nowhere else within Hungary, therefore they could be regarded as relict species from the last glacial. It is also worth mentioning that *Oconnorella tubifera*, which is widespread from Sweden to Italy in moist soils (Schmelz & Collado 2010), was found at first time in Hungary. We think, that it is possible that all 3 new species (*F. szoevenyii, Cernosvitoviella farkasi* and *Achaeta tothi*) are endemic to this region. The alder carr at a creekside near Paprét showed the highest diversity with 33 species. The second most diverse area was the mesophile montane hay meadow at Steier Houses with 27 species. Remarkable that in a mixed forest on a hillside only a single species (*Marionina clavata*) was found, probably due to the dense underwood of *Vaccinium myrtillus*.

Faunistic studies continued in **Keszthely Mts.** and the remnant hills around Lake **Balaton** was investigated also since 2015. Soil samples were collected from nearby sites; at the Inner Lake in Tihany, from the moorland near Raposka, and also from the alder carr, the rocky hillside of Szent-György Hill, at the columnar basalt site and from the small spring, from the vineyard at Szent-Gyönrgy Hill, and from the forests of Szigliget and Gulács. Up to now I have identified 37 species (12 genera) from the **Keszthely Mts.** Moreover, one terrestrial polychaeta (*Hrabeiella periglandulata*) and a terrestrial tubificid worm (*Rhyacodrilus falciformis*) were also found. From **the remnant hills around Lake Balaton**, up to now I have recorded 43 species (8 genera). The majority of these species are characteristic members of the Hungarian enchytraeid fauna. The most interesting was the detection of *Achaeta antefolliculata* Dózsa-Farkas & Boros, 2005 at Szent-György Hill, since this species was previously known only from Sas Hill and Szársomlyó Hills. The phenomenon that fauna of remnant hills around Lake Balaton contains more species but less genera as the fauna of Keszthely Mts., could be explained as the previous has drier habitats.

These results also contributed to the following other research projects:

The new species for Hungarian fauna *Euenchytraeus clarae* (Bauer, 1993) was found at a stream in alder wood in the Kőszeg Mountains, and Rax Mountain, and some other *Chamedrilus* (*Cognettia*) species were subjected to molecular analysis with **cooperation of Swedish colleagues**. The aim was to reveal the phylogenetic relations of these genera (Martinsson et al.2017).

The enchytraeid **fauna of Transylvania**, **Romania**, which contained also some results of my works supported by former HSRF grants (Boros & Dózsa-Farkas 2015).

Due to the he comprehensive study of Hungarian fauna, besides the description of new species occurring in the samples sent from **Korea**, common Hungarian species detected in Korea could be used for comparison and for resolving taxonomic problems like boundaries among interspecific and intraspecific character variation (Dózsa-Farkas et al 2018, Dózsa-Farkas et al 2019).

Since there was a decision made by the internationally-recognized taxonomists of Enchytraidae (including also myself) (Schmelz et al. 2017) that for the description of new species molecular analyses should be also performed (preferably from type material), with the help of my colleague, **Tamás Felföldi**, more emphasis was put on the comparative molecular analyses (which was also performed in our previous studies). A new PhD student (Hajnalka Nagy) also participated in the work. Unfortunately, this work also requires time, and in

several cases (but we do what would) not any result could be obtained, repetition is needed or the collection of new enchytraeid specimens is required. Studies with cryptic species (which were discovered on the basis of molecular analyses) also requires a lot of work.

Between 2014 and 2018 I have participiated in two international Symposium:

At the **11th International Symposium on Enchytraeidae** (Osnabrück, Germany, 25–27.07.2014). I have given an oral presentation (Dózsa-Farkas, K. & Felföldi, T. "Unexpected occurence of *Hemifridericia bivesiculata* in Hungary, a species presumed to be endemic in Devon Island, and its comparative analysis with *H. parva*"), and presented a poster, too (Boros, G. & Dózsa-Farkas, K. "The enchytraeid fauna of Transylvania, Romania").

At the **12th International Symposium on Enchytraeidae** (Tihany, Hungary, 27–29.06.2016.) I have given an oral presentation [Dózsa-Farkas, K. & Felföldi, T. "The Enchytraeid fauna (Enchytraeidae, Clitellata) of Rax mountain (Austria). Two new species and comparison of *Fridericia discifera* Healy,1975 and *F. alpica* sp. n."]. All presentation were published later.

In 2017 I did not participiated personally in 13th International Symposium on Enchytraeidae (4–6.06. 2018 Vesailles), but my PhD student presented a common poster about our recent research (Nagy, Dózsa-Farkas, Hong & Felföldi: Cryptic species from the genus *Bryodrilus*: morphologically similar *B. ehlersi* specimens from Korea and Europe).

The oral presentation of the importance of molecular methods in taxonomy at a scientific meeting of HAS (18.11.2013.) was published in 2014 (Dózsa-Farkas, K., Boros G., Felföldi T. & Cech G. 2014).

My database contains **3870 pieces of data** on the Hungarian fauna. Since 2014 I made **376 new slides of 29 species,** too. Similarly to my former studies, I took **photographic documentation** of the living and fixed specimens using a digital camera attached to a microscope (the camera was financed by my former OTKA projects). In this period **217 sequences** (CoI, ITS, H3) of the Hungarian enchytraeid species were also deposited in the GenBank database.

Some probably new species (a *Marionina* and some *Fridericia* species) were not described according to my earlier plans, because unfortunately they have not been found any more, however more samples were collected again from their localities.

My most important and final aim was the compilation of the "Enchytraeids of Hungary" volume in the Pedozoologica Hungarica book series on the basis the results of the 4 HSRF / NRDIO-projects. I have successfully achievined this goal, the book is in press. In the book, I summarize the results of the Hungarian enchytraeid research done so far. I have described 34 terrestrial species new to science from Hungary and registered altogether 124 species for the fauna of Hungary, which is about the half of the known European terrestrial enchytraeid species, and give a description of a new species (Enchytronia holochaeta sp. n.). First, I review the most important characters of enchytraeids used in identification and after give a key to the genera occurring in Hungary, which is followed by short descriptions of all species recorded so far from the country. Distribution data of each species are provided and distributions of the most important species are also shown in an UTM grid map. The results of molecular taxonomy of selected enchytraeid species are also presented. I give some micrographs on parasitae of the worms observed during my studies. All illustration are from my original colour micrographs.

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