Cent. Eur. J. Biol. • 7(1) • 2012 • 63-68
DOI: 10.2478/s11535-011-0096-7

Central European Journal of **Biology**

New associations of phoretic mites on *Pityokteines curvidens* (Coleoptera, Curculionidae, Scolytinae)

Research Article

Milan Pernek[1,*], Stefan Wirth[2], Stacy R. Blomquist[3], Dimitrios N. Avtzis[4], John C. Moser[5]

[1]*Croatian Forest Research Institute, 10450 Jastrebarsko, Croatia*

[2]*Museum für Naturkunde, Leibniz Institute for Research on Evolution and Biodiversity, Humboldt University Berlin, 10115 Berlin, Germany*

[3]*USDA Forest Service, Southern Research Station, 71360 Pineville, Louisiana, USA*

[4]*NAGREF-Forest Research Institute, 57006 Vassilika, Greece*

[5]*USDA Forest Service, Southern Research Station, 71360 Pineville, Louisiana, USA*

Received 01 July 2011; Accepted 23 November 2011

Abstract: The species composition and abundance of phoretic mites of the bark beetle *Pityokteines curvidens* caught in pheromone traps were investigated in Croatia. The *P. curvidens* trapping programs have been in an experimental phase in Croatia since 2004 as a possible monitoring and control system. The trapping program also permits the opportunity to sample phoretic mites found associated with the beetles. Beetles were caught using Curviwit pheromones in Theysohn traps placed in the Litorić region of Croatia. A total of 12 mite species were recovered, including *Schizostethus simulatrix*, *Dendrolaelaps quadrisetus*, *Histiostoma piceae*, *H.* cf. *varia*, *Paraleius leontonychus*, *Pleuronectocelaeno barbara, Tarsonemus minimax, Trichouropoda lamellosa, Uroobovella ipidis, Schwiebea* sp., *Phauloppia lucorum* and *Dolicheremaeus dorni*. Five species, *Pl. barbara, Schwiebea* sp., *H.* cf. *varia*, *Ph. lucorum* and *Do. dorni*, are identified for the first time in association with *P. curvidens*. These findings increase the number of mite species known to be phoretic on *P. curvidens* from 11 to 16. The present study also increases the number of known mite associates of *Pityokteines* spp. from 14 to 18.

Keywords: *Acarina • Scolytinae • Phoresy • Pheromone trap • Silver fir • Croatia*

1. Introduction

The fir bark beetle (FBB), *Pityokteines curvidens* (Germar) (Coleoptera, Curculionidae, Scolytinae), is a native pest of Silver fir (*Abies alba* Mill.) and occasionally other conifers [1,2]. It occurs in the Palaearctic region [2,3]. *Pityokteines curvidens* breeds in the lower trunk of Silver fir trees [4]. Overwintering adult beetles become active in early spring when they search for stressed or weakened Silver fir trees. Brood adults emerge in late spring or early summer after they have fed on phloem and have enough stored fat to search for new host trees. The next generation overwinters in the trees' phloem. The females and larvae make specific galleries within the phloem, which disrupts the water supply within the host tree. Together with the other two *Pityokteines* species native to Croatia (*P. spinidens* (Reitter) and *P. vorontzowi* (Jakobson)), these beetles have been reported as important factors in the decline of Silver fir since the beginning of 2000 [4-6]. This decline is observed in other European regions, and is caused by a variety of abiotic and biotic factors [1]. Only a few studies concerning FBB have been published recently; these addressed problems connected with the biology and ecology of these forest pests [4,6-10].

Bark beetles are known to be associated with diverse guilds of arthropods and microorganisms, of which phoretic mites and ophiostomatoid fungi are among the

* *E-mail: milanp@sumins.hr*

best-known [11-18]. Phoretic mites use bark beetles for transportation to new, suitable habitats [19]. It is of particular practical importance that some phoretic mites act as predators and parasitoids of the bark beetles' immature stages, especially egg and early larval stages. They are thus potential agents for the biological control of scolytine forest pests [11]. The first step in the search for mites that act as natural enemies of FBB is to compile a list of the associated mite species.

Phoretic mites of bark beetles are involved in complex symbiotic interactions with bark beetles and fungi, including ophiostomatoid species and their host trees [14,18,20]. Interactions range from mutualism, commensalism and antagonism between the various partners. The complex relationships between bark beetles, mites and fungi are best known for the southern pine beetle, *Dendroctonus frontalis* (Zimmermann) and its mite and fungal associates [13-15,20-23]. Similar interactions may also occur between other bark beetle species and their associates. Six ophistomatoid fungi found in *Pityokteines* galleries have been recently reported in Croatia and described as *P. spinidens* associations [9]. These fungi could also act as hyperphoretic associates on mites.

The increased importance of *Pityokteines* species as forest pests of *A. alba* in Croatia prompted research on the biology of these bark beetles species and on their mite and fungal associates. The species spectrum and abundance of phoretic mites associated with FBB caught in pheromone traps were studied in natural fir stands in Croatia, where eight species were obtained prior to this study [4].

The purpose of this paper is to offer new records of phoresy by mites on bark beetles, which may form the basis for further studies on the unknown interactions between FBB and their phoretic mites.

2. Experimental Procedures

On March 30, 2006, three Theysohn® traps (Trap 1-3) were established in mixed Silver fir and Beech forests (*Abieti-Fagetum*) at a locality in Litorić (550 m a.s.l., 45°27' N and 15°04´ E). The traps were placed ca. 15 m outside the natural Silver fir forests and were baited with the pheromone lure Curviwit® (Witasek, Feldkirchen, Austria), which is composed of methylbutenol and ipsenol ((S)-2-methyl-6-methyleneoct-7-en-4-ol). The three traps were spaced 20 m apart and suspended on wood beams 1 m above the ground.

FBB beetles caught in the pheromone traps were collected on April 8th, 15th and 20th 2006. The collected beetles were placed into plastic cups with 70% ethanol. Beetles were counted in the laboratory on the same day as collection. A sample of bark beetle individuals were randomly chosen and placed in vials with 70% ethanol for later mite inspection (Table 1). Beetle samples for mite inspection were transferred to lactophenol for clearing. Each beetle was then individually examined and any accompanying mites were counted and removed from the beetle's body. Mites were tallied separately according to whether they were: i) still attached to the beetle; ii) separated from the beetles in alcohol; or iii) separated from the beetles in lactophenol. Voucher specimens (slides) of all mite species detected in this study are stored in the collections of the author (M. Pernek).

3. Results and Discussion

Most beetle individuals in pheromone traps at the locality were caught on April 20, while the two previous collection dates of April 8 and 15 showed no or minimal catches of FBB. A total of 233 individual FBBs were collected, comprising 142 males (61%) and 91 females (39%) (Table 1). A sample of 60 bark beetle individuals (34 males and 26 females) were examined for phoretic mites, of which 58% possessed mites (Table 1). These values considerably underestimate the number of individuals actually carrying phoretic mites. This is suggested by the large number of mites that fell off the beetles, as indicated by their presence in the alcohol

Theysohn Trap	Number of males caught (number of beetles examined for mites)	Number of females caught (number of beetles examined for mites)	Total
Trap 1	44 (10)	35 (8)	79
Trap 2	36 (10)	17 (7)	53
Trap 3	62 (14)	39 (11)	101
Total number of beetles in traps	142 (34)	91 (26)	233 (60)
Number (percentage) of examined beetles possessing mites	24 (70%)	11 (42%)	35 (58%)

Table 1. Number of *Pityokteines curvidens* caught in pheromone traps and examined for mites.

and lactophenol sediments (Table 2). In total, 98 phoretic mites were extracted from 60 FBB adults (Table 2). The mites were located under the elytra (59%) and on the elytral declivity (20%), dorsal thorax (14%) and legs (7%). Of the 59 mites found in alcohol and lactophenol sediments, 90% were the same species as those attached to the beetles. The remaining 10% belonged to species represented by 1 to 3 specimens, and may be considered "accidentally" phoretic. These species were never found directly attached to the beetle's body, but rather were recovered from the sediments.

A total of 12 mite species were documented. Seven of these species were previously known to be phoretic: *Dendrolaelaps quadrisetus* (Berlese), *Histiostoma piceae* (Scheucher), *Paraleius leontonychus* (Berlese), *Tarsonemus minimax* (Vitzthum), *Trichouropoda lamellosa* (Hirschmann), *Uroobovella ipidis* (Vitzthum), *Schizostethus simulatrix* (Athias–Henriot). Five represented new associates: *Histiostoma* cf. *varia* (Figure 1a), *Schwiebea* sp. (Figure 1b), *Pleuronectocelaeno barbara* (Athias-Henriot) (Figure 1c and 1d), *Phauloppia lucorum* (C Koch) (Figure 1e) and *Dolicheremaeus dorni* (Balogh) (Figure 1f). Mites were either phoretic on *P. curvidens* as females (*Pl. barbara*, *T. minimax, Pa. leontonychus*), males (*Pl. barbara*, *Pa. leontonychus*) or as deutonymphs (*D. quadrisetus*, *T. lamellosa*, *U. ipidis*, *H. piceae, Histiostoma* cf. *varia*, *Schwiebea* sp., *S. simulatrix*) (Table 2). The most frequently observed mite species, representing 34.4% of all specimens, was *Pa. leontonychus*. *Dendrolaelaps quadrisetus* (20.4%), *U. ipidis* (14.6%), *T. minimax* (8.9%), and *Schwiebea* sp. (5.7%) were common, whereas the other six species were rare.

Mite species and phoretic stage	Location on FBB	On male	On female	Total number of phoretic mites on FBB	Number of mites in alcohol sediments	Number of mites in lactophenol sediments	Total number of mites	Percent of total mites found (n=157)
Uroobovella ipidis Deutonymph	Elytral declivity	3		22		1	23	14.6
	Leg		5					
	Dorsal thorax	1	13					
Paraleius leontonychus Female	Elytral declivity	6	7	20	3	31	54	34.4
	Under elytra	7						
Paraleius leontonychus Male	Leg	1	1	2			2	1.3
Tarsonemus minimax Female	Under elytra	8		8		6	14	8.9
Schwiebea sp. Deutonymph	Elytral declivity		1	2		7	9	5.7
	Under elytra	1						
Histiostoma piceae Deutonymph	Under elytra	2	3	5			5	3.2
Histiostoma cf. *varia* Deutonymph	Under elytra		3	3		4	7	4.5
Dendrolaelaps quadrisetus Deutonymph	Under elytra	20	10	30		2	32	20.4
Trichouropoda lamellosa Deutonymph	Elytral declivity	3		4			4	2.5
	Dorsal thorax		1					
Pleuronectocelaeno barbara Female	Under elytra	1		1			1	0.6
Pleuronectocelaeno barbara Male	Unknown				2		2	1.3
Schizostethus simulatrix Deutonymph	Elytral declivity	1		1			1	0.6
Phauloppia lucorum	Unknown				1		1	0.6
Dolicheremaeus dorni	Unknown				2		2	1.3
	Total	54	44	98	8	51	157	100.0

Table 2. Species spectrum and abundance of mites phoretic on 60 individuals of *Pityokteines curvidens* caught in pheromone traps.

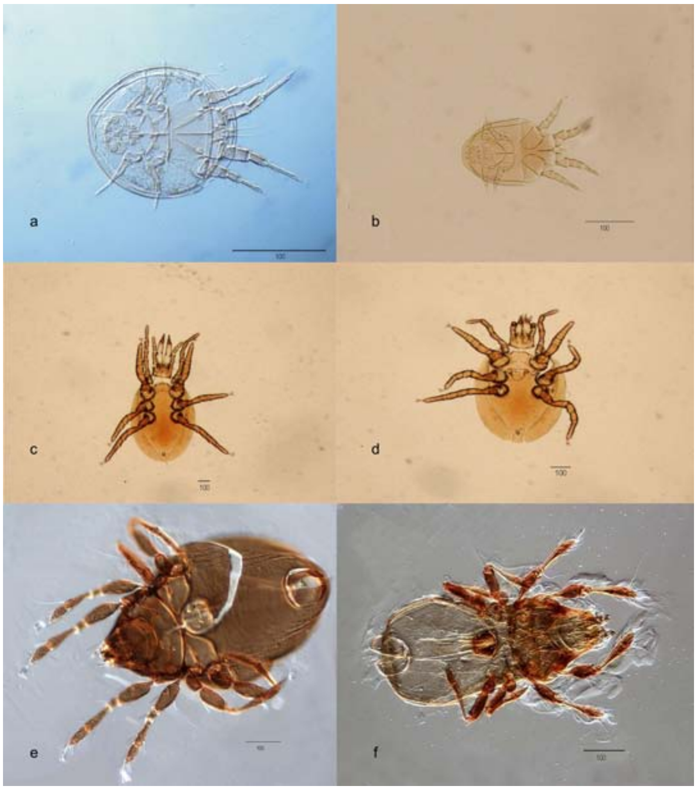

Figure 1. New phoretic mite associates found on *Pityokteines curvidens*: a) *Histiostoma* cf. *varia* b) *Schwiebea* sp. c) *Pleuronectocelaeno babara* male d) *Pleuronectocelaeno babara female* e) *Dolicheremaeus dorni* f) *Phauloppia lucorum*.

A combined total of 14 mite species have been detected as phoretic on FBB based on the results of this study and a previous study [4]. Eight of these species were reported earlier [4], but three species, *Nanacarus* sp., *Bonomia* sp. and *Pyemotes dryas*, have yet to be documented in Croatia [4,24]. Five new associates of *P. curvidens* found in this Croatian study include: *H.* cf. *varia*, *Schwiebea sp.*, *Pl. babara*, *Ph. lucorum*, *D. dorni.* These findings increase the total number of mite species phoretic on *P. curvidens* from 11 [4] to 16. Two of the eight mite species previously found from Croatia (*Pl. japonica* and *S. simulatrix*) did not occur in this study. The records in the present study also increase the number of known mite associates of *Pityokteines* spp. from 14 previously documented species [4] to 18.

The ecological roles of most of the phoretic mites associated with FBB are poorly known. Based on the knowledge of other bark beetle–mite–fungal systems and previous records of the biology of these mites in the scientific literature, we assume that the FBB biology and ecology are diverse [14,15,25]. All active instars of these mites feed on a wide variety of material, including living and dead plants and fungi, moss, lichens and carrion; many species are intermediate hosts of tapeworms, some species are predaceous, none are parasitic [26-28]. The Histiostomatidae usually filter-feed on bacteria [29]. According to O´Conner [30], *Schwiebea* sp. might be a typical mycetobiont and exploit and require fungi directly as food, while *Pl. barbara* may feed on nematodes [11].

Although *Pl. barbara* was found in very low numbers in this particular study – indicating that it may be “accidentally” phoretic – this mite has been documented to be phoretic on bark beetles belonging to the genera *Ips* and *Dendroctonus* [31].

There is little information available about phoresy in oribatid mites [26]. Oribatids are commonly known to disperse on their own or using wind gusts. Norton summarized the known records of possible phoretic oribatids [26], mentioning oribatid mites from specimens of 31 insect species. Norton describes *Mesoplophora’s* specific mechanism of attaching to a carrier arthropod: clasping insects’ hairs between the mite- rostrum of the aspis and the anterior portion of the genital plates. Specific morphological adaptations for phoretic transport are unknown for all other possibly phoretic oribatids [26]. This also concerns *Pa. leontonychus*, which was recorded in low abundance in Norton’s study [26]. In this study *Pa. leontonychus* was the most abundant species of all observed mites. Due to this high abundance, and its common association (in lower abundance) with different bark beetles, such as *Dendroctonus frontalis*, *Ips typographus* and *Pityokteines curvidens* [4,26], we conclude that this mite could be a regular phoretic associate of bark beetles. We exclude the possibility of an accidental appearance in our samples because specimens of *Pa. leontonychus* were found in both bark beetle galleries and attached to bark beetles in the pheromone traps. So far, details of the mechanisms by which they attach to the carriers are unknown; presumably the chelicerae are involved. The “phoretic” oribatids *Ph. lucorum* and *D. dorni* newly found in this study were rare and are likely be a result of accidental dispersal [26]. *Tarsonemus minimax* was the most frequent mite species in the study of Pernek *et al*. [4], whereas in the present study *T. minimax* was found to be less frequent. A large percentage (42%) of the individuals of *T. minimax* were found in lactophenol sediments, suggesting that this particular mite species is easily dislodged. This may explain why *T. minimax* was less abundant in previous studies, because beetles were collected by different methods (they were placed directly in vials as they emerged from stem sections that had been incubated in rearing cages [4]). Beetles in the present study were collected in pheromone traps which remained in the field a few days before being brought to the laboratory. Once in the laboratory, the beetles were transferred from the trap’s plastic cups to vials filled with alcohol. In both trapping methods, *T. minimax* mites could easily be dislodged from the beetles.

Other mite species occurred in abundances similar to those noted in previous studies. *Uroobovella ipidis* and *D. quadrisetus* were more commonly found on adult beetles than in the sediments, indicating these mite species were less easily dislodged.

The abundance of *Pa. leontonychus* on FBB raises questions concerning the large numbers of this mite. In a previous study [4], *Pa. leontonychus* was found in alcohol and lactophenol sediments; however, its abundance was rather rare in all three bark beetle species studied (*P. curvidens*, *P. spinidens*, *P. vorontzowi*).

Two species of the genus *Histiostoma* were discovered. The deutonymphs of *H.* cf. *varia* differ from those of *H. piceae* in the pattern of their ventral apodemes and the shape of their dorsal setae. Specimens of adult *Histiostoma* would be needed for a decisive taxonomic determination. The exact habitats in the bark for development of the *Histiostoma* species found in this study are still unknown.

The role of mites in transmitting vascular wilt disease could be more important than presumed. Moser *et al*. [18] showed that spreading of Dutch elm disease may be partly due to the association of bark beetles with a species of *Tarsonemus* and the hyperphoretic spores of *Ophiostoma novo-ulmi* (Ascomycota, Ophiostomatales). Further research may show that mites may also contribute to the transmission of Silver fir decline.

Acknowledgements

This research was supported by an USDA Forest Service Federal Financial Assistance Award (SRC 06-IC-11330129-038) and the Ministry of Science, Education and Sports of the Republic of Croatia. We thank Željko Kauzlarić, Matija Pleše and Mandica Dasović (Croatian Forests Ltd., Croatia) for their assistance in beetle sampling, Blaženka Ercegovac and Zoran Biljan (Croatian Forest Research Institute) for technical support and to Roy A. Norton for the identification of oribatid mites. Many thanks also to Walter Sudhaus for general biological discussions.

References

[1] Schwerdtfeger F., [Waldkrankheiten], 4th ed., Paul Parey Verlag, Hamburg, Berlin, 1981 (in German)

[2] Pfeffer A., [Zentral- und westpaläarktische Borkenund Kernkäfer, Pro Entomologia], Naturhistorisches Museum, Basel, 1995 (in German)

[3] Knizek M., A new species of Pityokteines (Coleoptera: Scolytidae) from Turkey, Klapalekiana, 1998, 34, 189-193

[4] Pernek M., Hrasovec B., Matosevic D., Pilas I., Kirisits T., Moser, J.C., Phoretic mites of three bark beetles (Pityokteines spp.) on Silver fir, J. Pest. Sci., 2008, 81, 35-42

[5] Hrasovec B., Pernek M., Matosevic D., Spruce, Fir and Pine Bark Beetle Outbreak Development and Gypsy Moth Situation in Croatia in 2007,Forstschutz Aktuell, 2008, 44, 12-13

[6] Pernek M., Matosevic D., Hrasovec B., Kucanic M., Wegensteiner R., Occurrence of pathogens in outbreak populations of Pityokteines spp. (Coleoptera, Curculionidae, Scolytinae) in Silver fir forests, J. Pest. Sci., 2009, 82, 343-349

[7] Urban J., Diagnostics of bark beetles of the genus Pityokteines Fuchs important in forestry, J. For. Science, 2002, 48, 329–341

[8] Pernek M., Avtzis D.N., Hrasovec B., Diminic D., Wegensteiner R., Stauffer C., et al., Novel morphological and genetic markers for the discrimination of three European Pityokteines

(Coleoptera: Curculionidae: Scolytinae) species, Periodicum biol., 2008, 110, 329-334

[9] Kirisits T., Diminic D., Hrasovec B., Pernek M., Baric B., First reports of Silver fir blue staining Ophiostomatoid fungi associated with Pityokteines spinidens, Croat. J. For. Eng., 2009, 30, 51-57

[10] Podlaski R., Borkowski A., Estimating stem infestation density of Pityokteines curvidens (Germ.) on windfalls: a statistical approach, J. Pest. Sci., 2009, 82, 357-365

[11] Kinn D.N., Mites as biocontrol agents of bark and sawyer beetles, In: Hoy M.A., Cunningham G.L., Knutson L. (Eds.), Proceedings of Conference (5-7 April 1982, Berkeley, USA), University of California, Special Publication Berkeley, 1983

[12] Kirschner R., [Diversität mit Borkenkäfern assoziierter filamentöser Mikropilze], PhD thesis, Eberhard-Karls-Universität, Tübingen, 1998 (in German)

[13] Moser J.C., Macias-Samano J.E., Tarsonemid mite associates of Dendroctonus frontalis (Coleoptera; Scolytidae): implications for the historical biogeography of D. frontalis, Can. Entomol., 2000, 132, 765-771

[14] Klepzig K.D., Moser J.C., Lombardero M.J., Ayres M.P., Hofstetter R.W., Walkinshaw C.J., Mutualism and antagonism, Ecological interactions among bark beetles, mites and fungi, In: Jeger M.J.,Spence N.J. (Eds.), Biotic Interactions in Plant-Pathogen Associations, CAB International, New York, 2001

[15] Lombardero M.J., Matthew P.A., Hofstetter R.W., Moser J.C., Klepzig K.D., Strong indirect interactions of Tarsonemus mites (Acarina: Tarsonemidae) and Dendroctonus frontalis (Coleoptera: Scolytidae), Oikos, 2003, 102, 243-252

[16] Kirisits T., Fungal associates of European bark beetles with special emphasis on the ophiostomatoid fungi, In: Lieutier F., Day K.R., Battisti A., Grégoire J.C., Evans H. (Eds.), Bark and wood boring insects in living trees in Europe, Kluwer Academic Publishers, Dordrecht, Boston, London, 2004

[17] Moser J.C., Konrad H., Kirisits T., Carta L.K., Phoretic mites and nematode associates of Scolytus multistriatus and Scolytus pygmaeus (Coleoptera: Scolytidae) in Austria, Agricult. Ecosys. Environ., 2005, 7, 169-177

[18] Moser J.C., Konrad H., Blomquist S.R., Kirisits T., Do mites phoretic on elm bark beetles contribute to the transmission of Dutch elm disease? Naturwissenschaften, 2010, 97, 219-227

[19] Lindquist E.E., Mites and regulations of bark beetle populations, In: G.O. Evans (Ed.),Proceedings of the 2nd International Congress Acarology (19-25 July 1967, Sutton Bonington, UK), Akademiai Kiado Budapest, 1969

[20] Hofstetter R.W., Cronin J.T., Klepzig K.D., Moser J.C., Ayres M.P., Antagonisms, mutualisms and commensalisms affect outbreak dynamics of the southern pine beetle, Oecologia, 2005, 147, 679-691

[21] Moser J.C., Use of sporothecae by phoretic Tarsonemus mites to transport ascospores of coniferous bluestain fungi, Trans. Br. Mycol. Soc., 1985, 84, 750-753

[22] Bridges J.R., Moser J.C., Relationship of phoretic mites (Acari: Tarsonemidae) to the bluestaining fungus, Ceratocystis minor, in trees infested by southern pine beetle (Coleoptera: Scolytidae), Environ. Entomol., 1986, 15, 951-953

[23] Magowski W.L., Moser J.C., Redescription of Tarsonemus minimax and definition of its species-group in the genus Tarsonemus (Acari:Tarsonemidae) with descriptions of two new species, Ann. Entomol. Soc. Am., 2003, 96, 345-368

[24] Nierhaus-Wunderwald D., [Liste der Borkenkäfer-Antagonisten, 3. Auflage. Phytosanitärer Beobachtungs- und Meldedienst der Eidgenössischen Forschungsanstalt für Wald, Schnee und Landschaft], Birmensdorf, 1997, (in German)

[25] Moser J.C., Perry T.J., Bridges J.R., Yin H.F., Ascospore dispersal of Ceratocystiopsis ranaculosus, a mycangial fungus of the southern pine beetle. Mycologia, 1995, 87, 84-86

[26] Norton R.A., Observations on phoresy by oribatid mites (Acari: Oribatei). Int. J. Acarol., 1980, 6, 121-130

[27] Krantz G.W., A manual of acarology, 2nd ed., Oregon State University Book Stores, Corvallis, 1978

[28] Behan-Pelletier V.M., Oribatid mite biodiversity in agroecosystems: role for bioindication, Agricult. Ecosys. Environ., 1999, 74, 411–423

[29] Wirth, S., Morphology and function of the Gnathosoma in the Histiostomatidae (Astigmata), Acarologia, 2006, 46, 103-109

[30] O´Connor B.M., Acarine-fungal relationships, the evolution of symbiotic associations, In: Wheeler Q., Blackwell M. (Eds.), Fungus-Insect Relationships, Perspectives in Ecology and Evolution, Columbia University Press, New York, 1984

[31] Kinn, D.N., A new Pleuronectocelaeno (Acari: Celaenopsidae) from Japan, with remarks on North American and European species, Int. J. Acarol., 1991, 17, 103-105