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FIRST RECORD OF THE MITE *Histiostoma ulmi* IN *SILVER FIR* AND INDICATION OF A POSSIBLE PHORETIC DISPERSAL BY THE LONGHORN BEETLE *Acanthocinus reticulatus*

PRVI NALAZ GRINJE *Histiostoma ulmi* NA OBIČNOJ JELI I NAZNAKA NJEZINOG MOGUĆEG FORETIČKOG ŠIRENJA PUTEM CVILIDRETE *Acanthocinus reticulatus*

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Abstract

The mite *Histiostoma ulmi* (Histiostomatidae), originally discovered by the describer within the bark beetle galleries of genus *Scolytus*, has been found to be phoretic on a tenebrionid beetle *Hypophloeus bicolor*. The first discovery of *H. ulmi* in the galleries of the longhorn beetle *Acanthocinus reticulatus* indicates the possibility of its phoretic association with several beetle carriers: tenebrionid, cerambycid and scolytid beetles. In Croatia, scolytids of the genus *Pityokteines*, occupying the same niche within their food plant, silver fir (*Abies alba*) could represent these additional carriers.

Acanthocinus reticulatus infested the bark of an *Abies alba* specimen as a pioneer taxon, as opposed to the usual earliest colonising beetles *Pityokteines spinidens* and *P. curvidens*. *Histiostoma ulmi* was reared from samples taken from the galleries of this longhorn beetle's larvae. As is typical for histiostomids, these mites feed on bacteria. They represent a monophyletic group of at least six very similar species. The morphological characters separating *H. ulmi* from similar species are expressed only in adult mites. Deutonymphs found attached to *Pityokteines* were previously determined to be *Histiostoma piceae*, but were never reared to adulthood. Due to a close resemblance between the deutonymphs of *H. piceae* and *H. ulmi*, incorrect assignments, relying only on deutonymphs for determination, are possible. *H. ulmi* was observed under laboratory conditions carrying spores of an undetermined mold fungus. Other phoretic mites, such as *Tarsonemus*, are known to carry spores of blue stain fungi (Ophiostomatidae) which could be of practical importance in silver fir decline. Future studies need to show, whether deutonymphs of *H. ulmi*, possible vectored by bark beetles, tenebrionids and longhorn beetles, also carry spores of blue stain fungi.

KEY WORDS: *Pityokteines*, Acari, Histiostomatidae, *Histiostoma ulmi*, *Histiostoma piceae*, *Acanthocinus reticulatus*, fungal spores, hyperphoresy

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Introduction
Uvod

Histiostomatidae are characterized by a short life cycle and can usually be easily cultured. This is why a wealth of biological data is permanently available. Astigmatid mites disperse as deutonymphs, which attach via a complex suckerplate to their phoretic carriers. They have no functional mouth. Adults are usually bacterial filter feeders (Wirth 2004). With about 500 named species, Histiostomatidae represent one of the largest groups within Astigmata. A number of histiostomatid species are known to be associated with bark beetles. Those from North America are *Bonomoia pini* Scheucher, 1957 and described by Woodring and Moser, 1970: *Bonomoia certa*, *Histiostoma conjuncta*, *H.insolita*, *H. media*, *H. sordida* and *H. varia* (Moser 1975). Those from Central-Europe are *Bonomoia pini*, *Histiostoma piceae*, *H. trichophorum* Oudemans, 1912, *H. ulmi*, *H. crypturgi*, *H. gordius* Vitzthum, 1923, *H. vitzthumi*, *H. dryocoeti*, *H. oudemansi* Womersley, 1941, *H. pini*, *H. gladiger* Vitzthum, 1926 and *H. abietis* (Scheucher 1957; Schwerdtfeger 1981; Pernek et al. 2012). They all are associated with different bark beetle species (Scheucher 1957). The European species have been considered a monophyletic group (Wirth 2004). *Histiostoma ulmi* Scheucher, 1957 is not phoretic on *Scolytus* beetles, but instead rides on the tenebrionid *Hypophloeus bicolor* Olivier, 1790 to the scolytid galleries (Scheucher, 1957).

Scheucher (1957) described several new species – *Histiostoma piceae*, *H. ulmi*, *H. crypturgi*, *H. dryocoeti*, *H. oudemansi*, *H. abietis*, *H. vitzthumi* and *H. pini* – but type material of her species does not exist. Therefore, *H. ulmi* could only be determined using her drawings of deutonymphs and adults.

Galleries of bark beetles (Curculionidae, Scolytinae), are known to host a substantial biodiversity of mites (e.g. (Scheucher 1957; Lindquist 1969). Different groups of mites are often present, and mostly stay in phoretic association with their corresponding beetle species. They use the beetles as carriers from one habitat to a new one. Members of Gamasida, Trombidiformes, Oribatida and Astigmata can be found attached to beetles, as well as free living associates in the galleries (Pernek et al. 2012). Sometimes the relationships between organisms can be more complex, such as when fungi become involved in these phoretic interactions.

Mite communities associated with Cerambycidae are poorly studied. Mites belonging to the Histiostomatidae (Astigmata) are very rarely found associated with longhorn beetles. Scheucher (1957) assumed the histiostomatid mite *B. pini* to be (besides other carriers) phoretically associated with *Acanthocinus aedilis* Linnaeus, 1758.

Mite communities associated with bark beetles (Scolytinae) and biological data about those beetles – which often act as forest pests – are much better investigated. For example the southern pine beetle *Dendroctonus frontalis* Zimmermann, 1868 carries phoretic mites of the genus *Tarsonemus* (Trombidiformes), which possess special morphological adaptations for a hyperphoretic transfer of fungal spores (Ophiostomatide) termed sporathecae (Moser 1985; Klepzig et al. 2001; Hofstetter et al. 2006). *Tarsonemus* mites phoretic on *D. frontalis* transport the fungus *Ophiostoma minus* (Hedgcock) H. and P. Sydow, on which they feed, directly into the bark beetle galleries. Bridges and Moser (1986) found a positive relationship between the occurrence of bluestain fungus and *Tarsonemus krantzi* Smiley and Moser, 1974 mites in *D. frontalis* outbreaks. A related fungus, *Ophiostoma novo-ulmi* Brasier, 1991, responsible for Dutch elm disease, is a very important threat to *Ulmus* spp. trees across European forests, and may also be carried by *Tarsonemus* mites (Moser et al. 2010). *Tarsonemus ips* Lindquist, 1969 indirectly inhibits reproductive success of beetles through interactions with the antagonistic bluestain fungi *O. minus* (Lombardero et al. 2003).

The aim of the present paper is to describe a new finding of *H. ulmi,* a histiomatid mite formerly not known to inhabit cortical tissue galleries of the phloemophagous beetles *Acanthocinus reticulatus* Razoumov, 1789 and possibly *Pityokteines curvidens* Germar, 1824 and *P. spinidens* Reitter, 1894 of silver fir (*Abies alba* Mill.). Some ecological and morphological information about *H. ulmi* is additionally presented.

Materials and methods
Materijali i metode

Two silver fir trees were felled in March 2011 in a natural silver fir stand in Otočac (15°13´50˝E and 44°50´57˝N). In this way they were exposed for colonisation by bark and wood living insects during the spring-summer. Before being felled, trees in different condition of health were selected: i) T1 with still green needles and no sign of any visible kind of infestation; ii) T2 characterized by red needles. The trunks were inspected in June and September. Small pieces of bark were cut out in order to identify the presence or absence of phloemophagous beetles. Two bolts of each tree were cut at levels of 10 m and 20 m per tree, each section was approximately 40 cm long, and 50 cm wide in diameter. They were brought into insect mesh covered cages in a climatic chamber and kept there at a humidity of 60 % and a constant room temperature of 20 °C and 16L:8D photoperiod. Sections were sprinkled daily with water to maintain optimal moisture in the bark.

Histiostomatid mites from those samples were cultured under conditions developed by Wirth and Moser (2010) as a standard method to rear species from their original substrate. Bark samples (2 square centimetres) were taken daily from the trees on September 12 until 29, 2011 using a knife.

The samples were put into Petri-dishes of 10 cm diameter. Dish bottoms were covered with tissue paper, which was humidified ca. every 3 days using faucet water. At least 5 pieces of peeled potatoes (ca. 0.5x1x1 cm) were distributed around the bark and on the top. They were moistened every 3rd day with water drops so that the potato's surface was slightly moistened. When the potato-pieces began decaying due to microorganism growth, the frequency of the regular moistening was reduced. Histiostomatid mites are microorganism/bacteria feeders, and usually develop on the surface of these potatoes, which represent a growth medium for the enrichment of bacteria and other microorganisms from the original habitat (i.e. the bark and the beetle galleries). More or less constant climatic conditions were enabled by storing these Petri dishes inside larger plastic containers. Proper air circulation was also needed for this arrangement of the dishes. The cultures were kept at room temperature (about 21 °C).

Results
Rezultati

The 10 m and the 20 m pieces of the trunk of T1 tree were only infested by the longhorn beetle *A. reticulatus*. The beetle's larvae were numerous, and distributed under the bark about 4–6 cm from one another. No signs of scolytid beetle galleries were noted. The longhorn beetle was apparently the first coleopteran to colonise this tree. The larvae finally formed pupal-chambers in the xylem. The galleries of *A. reticulatus* contained representatives of different mite groups.

The 10 m and the 20 m pieces of the T2 tree were heavily infested by *P. curvidens* and *P. spinidens*. Young beetles already had emerged from the bark before it was brought into the climatic chambers. The typical galleries of these bark beetle species and the areas around them were crowded with representatives of different mite groups. In these samples one histiostomatid species was found.

From two of the samples taken from the T1 tree at a level of 20 m, the mite *H. ulmi* was easily reared. About 10 days after taking the bark sample from the trunk piece, first adults, males and females, were observed feeding on the potato-surfaces. It is not yet known where exactly these histiostomatids were developing, but presumably they lived inside the galleries of the beetle larvae. *Histiostoma ulmi* needs about two weeks to develop from a larva to an adult. Detailed studies about its life cycle do not yet exist. The mites prefer developing in areas which are not too wet, which means that only a very thin film of moisture was visible, and they produce large numbers of deutonymphs in too dry conditions (on more or less dried out potato-pieces for example).

The mite was identified as belonging to the monophyletic *Histiostoma-piceae* group (Scheucher 1957; Wirth 2004). The drawings of Scheucher (1957) were used for identification of *H. ulmi* and light-microscopic slides with deutonymphs of *H. piceae* (collected on the original carrier and tree species in Germany) from the collection of J. Moser (USA) were used for morfological comparisons. This discovery documents for the first time the occurance of *H. ulmi* in silver fir and their possible phoretic association with *A. reticulatus*.

Emended diagnosis
Unaprjeđena dijagnoza

Deutonymph: The size of the deutonymphs was measured as an imaginary medial line beginning at the anterior outline of the proterosoma until the posterior end of the hysterosoma of mites, mounted on slides, in ventral view. Mean and range of three specimens 189 (185–193) µm. As in all species of the *H. piceae*-group, all dorsal setae (Figure 1C) are elongated and directed forwards. The median apodeme (st1), located directly posterior of the palposoma, does not touch the apodemes (bcx2), which run across between legs III; as is also the case in other *H. piceae*-group species (Figure 1A). A notable character of *H. ulmi* and at least *H. piceae* is the median apodeme st1 forming a "wide collar" (Figure 1A) around the palposoma. This character was discovered in the slide-material of *H. piceae*/Moser-collection, no official types or paratypes, but collected from the original carrier and the original trees as described by Scheucher (1957) and in the slides of *H. ulmi*/Wirth (the character is barely visible in Scheucher's drawings). The apodeme bcx2 of *H. ulmi*, *H. piceae*, *H. trichophorum*, *H. crypturgi* and *H. vitzthumi* is medially lineally running and laterally on both sides angled backwards (Figure 1A). Distinct (apomorphic) characters could not be found in the deutonymph of *H. ulmi*. Deutonymphs of *H. ulmi* and *H. piceae* look very similar to each other. Details about leg setation in deutonymphs are not visible in Scheucher's drawings. In a comparison between *H. ulmi* deutonymphs from our cultures and *H. piceae* deutonymphs of the Moser-collection, the leg setation looks quite similar.

Adults: The size of adult males and females was measured as an imaginary median line beginning at the level of the sternal apodemes a1 (the point where it touches the trochanters of legs I anteriorly) until the posterior end of the hysterosoma of mite specimens, mounted on slides, in ventral view.

Males: mean and range of three specimens 285 (253–264) µm. Females: mean and range of three specimens 355 (268–491) µm.

As in most histiostomatid species, males are smaller than females. Apodemes a2 in males (Figure 1E) that do not touch each other is a specific character of *H. ulmi*. The median endings of these apodemes are bulged posteriorly, a character not visible in Scheucher's drawings.

Dorsally, the females (Figure 1F) have three pairs of more or less distinctly elevated humps. They share this character with at least *H. piceae* and *H. trichophorum*. The posterior ringorgans (=osmoregulatory organs) are short-oval shaped (Figure 1B) and thus represent a specific *H. ulmi* character; they are not elongate-oval shaped as in *H. piceae*. The most distinct specific character of *H. ulmi* is the shape of the digitus fixus (Figure 1D) of the chelicera (easily visible in the females): the distal end is bulged downwards and clearly divided into three prongs with the dorsal one being longer than the following two prongs which have nearly the same length.

Voucher specimens of *H. ulmi* isolated from the galleries of *A. reticulatus* have been deposited at the Museum für Naturkunde Berlin under ZMB 48507 (deutonymphs), ZMB 48508 (adults) and ZMB 48509 (adults).

Fungal spores attached to mite deutonymphs
Spore gljiva pričvršćene za deutonimfu grinje

The colony was exposed to conditions assumed to be unfavourable to the mites, in order to induce the development of more deutonymphs than would be produced normally. Although it was found that they do not prefer a wet or very moist habitat, but slightly drier conditions, for these purposes all kinds of moistening were ceased for about six days. The surfaces of the bark and the potatoes partly dried out.

Later, many deutonymphs were visible crawling around in larger aggregations in these drier areas of potatoes and bark. As is usual for histiostomatid deutonymphs, they preferred to occupy elevated structures such as the edges of potatoes or protruding bark splinters. There they performed – also typical for many other histiostomatid species – a behaviour during which they were fixed with their sucking plates on the ground with the whole body in an upright position and then moved alternating the body to the right and to the left. During this procedure they alternately moved the first pairs of legs. This behaviour is interpreted as olfactory carrier-searching behaviour (Wirth 2005).

Some of these deutonymphs were covered with fungal spores (Figure 1A). In the still moist areas, where the resting adults and non-deutonymphal nymphs remained, no fungus growth took place. Undetermined mold fungi grew only outside these areas. Sometimes the border between mite-development-area and fungus-area formed a sharp, distinct border. This is because histiostomatids are assumed to produce chemical fungicides in their opisthonotal glands (e.g. Wirth and Moser 2010; Koller et al. 2012). The whereabouts of the deutonymphs during the carrier-search often were inside or close to the fungal-areas, which is due to the fact that deutonymphs of Histiostomatidae in most species seem to separate themselves under laboratory conditions from the rest of their population to use elevated areas for a better perception of the olfactory particles of their carriers and better access by which to attach themselves (Wirth 2005). Some of them were visibly covered with fungal spores. Due to a 'sticky' cuticle surface of the mites, spores could adhere to the whole mite bodies. During the procedure of mounting such deutonymphs on light microscopic slides, many spores fell off. Lumps of spores obviously had a better hold in the areas between the dorsal shield and leg-trochanters I + II and in ventral areas, where the hysterosoma-shield laterally bulges downwards, and there remained visible also on the mounted objects (Figure 1A).

Observed deutonymphs could walk while covered with fungal spores. It is unknown whether they are able to attach to a carrier under these conditions and whether they would be successfully able to reach a new bark habitat by phoretic transport. The phenomenon of mite deutonymphs carrying fungal spores could be of an applied importance if it could be shown that they are able to disperse germinable entomopathogenous fungi such as the blue-stained fungus. Further examinations are needed.

Discussion
Rasprava

Discovering *Acanthocinus reticulatus* as the first bark infesting settlers was unexpected. Usually bark beetles of the genus *Pityokteines* were first observed infesting the bark of silver fir, with insects such as the Cerambycidae arriving later. More rarely, *Pityokteines* and Cerambycidae arrived at the same time (pers. obs. M. Pernek). Our T1 tree indicated for the first time that Cerambycidae can also sometimes arrive as the first bark-damaging pests. It is unknown whether this specifically concerns silver fir and *A. reticulatus*. Quantitative studies as a specific investigation of this phenomenon still need to be done. It can also happen that different parts of the tree such as the crowns can have different insect invaders than the lower trunk (pers. obs. M. Pernek). This is why we observed pieces at different levels of the trunk, but different levels of the crowns remained unobserved. Besides galleries of *P. curvidens* and *P. spinidens*, galleries of *A. reticulatus* were not visible in our T2 tree. It is unknown whether the longhorn beetle in this tree also appeared as the first invader before *Pityokteines* or not.

In previous studies, deutonymphs collected directly from beetles of *P. curvidens* and *P. spinidens* were determined as *H. piceae* (Pernek et al. 2012). These deutonymphs were not reared to adults to provide a direct comparison with *H. ulmi* from *A. reticulatus*. Due to the morphological similarities of *H. ulmi* and *H. piceae*-deutonymphs, it is possible that both species appear in the silver fir bark of the studied area in Croatia. Perhaps *H. ulmi* rides on *A. reticulatus* and *H. piceae* on *Pityokteines*, or perhaps the deutonymphs found

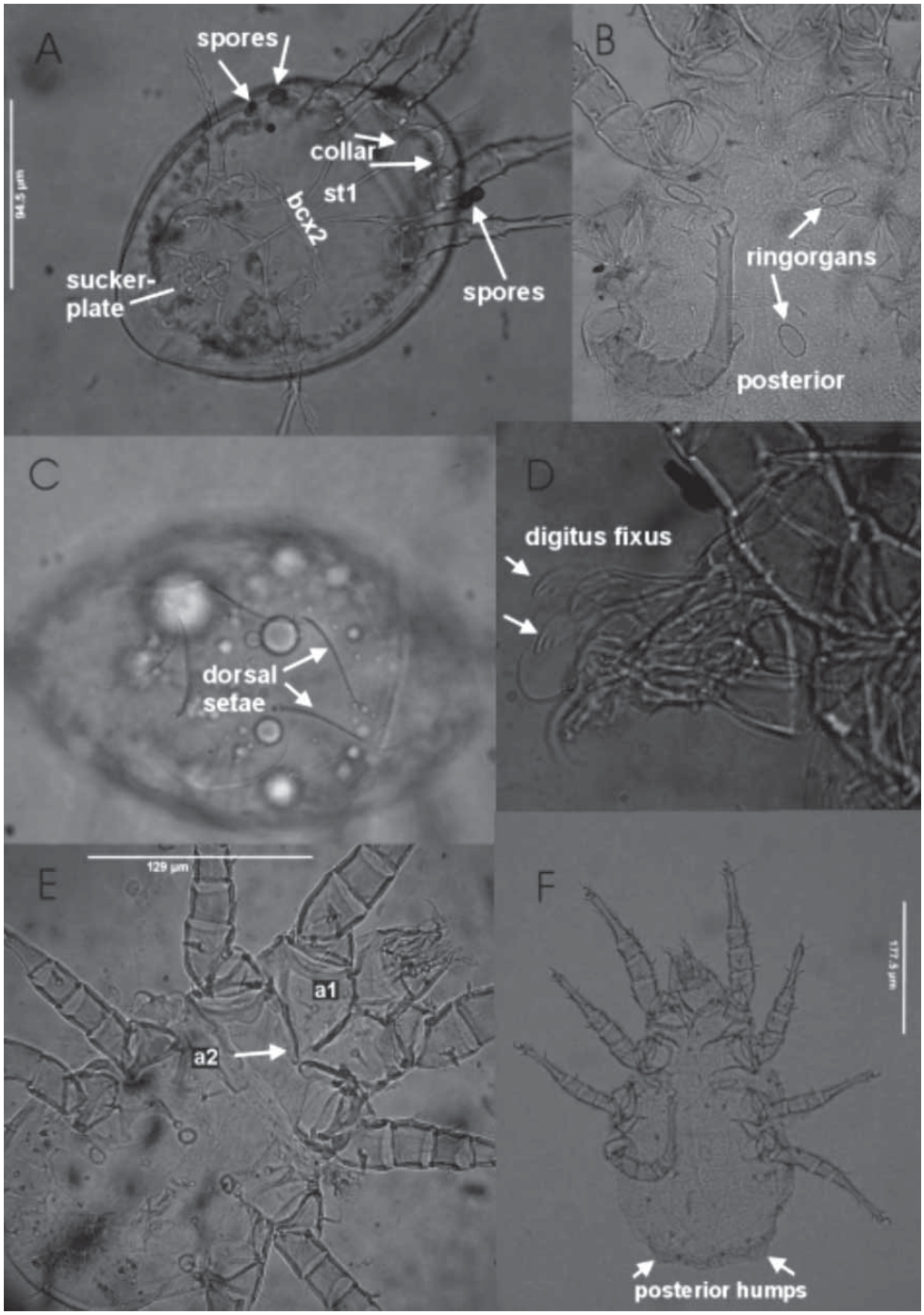

Figure 1. *Histiostoma ulmi*: A = deutonymph in ventral view, B = middle part of the female in ventral view, C = deutonymph in dorsal view, D = mouthparts of female in lateral view, E = male in ventral view, F = total female in ventral view, humps barely recognizable

Slika 1. *Histiostoma ulmi:* A = deutonimfa ventralno, B = srednji dio ženke ventralno, C = deutonimfa dorzalno, D = usta ženke lateralno, E = mužjak ventralno, F = cijela ženka ventralno, teško prepoznatljive grbice

on specimens of *P. curvidens* and *P. spinidens* represent *H. ulmi*? In the latter case, *H. ulmi* could arrive on the bark using *A. reticulates* as a primary phoretic carrier and could, in addition, switch to *P. curvidens* or *P. spinidens* as secondary carriers. Alternatively *Pityokteines* could be the usual primary and *A. reticulates* only the usual secondary carrier, which then in our T1 tree only exceptionally arrived first with the mite deutonymphs attached.

An analysis of how species of the *H. piceae*-group are phylogenetically related to each other is not presented here, because there are not enough characters available for a meaningful phylogenetic reconstruction. Perhaps *H. ulmi* and *H. piceae* are sister-species due to their very similar deutonymphs.

The results of these studies show that rearing histiostomatid mites until adulthood is often important for a precise species determination. This is because closely related species such as members of the *H. piceae*-group may have nearly identical deutonymphs.

With *H. ulmi*, it is surprising that we found the mites neither on the carriers (*Hypophloeus bicolor* inside *Scolytus* sp. galleries) nor on the trees (*Ulmus* sp.), where they were ori-

ginally discovered by the describing author Scheucher (1957). Our studies might indicate that *H. ulmi* is switching between carriers of two beetle groups. This agrees with the original findings by Scheucher (1957) that besides Scolytinae the mite also switches to another bark-inhabiting beetle, which in our case is a cerambycid. This beetle species, as well as the *Pityokteines*-species, were never previously found associated with deutonymphs of *H. ulmi*. Our new findings might indicate that the mite has a wider range of carriers and tree hosts than assumed by Scheucher (1957).

Histiostoma ulmi was not found in samples from the T2 tree. This might suggest that most specimens had already left the tree together with newly hatched *Pityokteines* beetles and *A. reticulatus* (in case it was also there). Alternatively, it might indicate that this tree had never been colonized by *H. ulmi*.

However, because the real biodiversity of histiostomatid species in general has not been fully discovered worldwide, new species could also appear among these bark beetle associates. Cryptic species-groups are characterized by very similar looking species (Wirth 2004). The *H. piceae*-group is such a group containing cryptic species. Due to the morphology of apodeme a1 of the mite that we determined as *H. ulmi*, looking slightly different from that which Scheucher (1957) depicted, the mite might also represent a subspecies of *H. ulmi*, or could even be a new species very closely related to *H. ulmi*. Were doubts about the species status to remain in future, the species could in further papers be named *Histiostoma* cf. *ulmi*. Different kinds of characters such as ecological, morphological, biochemical and genetic information would help to clarify the species status.

The cuticle surface of *H. ulmi* appeared to be sticky. This may be due to secretions of dermal glands containing long-chain hydrocarbons and fatty acids, as was discovered by Leal and Kuwahara (1991) for some Astigmata, which produced in detail ester, medium sized hydrocarbons, higher hydrocarbons and sequestered compounds from food. This 'sticky' cuticle surface in deutonymphs maybe intensifies the natural adhesiveness of the fungal conidia and could ensure a permanent fixation of spores in some ventral body areas 'full of nooks and crannies'.

Phoretic deutonymphs of *H. ulmi* mites were observed carrying fungal spores under culture conditions. If future studies could prove that they also carry blue stain fungi (Ophiostomatidae), then this could be of practical importance in fir decline. Considering that *H. ulmi* has been vectored by bark and longhorn beetles, future studies need to show how and whether this knowledge could be used in forest protection management.

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Sažetak:

Grinja *Histiostoma ulmi* (Histiostomatidae) izvorno je opisana kao vrsta koja se nalazi u hodnicima potkornjaka roda *Scolytus* s kojima nema foretički odnos, za razliku od kornjaša *Hypophloeus bicolor* iz porodice Tenebrionidae. Prvi nalaz grinje *H. ulmi* u hodnicima strizibuba *Acanthocinus reticulatus* indicira mogućnost foretičke asocijacije s nekoliko kornjaša iz porodica Tenebrionidae, Cerambycidae i Curculionidae. Na običnoj jeli (*Abies alba*) u Hrvatskoj potkornjaci roda *Pityokteines* zauzimaju istu hranidbenu nišu kao i *A. reticulatus* te su također mogući prijenosnici spomenutih grinja.

Acanthocinus reticulatus napadaju koru jele debla kao pionirski takson, suprotno od dosadašnjih saznanja kako *Pityokteines spinidens* i *P. curvidens* dolaze prvi.

Histiostoma ulmi sakupljana je s uzoraka uzetih od hodnika larvi strizibuba. Histiotomatidae koje se hrane bakterijama, predstavlja monofiletičku grupu sastavljenu od šest vrlo sličnih vrsta. Morfološke razlike *H. ulmi* sa sličnim vrstama vidljive su isključivo u stadiju imaga.

Deutonimfe nađene na vrstama roda *Pityokteines* u ranijim istraživanjima determinirane su, bez razvoja odraslih oblika, kao *Histiostoma piceae*. Glede velike sličnosti između deutonimfi *H. piceae* i *H. ulmi*, moguće je da su napravljene pogreške u identifikaciji. *Histiostoma ulmi* uzgajana u laboratorijskim uvjetima na tijelu je nosila neidentificirane gljive. Grinje kao primjerice *Tarsonemus*, poznate su kao prenositelji gljive plavila (Ophiostomatidae) koje mogu imati važnu ulogu kod odumiranja jele. Buduća istraživanja trebaju pokazati da li deutonimfe *H. ul*mi kojima su mogući vektori kukci rodova Cerambycidae, Tenebrionidae ili Curculionidae, također prenose spore gljive plavila.

KLJUČNE RIJEČI: *Pityokteines*, Acari, Histiostomatidae, *Histiostoma ulmi*, *Histiostoma piceae*, *Acanthocinus reticulatus*, spore gljiva, hiperforezija