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First Record of the Family Penthaleidae (Acari) in Hungary: Morphological and Molecular Approaches of the Hungarian *Penthaleus cf. major* (Dugès, 1837)

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The first Hungarian record of the family Penthaleidae, the occurrence of blue oat mite [*Penthaleus cf. major* (Dugès, 1837)] in Hungary is presented. This pest mite species was collected on lettuce in greenhouse. Notes to the morphology and the three (cox 1, 18sRNA, ITS2) sequences of the Hungarian specimens are given.

Keywords: Pest mite, *Penthaleus cf. major* (Dugès, 1837), new occurrence, DNA barcoding.

The Hungarian mite fauna seems to be relatively well-known (Horváth et al., 2010), but numerous groups are absolutely neglected, like some groups of order Trombidiformes. The last some years several poorly known, but large and easy to recognize phytophagous and pest mite species are discovered from Hungary (like the tetranychid *Eurytetranychus latus* (Canestrini and Fanzago, 1876) or *Petrobia harti* (Ewing, 1909) (see: Kontschán, 2015; Kontschán and Molnár, 2016). In February 2017, numerous specimens of unusual mite were collected from lettuce plants in a greenhouse production located at Forráskút, Southern Hungary, which resembles to the blue oat mite (*Penthaleus cf. major* (Dugés, 1837)) which was not reported till today from Hungary.

The family Penthaleidae currently contains four genera, namely *Penthaleus* Dugès, 1834, *Linopenthaleus* Willmann, 1951, *Linopenthaloides* Strandtmann, 1981 and *Turanopenthalodes* Barilo, 1988 (Khaustov, 2016). Only one genus from the latter mentioned ones has wider distribution, species of the genus *Penthaleus* are recorded from Europe, Asia, Australia, North- and South-America. The distribution areas of the other three genera are smaller, like *Linopenthaleus* species occurs in Austria and Switzerland, *Linopenthaloides* in New Zealand, and *Turanopenthalodes* in Uzbekistan (Willmann, 1951; Strandtmann, 1981; Khaustov, 2016). The genus *Penthaleus* contains four species: the Australian *P. falcatus* Qin and Halliday, 1996 and *P. tectus* Halliday, 2005 and the *P. minor* Canestrini, 1886 which occurring in Europe and Australia. The fourth species,

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the blue oat mite or winter grain mite [*P. major* (Dugès, 1837)] is an economically important agricultural pest. The blue oat mite probably originates from Europe, from where this species was introduced to North-America (Narayan, 1962), to South-Africa (Womersley, 1933), to Australia (Qin and Halliday, 1995) and to China (Ren et al., 2008) as well. In Europe, beyond the original type locality (France), the blue oat mite was mentioned from the Netherlands, Germany, England (Narayan, 1962), from Norway (Johansen and Haug, 2002), and from Iceland (Gudleifsson and Ölafsson, 1987). Only two South-American records are published, the first one from Argentina (Grasso, 1958) and the second one from Brazil (Pereira et al., 2017). During these studies the blue oat mite was mentioned in the following names: *Tetranychus majeur* (Dugès, 1834), *Rhynocolophus major* (Dug.) (Murray, 1877), *Notophallus bicolor* (Froggatt) (Womersley, 1933) and *Penthaleus major* (Dugès) (Thor, 1930; André, 1932; Womersley, 1935, 1941; Thor and Willmann, 1941; Grasso, 1958; Meyer and Ryke, 1960; Narayan, 1962; Strandtmann, 1964; Meyer, 1981; Qin and Halliday, 1995, 1996; Ren et al., 2008; Pereira et al., 2017). Some re-description (Narayan, 1962; Qin and Halliday 1996; Pereira et al., 2017) were added, but some characters were observed in different shapes as well. The Hungarian specimens of blue oat mite (*Penthaleus cf. major* (Dugès, 1837)) differ in several characters from the previously described ones. Therefore we give a description of the Hungarian specimens accompanied with three different Genebank sequences.

Materials and Methods

Collecting methods

The mite specimens were collected from the surface of lettuce (*Lactucea sativa* L.) by brush and placed into vials with 96% ethanol.

Methods of morphological studies

The collected mites were cleared in lactic-acid and mounted in Hoyer's medium on slides. The investigated specimens were studied under light microscope (Leica DM1000). The drawings were made by using camera lucida of Leica DM1000. Some specimens were spatter coated by gold-palladium and studied with HITACHI SN 2600 scanning electron microscope in the Hungarian Natural History Museum, Budapest. Other photos were taken with Keyence VHX5000 digital microscope. The investigated specimens are deposited in the Department of Zoology, Plant Protection Institute, Centre for Agricultural Researches of Hungarian Academy of Sciences and the Soil Zoology Collection of the Hungarian Natural History Museum. The terminology follows Baker (1995).

DNA extraction, PCR amplification, cloning and sequencing

Total genomic DNA was extracted from each individual using the REDExtract-N-AmplTM Seed PCR Kit (Sigma) following the manufacturer instructions: the

bodies were covered by 15 μl of extraction solution and incubated at 55 ºC for 10 minutes, and 3 minutes at 95 ºC. Then, the same volume (15 μl) of neutralization solution was added to the sample and mixed by vortexing. One microliter from this mixture was used as DNA matrix in the PCR. Amplification was performed in 25 - 50 μl volume containing the PCR buffer (10 mM Tris-HCl, pH 9.5, 2.5 mM $MgCl_2$, 50 mM KCl, 0.1 % Triton X100), 100 ng each of dATP, dCTP, dGTP and dTTP, 0.1 nM of each sense and antisense primers and 5 U Taq polymerase (Invitrogen). The used primer sequences for the 18sRNA were the follows: 5'-GCAGTCTGGTGCCAGCAGCC-3' and 5-CTTCCGTCAATTCCTTTAAG-3'., for cox 1 gene: 5'-ttgatttttggdcayccwgaagt-3' and 5-ccwvytardcctarraartgttg-3'), for the ITS2: 5'-atatgcttaaattcagcggg-3' and 5'-gggtcgatgaagaacgcagc-3'.

Thirty-five PCR cycles were performed (Eppendorf Mastercycler gradient) with the following parameters: initial denaturation at 95 ºC for 4 min; denaturation at 94 ºC for 60 s, hybridization at 51 °C for 60 s and elongation at 70 ºC for 90 s; final extension at 70 ºC for 7 min. PCR products were observed in a BioDoc-ItTM system (UV Transilluminator, UVP, USA) after electrophoresis in a 1 % agarose gel. PCR Products were purified using Silica Bead DNA Gel Extraction Kit (Fermentas) or High Pure PCR Product Purification Kit (Roche) and subsequently cloned into CloneJet (Fermentas) or pGEM-T Easy Vector (Promega, Madison USA) according to manufacturer instructions. The recombinant plasmids were transformed into *Escherichia coli* DH5α (Sambrook et al., 1989). All clones containing the expected inserts were sequenced by BAYGEN (Szeged).

The obtained sequences were analyzed with MEGA 7.0 program with maximum likelihood methods, Juke-Cantor (18sRNA) and Tamura-Nei (cox 1) models (using by the MEGA 7.0 model selection), and 1000 bootstraps.

Results

Penthaleus cf. major (Dugès, 1837)

Material examined. 15 females. Hungary, Csongrád county, Forráskút, collected on lettuce (*Lactuca sativa* L.). February, 2017, Almási, K. coll.

Description of the Hungarian specimens. *Female* (Figs 1–4).

Idiosoma. 770–800 long, 540–550 wide. Color green in alcohol, body darker than legs (living specimens were not available). Surface covered by small oval globulars arranged in rows. *Dorsal idiosoma* (Fig. 1a). Naso 30–35 long and 70–76 wide. Internal vertical setae (*v1*) smooth and situated far from each other close to anterior margin of naso. Trichobothria (*sc1*) long (45–55), smooth and narrower than other dorsal setae. 14 setae (length: 40–45) situated on dorsal hysterosoma posterior to naso. Hysterosomal setae barbed (Fig. 1b and Fig. 4a) and situated scattered positions, and forming six longitudinal rows. Lyrifissures (*ia, im, ip*) oval, their positions illustrated in (Fig. 1a). Anal opening

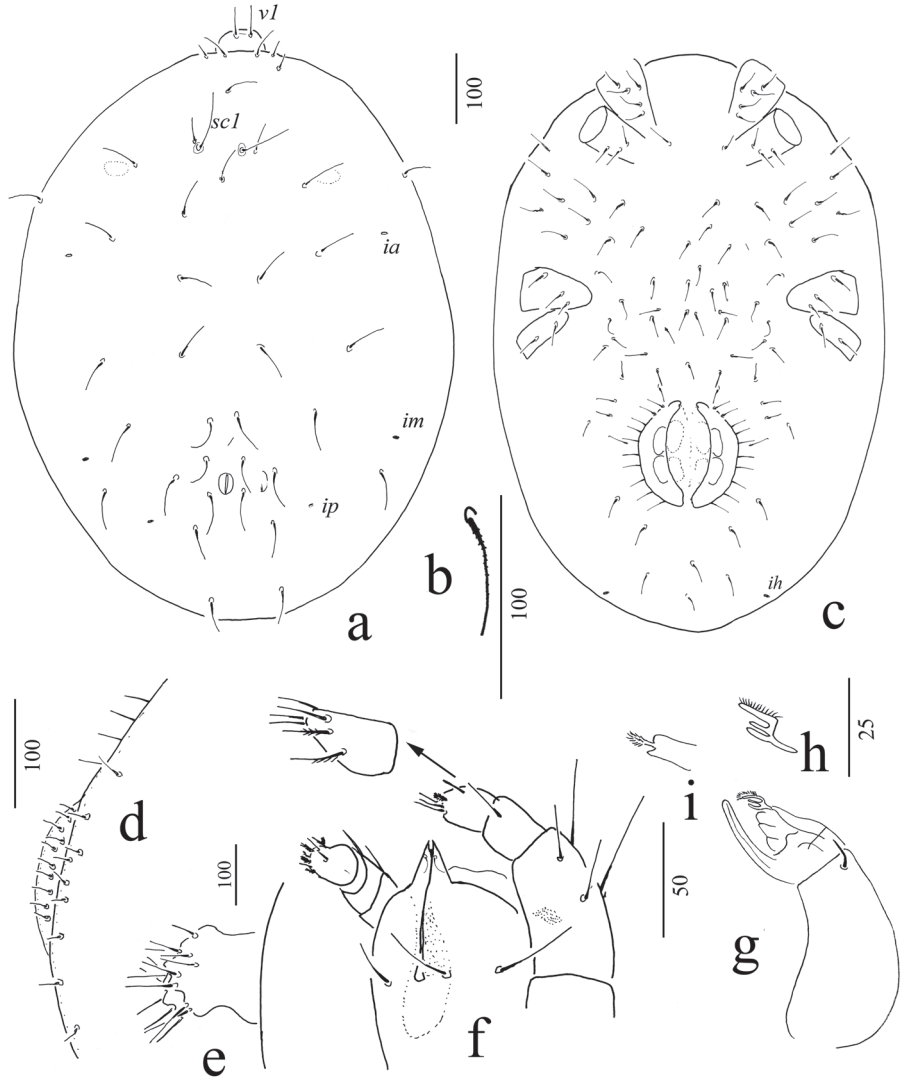

Fig. 1. *Penthaleus cf. major* (Dugès, 1837), from Hungary, female: a: dorsal view of idiosoma, b: hysterosomal seta, c: ventral view of idiosoma, d: lateral view of genital valves, e: eugenital setae, f: ventrolateral view of gnathosoma and palps, arrow shows palpal tarsus, g: lateral view of chelicera, h: fixed digit of chelicera, lateral view, i: fixed digit of chelicera, dorsal view

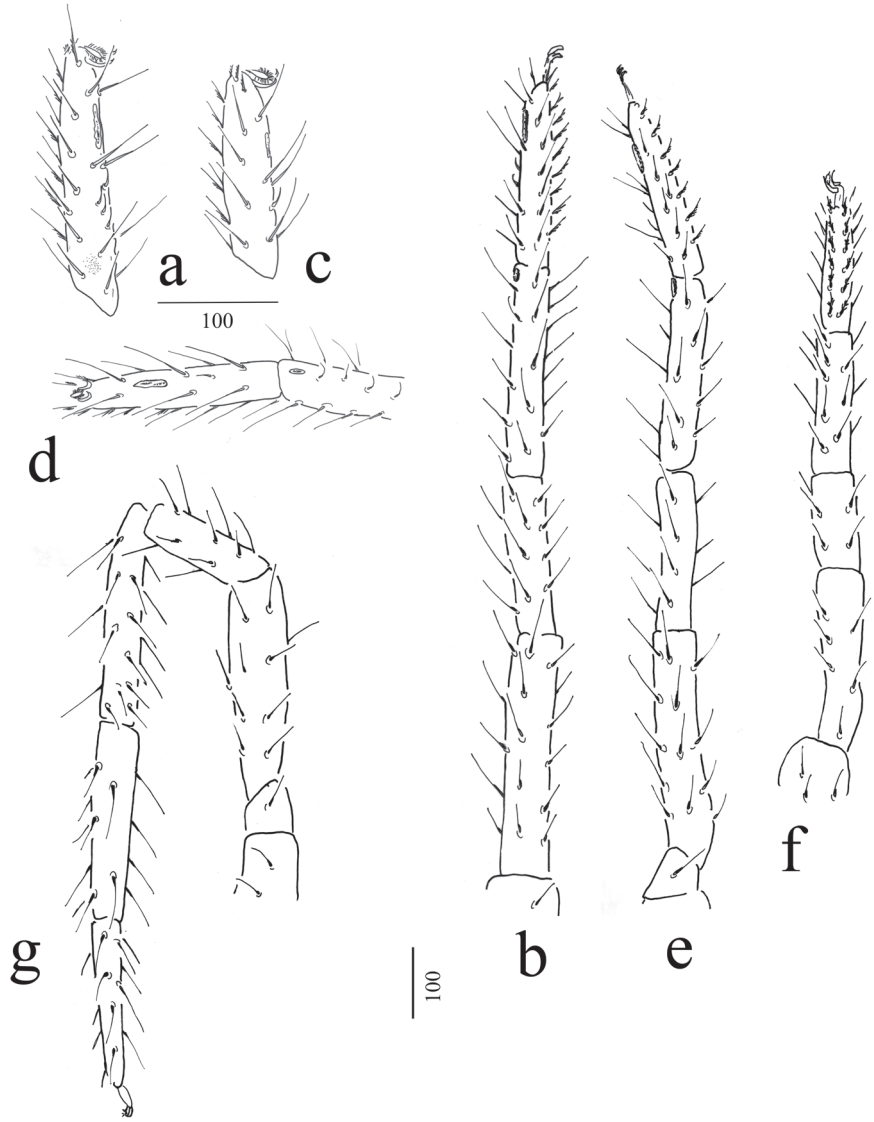

Fig. 2. *Penthaleus cf. major* (Dugès, 1837), from Hungary, female: a: laterodorsal view of tarsus I, b: laterodorsal view of leg I, c: laterodorsal view of tarsus II, d: dorsal view of tarsus and tibia of leg II, e: laterodorsal view of leg II, f: ventral view of leg III, g: laterodorsal view of leg IV

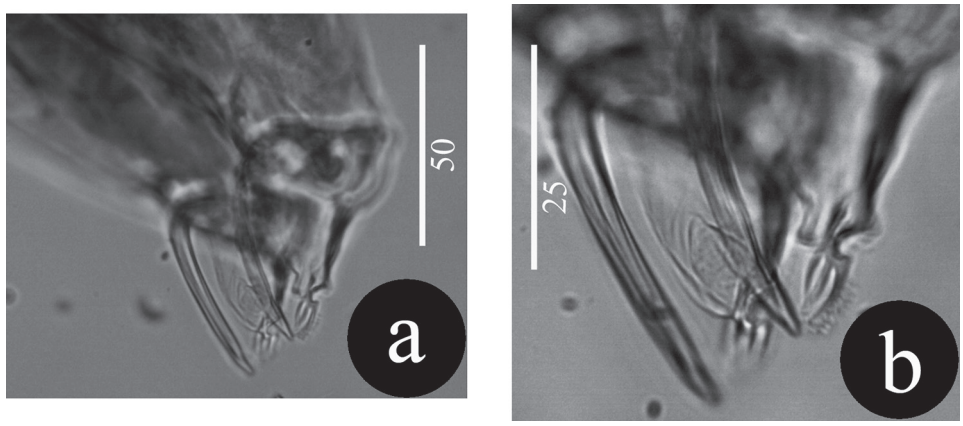

Fig. 3. Photos of chelicerae of *Penthaleus cf. major* (Dugès, 1837), from Hungary, female (a and b in different magnifications)

on dorsal side, tube-like and covered by anal valves (30–35 long, 12–14 width) (Fig. 4b). Three pairs curved pseudanal setae (length: 65–70) situated around anal opening.

Ventral idiosoma (Fig. 1c). Ventral idiosoma neotrichous, ventral setae smooth and 30–35 long. Coxae I with five, II-III with three, IV two smooth and needle-like setae. Posterior to coxae II more than 30 smooth and 30–35 long setae situated. Genital shields covered with 10 smooth and 18–25 long setae (Fig. 1d), eight pairs of eugenital setae smooth and need-like (74–78) (Fig. 1e). Lyrifissures (*ih*) situated close to caudal margin of ventral idiosoma.

Legs (Fig. 2a-g). Length of legs (with coxae): I 940–950, II 690–700, III 780–790, IV 930–940. All legs with smooth setae, only ventral surface of tarsi bearing ciliate setae. All tarsi with one pair of simple claws and a tongue-like empodium covered by numerous hair-like, long projections. Papillae present on legI and II. Rhagidial organs visible on tibia and tarsus of leg I and II, with 1-1 visible rhagidial solenidion. Tarsus I with four, tarsus II with one dorsal famulus.

All leg setae smooth except ventral setae on tarsi, which are ciliate. Setal number variable, trochanters I, II and IV with 1 seta, thochanter III with three setae; femur I with 21-24, II 15-17, III 13-15 and IV 12-14 setae; genu I with 16-17, II 10-12, III 10-11 and IV 10-12 setae; tibia I with 24-26, II 15-18, III 15-17 and IV 20-23 setae; tarsus I with 39-40, II 30-32, III 30-32 and IV 28-30.

Gnathosoma (Fig. 1f and Fig. 4c). One pair of adoral setae curved and smooth, (10–12 long). Subcapitular setae smooth, *sbc1* 30–35, *sbc2* 28–34 long. Palp 85–95 long, palp setal formula 0-4-3-9, two setae on palpal tarsus ciliate, other visible setae smooth. Chelicerae (Fig. 1g) 90–100 long and 40–45 wide, movable digit 50 long narrow, sword-like. Fixed digit trifurcated, upper branch bearing numerous hair-like projection (Fig. 1h-i and Fig. 3a-b). Cheliceral setae present.

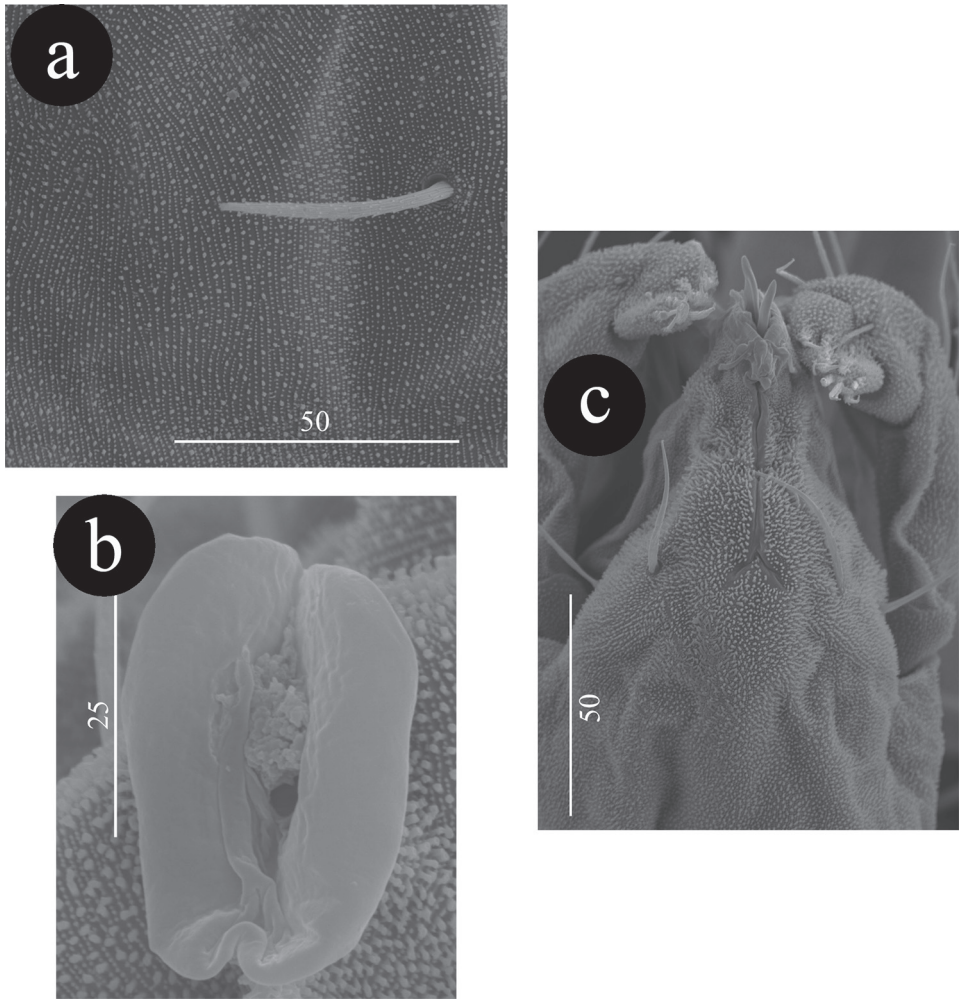

Fig. 4. Scanning micrographs of *Penthaleus cf. major* (Dugès, 1837), from Hungary, female: a: hysterosomal seta, b: anal opening and valves, c: ventral view of gnathosoma

Molecular studies. The 18s RNA sequences of the two Hungarian specimens of blue oat mite were identical (similarity 100%), and they had the highest similarity to the "*Penthaleus cf. major*" (GQ864271) from Poland and "*Penthaleus minor"* (AY620909) from USA while less similarity was found to "*Penthaleus* sp." (KU253785) from USA, WA (Table 1). Compared our cox 1 sequences with the by the previously uploaded sequences into the Genebank we did not find similarity with the Genebank *"Penthaleus*" cox 1 genes (GQ864391, GQ864389 from Poland and JX838308, JX836182, KM832689 from Canada) Higher similarity was found only with the penthalodid mites (i.e. three species of the genus *Stereotydeus* Berlese) (Table 2). The three investigated sequences (cox 1, 18s RNA, ITS2) are presented in (Table 3).

Table 1

Pairwise distances between the 18sRNA sequences (using by Juke-Cantor model in the Mega 7.0)

Penthaleus_ cf. major _Hungary2							
Penthaleus_ cf. major _Hungary1	0,000						
*Penthaleus*_sp._KU253785_USA_WA	0,069	0,069					
*Penthaleus_cf._major*_GQ864271_Poland	0,004	0,004	0,074				
*Penthaleus_minor*_AY620909_USA	0,004	0,004	0,074	0,000			
*Eupodidae*_sp._GQ864273_Poland	0,118	0,118	0,150	0,122	0,122		
*Linopodes_motatorius*_GQ864270_Poland	0,174	0,174	0,194	0,179	0,179	0,146	
*Linopodes*_sp._GQ864274_Poland	0,165	0,165	0,184	0,170	0,170	0,150	0,024

Table 2

Pairwise distances between the cox 1 sequences (using by Tamura-Nei model in the Mega 7.0)

Penthaleus_ cf. major _Hungary2						
Penthaleus_ cf. major _Hungary1	0,000					
*Stereotydeus_villosus*_DQ309578	0,127	0,127				
*Stereotydeus_shoupi*_DQ309575	0,139	0,139	0,141			
*Stereotydeus_belli*_DQ309577	0,153	0,153	0,124	0,135		
Rhagidiidae sp._AF142142	0,196	0,196	0,196	0,204	0,186	
*Eueremaeus_silvestris*_KF199393	0,206	0,206	0,202	0,218	0,194	0,222

Observed damage and behavior. Due to the damage of mites on the plants, silver and frosted spots were observed on the studied lettuces. The first damages were observed few weeks after planting and later all over the vegetation period. It was no differences between feeding of mites on young and old, or distressed plants. At the beginning, feeding damages were more frequent in the corners of the greenhouse close to the entrance due to the low humidity.

After the population build-up, blue oat mite spread quickly, they moved very fast on the plants and they were observed everywhere. If upper irrigation were used the mites tried to avoid the water drops and tried to search refuge among the leaves.

Discussion

The hereby observed Hungarian specimens of blue oat mite have an unusual character which was not mentioned in the detailed re-description of Qin and Halliday (1996). Smooth upper branch of trifurcated fixed digit of chelicerae was illustrated in Qin and Halliday (1996 on Fig. 26) and Pereira et al. (2017 on Fig. 6), contrary, the Hungarian specimens have numerous short hair-like projection on upper surface. Similar character state is visible in North-American (USA, Kansas) specimens (see Narayan, 1962 on

Table 3

Sequences of the investigated genes

Cox 1	specimen 1	GATTGGATATTTCTCATATTATCAGATTCTATTCTGGAAAAAAAGAACCATTCGCG- CATTAGGTATAATCTATGCTATAATATCAATTGGATTTTTAGGATTTATTGTATGACA- CATCATATGTTTACAGTAGGTATAGATATTGATACACGAGCTTATTTTACAGCCGCT- CAATAATTATTGCTATCCCAACAGGTATTAAAATTTTTAGATGAATAGCAACTATGT- CAGATCTCATATTTCAATAGATACTCCCATGTTATGAGCATTAGGATTCGT- TTTTTTATTTATGTAGGAGGATTAACTGGAGTAATTTTAGCTAATTCATCCATTGA- TATTATTTTACATACACATACTACGTGGTAGCACATTTCCATTACGTTCTTTCAATAG- GAGCAGTATTTGCTTTATAGCCGGTTTAACCCATTGAGTACCCATAATCTTCGAAAT- CAATATAAATCCAAAGACTAAAAATTCATTTTTTTCTAATATTTCTAGGAGTTAA- CATAACCTTTTTTCCA
	specimen 2	GATTGGATATTTCTCATATTATCAGATTCTATTCTGGAAAAAAAGAACCATTCGCG- CATTAGGTATAATCTATGCTATAATATCAATTGGATTTTTAGGATTTATTGATGAGCA- CATCATATGTTTACAGTAGGTATAGATATTGATACACGAGCTTATTTTCAGCCGCTA- CAATAATTATTGCTATCCCAACAGGTATTAAAATTTTTAGATGAAAGCAACTATGT- CAGGATCTCATATTTCAATAGATACTCCCATGTTATGAGCATTGGATTCGT- TTTTTTATTTACTGTAGGAGGATTAACTGGAGTAATTTTAGCTAATCATCCATTGA- TATTATTTTACATGACACATACTACGTGGTAGCACATTTCCATTAGTTCTTTCAATAG- GAGCAGTATTTGCTATTATAGCCGGTTTAACCCATTGAGTACCATAATCTTCGAAAT- CAATATAAATCCAAAATGACTAAAAATTCATTTTTTTCTATATTTCTAGGAGTTAA- CATAACCTTTTTTCCA
18s RNA	specimen 1	CTGGGCATTTTACCGAGCCGTCTCTTGATGCTCTTTACCGAGTGTCTTGAG- CGATCGGTACGTTTACTTTGAAAAATTAGAGTGCTCAAAGCAGG- CGACCCGCCCGAATAATCTTGCATGGAATAATGGAATAGACCTCGGTTCTAT- TTTGTTGGTCTTCGGAACCCGAGGTAATGATTAAGAGGGACAGACGGGGCATTCG- TATTGCGGCGCTAGAGGTGAAATTCTTGGACCGTCGCAAGACGAACTACG- CGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGGT- TCGAAGCGATCAGATACCGCCCTAGTTCTAACCATAAACGATGCCAACCAGCGAT- CAGCCTGAGTCTTAATATGACTCGGCTGGCAGCTTCCGGGAAACCAAAGTTTTTCG- GTTCCGGGGGAAGATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGA
	specimens 2	CTGGGCATTTTACCGAGCCGTCTCTTGATGCTCTTTACCGAGTGTCTTGAG- CGATCGGTACGTTTACTTTGAAAAATTAGAGTGCTCAAAGCAGG- CGACCCGCCCGAATAATCTTGCATGGAATAATGGAATAGACCTCGGTTCTAT- TTTGTTGGTCTTCGGAACCCGAGGTAATGATTAAGAGGGACAGACGGGGCATTCG- TATTGCGGCGCTAGAGGTGAAATTCTTGGACCGTCGCAAGACGAACTA- CACGAAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTAGAGGT- TCGAAGGGATCAGATACCGCCCTAGTTCTAACCATAAACGATGCCAACCAGCGAT- CAGCCTGAGTTTTAATATGACTCGGCTGGCAGCTTCCGGGAAACCAAAGTTTTTCG- GTTCCGGGGGAAGTTGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGA
ITS 2	specimen 1	ACCACTACCGAACGTGCATAGAACGCCGAGCACTTGATTTTCGAACGCACAT- GACGGCTACGGGGTTTCCCGTAGCCTTGTCTGTCTGAGGGTCGGATAATAAGAT- TAACTATAATGCAGGTCTGCATCGAGGGGACCGGAGCTGTCTCTCTGT- TTTTCGGCCTTAACCGGCAATAGCAAGAGTTGCCCCAGGACCTCTCGTCAGGCT- TTGGATTGCGGTTGCTTGCCTCCTGCCTGACGGTGTTTGCCCATGTCAGCACAGCT- GTTGCTGGTTCATGGTGTCGGACGTTGCCTGACGGTAGTAGAAGGGGACTTGT- GTGCAGCACTGCCCGTGGTCTCACTTGCCGTAAGGGTGCCTTCCGGACTTTCGG- GTCTGGTTGCGTACCTGGCAAGTTGTTGTACCAACCTATTGATCCGTGTTGTGTG- GTCTTGGTTGTAGCCGGTTAAGCGGACAACTAGGACCCTGAGCGGGGAGATCT- CATTATCCCATTTGTCGACCTCAGATCAGGCAAGATTACCCGCTGAATTAAGCATAT
	specimens 2	CAATAAATTGGCAGAGAACACGCCGGAGCACTTGATTTTCGAACGCACATT- GACGGCTACGGGGTTTCCCGTAGCCTTGTCTGTCTGAGGGTCGGATAATAAGAT- TAACTATATGCAGGTCTGCATCGAGGGGACCGGAGCTGTCTCTCTGT- TTTTCGGCCTTAACCGGCCATAGCAAGAGTTGCCCCAGGACCTCTCGTCAGGCT- TTGGATTGCGGTTGCTTGCCTCCCTCCTGACGGTGTTTGCCCATGTCAGCACAGCT- GTTGCTGGTTCATGGTGTCGGACGTTAGCTGACGGTAGTAGAAGGGGACTTGT- GTGCAGCACTGCCCGTGGTCTCACTTGCCGTTAGGGTGCCTTCCGGACTTTCGG- GTCTGGTTGCGTACCTGGCAAGTTGTTGTACCAACCTGATGATCCGTGTTGTGTG- GTCTTGGTTGTAGCCGGTTAAGCGGACAACTAGGACCCTGAGCGGGGAGATCT- CATTATCCCATTTGTCGACCTCAGATCAGGCAAGATTACCCGCTATTTAAGCATAT

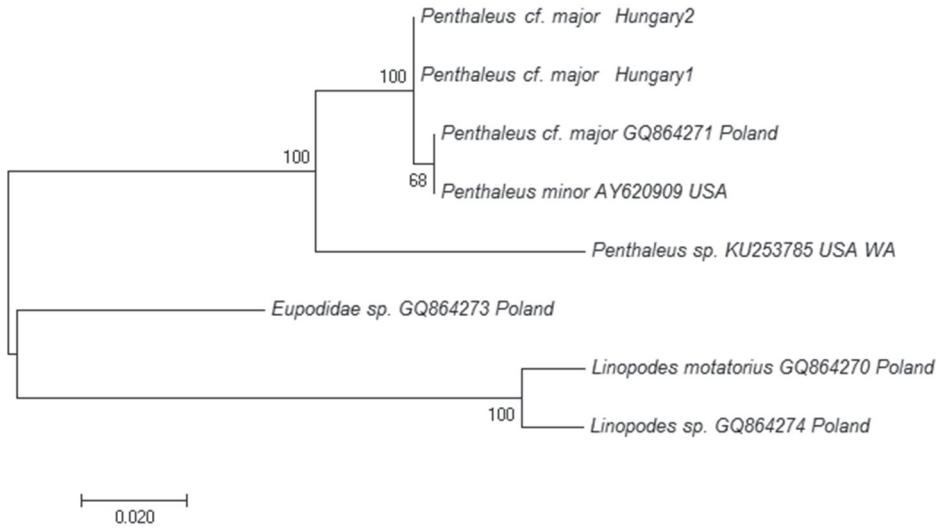

Fig. 5. Phylogenetic tree with the Hungarian specimens of *Penthaleus cf. major* (Dugès, 1837), based on 18sRNA gene

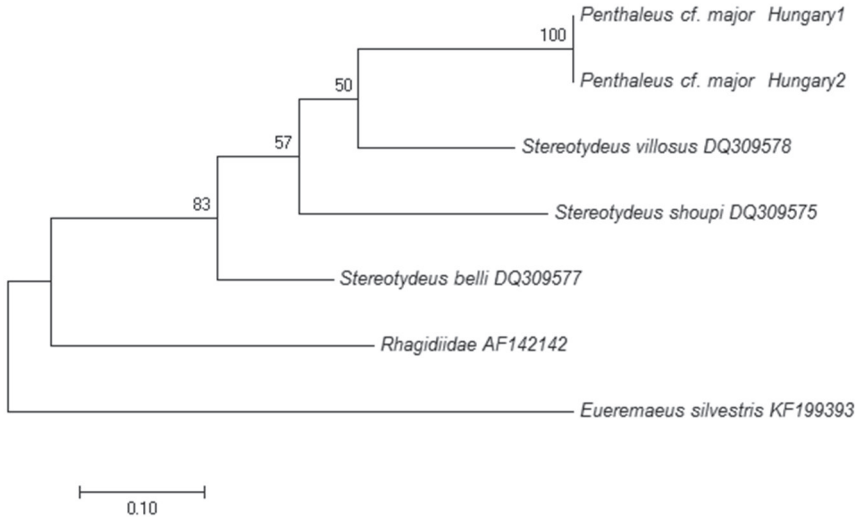

Fig. 6. Phylogenetic tree with the Hungarian specimens of *Penthaleus cf. major* (Dugès, 1837), based on cox 1 gene

Fig. 3, Walter, 2006) and specimens from France (André, 1932 on Fig. 8). No other information is available about this character state of the previously presented blue oat mite specimens, this difference can be explained by the existence of two morphovarieties having distinct geographical distributions. Specimens with hair-like process on fixed digit are presented till today only from the Northern Hemisphere while specimens with smooth fixed digit were mentioned on Southern Hemisphere.

Other difference between the Hungarian and other specimens is the shape of the hysterosomal setae. The Hungarian specimens bear barbed setae, contrary the previously published ones, where smooth setae are illustrated (see André, 1932; Narayan, 1962; Qin and Halliday, 1996). There are also differences in the number of the dorsal setae in different author's works. Narayan (1962 on Fig. 3) and André (1932 on Fig. 1) presented dorsal setae in larger number than what we found in the Hungarian specimens or what was found in the Australian specimens Qin and Halliday (1996 on Fig. 21).

Concerning our short analyses using by maximum likelihood methods and Juke-Cantor model for 18sRNA sequence, the investigated *Penthaleus* species clustered separately from Eupodidae/*Linopodes* spp. line (Fig. 5). This finding confirms that Penthaleidae is a separated family from Eupodidae (earlier the *Penthaleus* species were placed into the family Eupodidae, like: Wallace and Mahon, 1971). Within the Penthaleidae clad, the Hungarian specimens differ from the others. The unidentified "*Penthaleus* sp." (KU253785), from USA, WA can be an undescribed genus or species. The other two ("*Penthaleus cf. major*" (GQ864271) from Poland and "*Penthaleus minor"* (AY620909) from USA) species situated closer to the Hungarian specimens, but the Polish "*Penthaleus cf. major*" (GQ864271) seems to be more similar to the "*Penthaleus minor"* (AY620909), than to the Hungarian specimens. Regarding of the cox 1 gene (Fig. 6), the Hungarian *Penthaleus* specimens show higher similarities with the penthalodid *Stereotydeus* species than the members of family Eupodidae. We need to suppose, beside the morphological similarities, that the families Penthaleidae and Penthalodidae are close related with each other, and their sister group can be the family Eupodidae.

The Hungarian specimens of blue oat mite originated from greenhouse from lettuce. Currently there is no information about the origin of these specimens, maybe they were introduced to Hungary by soil or plant transportation or native Hungarian populations colonized the greenhouse from the neighboring habitats. Therefore, in the future, we need to confirm the occurrence of this species in natural, seminatural or agricultural habitats in Hungary.

On the basis of the Hungarian specimens and the earlier published illustrations and descriptions about the species, we need to suppose that the specimens published under the name of *Penthaleus major* belong to more than one different species.

Acknowledgements

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