

Syninclusions of the Eocene winter ant *Prenolepis henschei*  
(Hymenoptera: Formicidae) and *Germaraphis* aphids  
(Hemiptera: Eriosomatidae) in the Late Eocene Baltic  
and Rovno ambers: some implications

Сининкклюзы эоценового зимнего муравья *Prenolepis henschei*  
(Hymenoptera: Formicidae) и тлей *Germaraphis*  
(Hemiptera: Eriosomatidae) в позднеэоценовых балтийском  
и ровенском янтарях: некоторые выводы

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КЛЮЧЕВЫЕ СЛОВА: сининкклюзы, Aphidinea, Formicidae, эоцен, янтарь, Украина, Россия, Дания, дивергенция экологических ниш.

ABSTRACT. *Prenolepis-Germaraphis* syninclusions are reported for the first time from the Baltic and Rovno ambers. The number of syninclusions of those taxa in the representative Kalandyk collection of the Baltic amber (half of all samples with *Prenolepis*) indicates close lifetime relationship between *Germaraphis* and *Prenolepis*. We hypothesize that ants *Prenolepis henschei* were not dendrobiotic, i.e. they foraged on the resin-producing trees mainly looking for homopterans, particularly aphids which they did not keep in their nests. The relationship between *P. henschei* and *Germaraphis* was not obligatory since it depended on the abundance of the aphids. Activity of the resin traps in the amber forests is supposed to be time-limited, and we hypothesize that the resin traps in the Baltic amber forest were active at lower temperatures if compared to the forests that produced the Scandinavian and Rovno ambers. This hypothesis explains the low proportion of workers and the high numbers of sexuals of *P. henschei* in the Scandinavian and Rovno ambers as compared to the Baltic one.

РЕЗЮМЕ. Сининкклюзы *Prenolepis* и *Germaraphis* впервые найдены в балтийском и ровенском янтаре. Число сининкклюзов этих таксонов (половина всех образцов с *Prenolepis*) в репрезентативной коллекции Каландыка (балтийский янтарь) указывает на тесную прижизненную связь *Germaraphis* и *Prenolepis*. Мы предполагаем, что муравьи *Prenolepis henschei* не были дендробионтами: они лишь фуражировали на деревьях, производящих смолу,

главным образом в поисках равнокрылых, в частности, тлей, которых они не держали в своих гнездах. Отношения *P. henschei* и *Germaraphis* не были облигатными и зависели от численности тлей. Предполагается, что активность смоляных ловушек в янтарных лесах была ограничена во времени, и в балтийском янтарном лесу смоляные ловушки начинали действовать при более низких температурах, чем в лесах, послуживших источниками скандинавского и ровенского янтарей. Эта гипотеза объясняет низкую долю рабочих и многочисленность крылатых муравьев в скандинавском и ровенском янтарях по сравнению с балтийским.

## Introduction

The term “syninclusions” was coined by the famous Polish entomologist Jan Koteja who predicted the key role of multiple organic inclusions in the same pieces of amber for understanding the biotic structure of past environments [Koteja, 1989]. The significance of syninclusion analysis as one of the key ways of revealing the structure of biocenoses of the fossil forests is becoming more obvious now [Kutscher, Koteja, 2000; Sontag, 2003; Perrichot, Girard, 2009; Weitschat, 2009; Wichard, 2009; Rasnitsyn, 2011]. This work follows our previous studies in this field (summarized in [Perkovsky et al., 2010a]) based mainly on the inclusions in the Rovno amber, a southern coeval analog of the Baltic amber with the same age of origin

(see reviews in [Perkovsky et al., 2007, 2010b] for details).

Previous studies have shown that composition of the inclusions depended on the weight (= size) of the pieces of amber [Perkovsky, 2009]. It was demonstrated, for example, that the specialized dendrobionic ants, like *Ctenobethylus goepperti* (Mayr, 1868), occupied a higher proportion of the ant assemblage in smaller pieces of amber (net weight less than 1.5 g) than in the larger ones, while the more generalist (mainly herpetobiotic) ants, *Lasius schiefferdeckeri* Mayr, 1868, showed an opposite tendency. The larger pieces of amber are, the higher is the probability that they have originated from the trunk and not from thin branches of the amber tree. The above mentioned trend could therefore be explained by the situation when *C. goepperti* was a frequent visitor to the tree crown, whereas *L. schiefferdeckeri* was probably more or less restricted to trunks and perhaps also to thick branches.

Distribution of families of the order Diptera is found to depend on the weight of the pieces of amber as well [Perkovsky et al., 2010a]. We can generally infer that small pieces were widely distributed over the amber tree and they served as better traps than the larger ones for both insects of the air plankton as well as other arthropods which are not confined to tree trunks.

Syninclusions of ants and the dominant aphid, the longbeaked aphids *Germaraphis* Heie, 1967 are of particular interest, since modern analogs of *Germaraphis*, i.e. aphids of the genus *Stomaphis* Walker, 1870, are obligate ant symbionts [Heie, Wegierek, 2009]. The proportion of *Germaraphis* among all insect inclusions of the different weight fractions of the Rovno amber reveals the same pattern. For the larger pieces (with the net weight not less than 6.5 g), *Germaraphis* comprises 2.9% of all insects identified to the order level (excluding Entognatha), and the corresponding share is only 1.45% for the smaller pieces. Basing on the same material, worker ants occupy 4.4% of all insects (excluding Entognatha) in the larger pieces, and 6.2% in the smaller ones [Perkovsky, 2010]. At present, this pattern cannot be unequivocally explained by the presence or absence of particular species of amber ants, except that *C. goepperti* occupies 1.2% of all insects in the larger pieces, and 2.3% in the smaller ones, while the proportion of *L. schiefferdeckeri* in the larger pieces is slightly higher than that in the smaller ones [Perkovsky, 2010].

## Material and methods

The collection of the Baltic amber from the East Coast of the Bay of Gdańsk (Sambia) donated by Wojciech Kalandyk to the Museum of Amber Inclusions at the Department of Invertebrate Zoology, University of Gdańsk [Sontag, 2003] was examined. The Kalandyk collection mainly contains large pieces of amber. Specifically, only 1824 pieces with zooinclusions, approximately one third weights less than 10 g

[Sontag, pers. comm.]. Ant-aphid syninclusions were studied in the Rovno amber collection of Schmalhausen Institute of Zoology of the National Academy of Sciences of Ukraine, Kiev (SIZK) as well. Photographs were taken at the Paleontological Institute, Russian Academy of Sciences (Moscow) using the microscopes Leica MZ 16 and Leica M 165.

The statistical significance of correlation between components of the amber arthropod assemblage was assessed using  $\chi^2$  test. For the direct assessment of correlations between syninclusion components, we used the ratio of the expected and observed shares of syninclusions with regard to their reliability. The expected share is calculated as the product of the actual shares of the components, i.e. the taxa forming the syninclusion, multiplied by each other. Values substantially exceeding unity indicate a positive correlation between the components, i.e. either the preference of the same conditions or their direct ecological interdependence, e.g. certain form of symbiotic relationships [Perkovsky et al., submitted]. When  $\chi^2$  value is higher than 6.63, the probability of an occasional coincidence of the respective events is less than 0.005, and the difference between the observed and expected values is therefore termed highly significant.

## Results

During the study of ant-*Germaraphis* syninclusions in the Kalandyk collection, we have unexpectedly revealed that this kind of co-occurrence was the most characteristic of the ant *Prenolepis henschei* Mayr, 1868, the fifth in number in the Eocene ambers (as estimated by Dlussky and Rasnitsyn [2009]), and not of the most common amber ants *C. goepperti* and *L. schiefferdeckeri* (Table 1).

Five or six pieces of amber with the specimens of *Prenolepis* Mayr, 1861 weighted above 10 g; and three out of those five pieces also contained four small *Germaraphis* larvae and two coccids as syninclusions (Table 1). An average weight of *Prenolepis* syninclusions with arthropods that could be identified to the order level was 14.4 g. On the other hand, an average weight of *Prenolepis*-*Germaraphis* syninclusions was 11.9 g, which was nearly equal to an average weight of the pieces with animal inclusions in the Kalandyk collection, i.e. 12.2 g.

Ant-aphid syninclusions were also studied in the collection of the Rovno amber of Schmalhausen Institute of Zoology of NAS of Ukraine, Kiev (SIZK). We have found aphids in five out of 22 syninclusions (22.7%) with ants from two different genera (see below).

Most pieces of the Rovno amber with *Prenolepis* inclusions were much smaller than those from the Kalandyk collection (see below). Syninclusions in those small pieces of amber were relatively rare (139 out of 1339 pieces of the Rovno amber [Perkovsky et al., 2010a, Table 4]), and ant-aphid syninclusions were

Table 1. Syninclusions of *Prenolepis henschei* in the Kalandyk collection.  
Таблица 1. Состав сининклюзов янтарей с включениями *Prenolepis henschei* в коллекции Каландыка.

Inventory number	Other syninclusions	<i>Prenolepis</i> , specimens	<i>Germaraphis</i> , specimens	Weight, g
224	Aranei Acari (Oribatei) Coleoptera: Scaptiidae: Anaspidinae Diptera: Dolichopodidae Collembola, Arthropleona	1		14.2
816	Diptera: Chironomidae – 2 females, male; Sciaridae, male; Ceratopogonidae: <i>Forcipomyia</i> , female; larva Collembola: Sminthuridae Thysanoptera – 2 Acari (2, Oribatei – 1) stellate hairs	2	1	10.9
1684	Acari (Oribatei) stellate hairs	2		15.15
1813	Diptera: Chironomidae, female; Cecidomyiidae; larva Coleoptera: Elateridae Aranei Coccinea Collembola, Arthropleona Acari (3, Oribatei – 1)	1	2	13.85
1905	Leg of insect	1		3.7
1920	Diptera: Sciaridae (2); Chironomidae, female Acari (3, Oribatei – 1) Coccinea Aranei	1	1	17.7

almost unique among small pieces of amber (with net weight less than 6.5 g [Perkovsky, 2010]). The sample SIZK K-25828 was the first known ant-aphid syninclusion in a small piece of the Rovno amber (Fig. 1).

## Discussion

The ratio of the observed and expected shares of *Prenolepis*–*Germaraphis* syninclusions among 1824 pieces of amber with animal inclusions from the Kalandyk collection (Table 1) is 16.888 ( $\chi^2=46.365$ ,  $P \ll 0.001$ ). Consequently, the probability of the occasional co-occurrence of those insects in the same piece of amber is extremely small. The high number of syninclusions therefore indicates the close relationship between *Germaraphis* and *Prenolepis*.

Longbeaked *Germaraphis* aphids were undoubtedly tended by ants. Thirteen out of 54 pieces with *Germaraphis* in the Kalandyk collection also contained ant inclusions (the ratio of the observed and expected shares of syninclusions of the worker ants and *Germaraphis* is 3.85:1). In addition to syninclusions shown in Table 1, *Germaraphis* aphids were found in five pieces of amber together with *L. schiefferdeckeri* (the ratio of the observed and expected shares for *Lasius*–*Germaraphis* syninclusions is 5:1), in four pieces with *C. goepperti* (the ratio of the observed and expected shares is 2.8:1),

and in one piece with *Formica flori* Mayr, 1868. It is noteworthy that in all those cases the ratio of the observed and expected shares is relatively low if compared to that of *Prenolepis*–*Germaraphis* syninclusions.

In the Rovno amber, *Prenolepis*–*Germaraphis* syninclusions were unknown until recently. It should be noted that the share of *Germaraphis* itself among all insects (excluding Entognatha) in the Rovno amber is several times less than that in the Baltic amber from the Bay of Gdańsk [Perkovsky et al., 2007]. Until now, in SIZK collection of the Rovno amber there were 29 pieces with *Prenolepis* workers (as compared to six pieces in the Kalandyk collection), but only four pieces of the Rovno amber with *Prenolepis* were large enough. The other pieces were small (usually not exceeding the piece no. 1905 in the Kalandyk collection; Table 1), and rarely had Entognatha as syninclusions. The ant-*Germaraphis* syninclusion that was found only in 2010 contains the thermally modified ant which “can belong only to *Prenolepis* among the known genera” [Dlussky, pers. comm.] (SIZK, specimen K-25828, net weight 1 g; Fig. 1). Another similar syninclusion of an aphid and the incomplete remains of *Prenolepis* (thermally unmodified in that particular case) in the Rovno amber is described below.

*Prenolepis* accounts for 6.2–6.7% of all ant specimens in the representative collections of the Baltic



Fig.1. *Prenolepis*–*Germaraphis* syninclusion in a small piece of the Rovno amber (SIZK, K-25828).

Рис.1. Сининклюз *Prenolepis* и *Germaraphis* в мелком куске ровненского янтаря (коллекция Института зоологии НАН Украины; ИЗАНУ, K-25828).

amber, 6.3% in the Scandinavian amber (that is unique by having half of all specimens as males), 3.9% in the Rovno amber, and 0.6% in the representative collection of the Bitterfeldian amber. Rarity of *P. henschei* in the amber from Bitterfeld can be explained by a very small size of the studied pieces of the Bitterfeldian amber with ant inclusions.

W.M. Wheeler [1930, p. 1] noted strong morphological similarity of *P. henschei* to *Prenolepis imparis* (Say, 1836) and *P. nitens* (Mayr, 1853) (“*imparis* ... is scarcely more than variety of extinct *henschei*”). The males of *P. henschei* and *P. imparis* are likewise remarkably similar, with an important difference in the structure of the parameres [La Polla, Dlussky, 2010]. Wheeler [1930] emphasized that neotropical and palaeotropical *Prenolepis* species “are quite different from the group comprising the very closely interrelated *imparis*, *nitens* and *henschei*”. Mentioned modern species of this group are distributed in the Nearctic and West Palaearctic Regions. Consequently, this group together with the Late Eocene European ambers indicate that *Prenolepis* together with *Formica* Linnaeus,

1758 and *Lasius* Fabricius, 1804, is characteristic of the Palaearctic and Nearctic Regions. Latter genera are only scarcely represented in the tropical areas, where they are exclusively restricted to the mountains [Dlussky, Rasnitsyn, 2009].

*Prenolepis imparis* and *P. nitens* are very similar to each other in their ecology. The former species is the only Nearctic member of *Prenolepis*, also known as the false, winter or small honey ant. It is widely distributed, very common and well-studied. Winter ants initiate foraging when the temperature rises just above the freezing and discontinue this activity at about 26°C in Maryland [Lynch et al., 1980], and at 18.5°C in Missouri. They often build their nests deep underground staying inactive during the summer months. When the ants resume their activity during the cool season, they can forage even at 0°C and below, often being the only active ants. In North Florida, the ant is absent above the ground for 7 to 8 months, foraging only during the winter [Tschinkel, 1987] (e.g. for two months in north USA). However, their distribution range does not extend far to the North despite their tolerance to low



temperatures [Wheeler, 1930]. Interestingly enough is that their range does not extend into the far north as might be expected from their cold tolerance. The distribution range of *P. imparis* almost precisely coincides with that of the oak [Wheeler, 1915, 1930]. Sexu-als of *P. imparis* overwinter in the nests, and mating flights take place on the first warm day of spring [Tschinkel, 1987], towards the end of the season of the aboveground activity of the ants.

It was found that the winter ant is the most active at 15–19°C in Maryland; however, it is often also active at temperatures below 10°C, while other ant species are the most active at 25–29°C and inactive at 10°C [Lynch et al., 1980]. In spring, *P. imparis*, in contrast to the other species, is the most active in the early morning. The unusual seasonal and circadian activity pattern of *P. imparis* results in a relatively low degree of the annual niche overlap between this species and its main competitors [Lynch et al., 1980]. This niche divergence might occur in the Late Eocene amber forests as well. The ability of *Prenolepis* foragers to individually collect up to 4 g of syrup could be their additional advantage [Lynch et al., 1980] because a single forager was therefore able to collect the entire honeydew crop from the colony of small *Germaraphis* larvae (having body length of 0.4 to 0.6 mm in the syninclusions with *Prenolepis*). The general aphid survey performed in Great Smoky Mountains National Park (Tennessee, North Carolina) showed that *P. imparis* tended six species of aphids, i.e. more than any other ant species except for *Formica subsericea* Say, 1836 [Favret et al., 2010]. The complete absence of myrmecophilous beetles in the nests of *P. imparis* [Wheeler, 1930] can probably be explained by the fact that the life cycle of the latter species significantly differs from those of a vast majority of other ants for a very long time.

Unlikely most native ants, *P. imparis* can survive in the presence of the invasive Argentine ant *Linepithema humile* (Mayr, 1868) from the subfamily Dolichoderinae [Sorrels et al., 2011]. *P. imparis* has been found to be relatively unaffected by the presence of *L. humile* due to the higher seasonal activity of *P. imparis* during the more cool and wet season when *L. humile* reduces its foraging intensity [Ward, 1987; Suarez et al., 1998; Sanders et al., 2001]. The coexistence of *L. humile* and *P. imparis* has been explained by the temporal niche separation, but it appears that *P. imparis* also has an effective defensive secretion [Sorrels et al., 2011]. *P. imparis* is therefore likely to have been preadapted for the competition with *L. humile*. The change in the seasonal activity in an ancestor of *P. imparis* was probably caused by the need to defend against very numerous and aggressive thermophilous ants. At present, this adaptation, together with the defensive secretion, allows *P. imparis* to successfully compete even with *L. humile* which is able to form so-called supercolonies.

Wheeler considered that foraging workers of *P. imparis* and *P. nitens* had greatly distended gasters [Wheeler, 1930, Fig. 3]. He also supposed that they

were real “repletes” like those of the true honey ants of the Southwest USA and Mexico. However, W.R. Tschinkel [1987] ascertained that the gasters of those “replete” workers in fact contained enormously hypertrophied fat bodies, not accumulated carbohydrate solutions characteristic of the plerergates of the honey ants.

According to Tschinkel [1987], brood rearing by *P. imparis* in Florida during the summer highly depended on large amounts of fat and other nutrients stored by the corpulent workers. The weight of the corpulent (obese) workers ranged up to 8 mg (with an average of 5.4 mg) and the weight of foragers was 2.0–2.2 mg [Tschinkel, 1987]. In winter, foragers fed young (callow) workers. Corpulent young workers were losing their weight during the summer while rearing brood in the sealed nest, and they became foragers in winter, with their abdomens were not bloated any more. Those foragers had very dark body colour.

Gasters of some workers of the Priabonian *Prenolepis henschei* seem distended (Figs 2, 3), but these ants are not corpulent. We believe that the life cycle of *P. henschei* could be similar to that of *P. imparis*. Callow workers of *P. henschei* have never been found, although individuals of this species are rather common (see above). Moreover, inclusions of callow workers of other common amber species are well-known, including those in the Rovno amber.

Similarity of the life cycles of *P. henschei* and *P. imparis* could explain the abundance of the males of *P. henschei* in certain representative collections, e.g., in the Scandinavian amber, where they are as numerous as the workers [La Polla, Dlussky, 2010]. The abundance of the male ants in amber is unusual and is also known only for the subfamily Ponerinae and *Nylanderia pygmaea* (Mayr, 1868) [Dlussky, Rasnitsyn, 2009; La Polla, Dlussky, 2010]. However, the situation with those groups is better explained by the scarcity of the workers rather than by the abundance of the alates. This is because of predominantly geobiotic foraging of the workers of the Ponerinae and *Nylanderia pygmaea* who rarely visited trees [Dlussky, Rasnitsyn, 2009]. On the contrary, this is less obvious for *P. henschei*, although we suggest a corresponding hypothesis that is given below.

Wheeler pointed out the unusual combination of “two distinct faunal components, one closely related to our present circumpolar, north temperate species, the other closely related to the tropical and subtropical species of Indomalayan and Australian Regions” [Wheeler, 1930, p. 14] in the myrmecofauna of the Baltic amber. This phenomenon could be explained by the low climatic seasonality in the first half of the Priabonian as compared to the modern times [Archibald, Farrell, 2003]. The detailed analysis of the above mentioned faunal components is given below. Wheeler [1930, Figs 1, 2] confirmed that *P. imparis* and *P. nitens*, on one hand, and the genus *Liometopum* Mayr, 1861, on the other hand, have similar distribution in the



Fig.2. *Prenolepis henschei* in Baltic amber (the Kalandyk collection, No.1905 in the Museum of Amber Inclusions of the Department of Invertebrate Zoology, University of Gdańsk), dorsal view.

Рис.2. *Prenolepis henschei* в балтийском янтаре (коллекция Каландыка, No.1905 в Музее янтарных включений кафедры беспозвоночных Гданьского университета), вид сверху.

Nearctic and Western Palaearctic Regions. *Liometopum* is found in the Baltic amber and it was also a dominant species in the North American Florissant (terminal Eocene). It is associated with oak trees even more than *P. imparis* [Wheeler, 1930], and should also be considered as a temperate element in the Priabonian myrmecofauna. It is interesting that the European species of this genus is reported to have symbiotic relationship with *Stomaphis* aphids (like *Lasius*, see Petráková, Schlaghamerský, 2011). Moreover, distribution of modern species of the *Plagiolepis pygmaea* (Latreille, 1798) group is now limited to the Europe, Korea and Japan; four species of that group are recorded in the Late Eocene European ambers [Dlussky, 2010]. In addition, five species of the *Dolichoderus quadripunctatus* (Linnaeus, 1771) group are now distributed in the Europe, Asia Minor, Caucasus, Far East and North America [Dlussky, 2002]. Dlussky and Rasnitsyn [2009] refer to the genus *Myrmica* Latreille, 1804, together with *Formica* and *Lasius*, as Palaearctic groups. However, the recent revision [Radchenko, Elmes, 2010] reveals 62 Oriental endemics out of 142 *Myrmica* species

known in the Old World. This genus therefore cannot be considered as the entirely Palaearctic group any more.

Species of *Formica*, *Lasius*, *Prenolepis*, *Liometopum*, the *Plagiolepis pygmaea* group, the *Dolichoderus quadripunctatus* group, and *Stenammas* Westwood, 1839 are considered here as “Holarctic” (“Palaearctic” sensu Dlussky and Rasnitsyn [2009]). Alternatively, there are 17 “tropical” genera, viz., 14 genera listed by Dlussky and Rasnitsyn [2009], together with *Ochetellus* Shattuck, 1992 (found in the Rovno amber, but with the present tropical distribution range) as well as with some undescribed members of the other genera from the Rovno amber, i.e. of *Pachycondyla* Smith, 1858 (now with predominantly tropical distribution, with a few exceptions [Yashiro et al., 2010]) and *Pheidole* Westwood, 1839 [Dlussky, pers. comm.]. The total species list of ants in the Rovno amber is updated to include a new species of *Monomorium* Mayr, 1855 [Radchenko, Perkovsky, 2009] as well as five additional undescribed members of other genera [Dlussky, Radchenko, pers. comm.]. As a result, the “Holarctic”



Fig.3. *P. henschei* in the Rovno amber (SIZK, K-7777), lateral view.

Рис.3. *P. henschei* в ровненском янтаре (коллекция Института зоологии НАН Украины; ИЗАНУ, К-7777), вид сбоку.

and “tropical” genera account for 21 (16.9%) and 22 (17.7%) species respectively out of 124 species in the Baltic amber, 12 (16.9%) and 19 (26.8%) species respectively out of 71 species in the Bitterfeldian amber, 7 (18.9%) and 9 (24.3%) species respectively out of 37 species in the Scandinavian amber, and 11 (17.2%) and 17 (26.6%) species respectively out of 64 species in the Rovno amber. In the Baltic amber, the number of species of the “tropical” genera is nearly the same as that of the “Holarctic” ones. However, it is 1.3 times more in the Scandinavian amber and 1.6 times more in the Bitterfeldian and Rovno ambers. Out of the five commonest ant species in the Late Eocene ambers, four species belong to modern genera, three of which are Holarctic and one is tropical. In terms of ant specimens found in the Late Eocene ambers, *L. schiefferdeckeri* is more numerous than all “tropical” ants combined [Dlussky, Rasnitsyn, 2009]. The abundance of the latter group is 3.2, 3.3, 3.8–9.4 and 2.8 times less than that of the “Holarctic” species in the Rovno and Scandinavian ambers as well as in the representative collections of the Baltic amber and in the Berlin collection of the Bitterfeldian amber respectively. Since the propor-

tion of *L. schiefferdeckeri* in the collections of amber ants strongly depends on the size of the pieces of amber [Perkovsky, 2009], we attempted to calculate the abundance of Holarctic genera excluding that species. With this exclusion, the ratio of specimens of the Holarctic vs. tropical genera is 2.3–3.6, 1.5, 1.2 and 0.9 in the representative collections of the Baltic amber, the Scandinavian and Rovno ambers as well as in the Bitterfeldian amber (the representative collection of Humboldt Museum, Berlin) respectively.

The above results are likely to reflect respective climatic and geographic differences of the source areas of the respective ambers. Specifically, the Scandinavian amber comes from the South Swedish Eocene forest [Larsson, 1978]. Moreover, according to the palaeogeographic reconstructions by Popov et al. [2009], the southwestern part of the Fennoscandian Upland is the putative initial site of origin of the Scandinavian amber (with the subsequent southwestern drift). The territory of the large deposits of the Baltic amber near the present Bay of Gdańsk was occupied by deltas with a palaeochannel situated further to the North and with the subsequent drift to the South [Popov et al.,



2009]. The territory of the contemporary Baltic Sea (from Gotland to the West and the Gulf of Finland to the East) was represented by a plain. Huge stocks of the Baltic amber in Sambia suggest the former action of river flows from large territories of the eastern part of the Fennoscandian Upland that was situated further to the North (and more distant from the coast) than the putative sources of the Danish, i.e. Scandinavian, amber in Southern Scandinavia. The age and drift direction of the Bitterfeldian amber coincide with those of the eastern Baltic one [Ritzkowski, 1999]. This conclusion agrees with the low degree of difference between the faunas of those ambers, contrary to Dlussky and Rasnitsyn [2009]. The number of individuals of the ant species unknown from the eastern Baltic amber in the representative collection from Bitterfeld (Humboldt Museum) is as low as 1.5% of all ants identified to the genus level, in contrast to 5% in the Rovno amber and 8.3% in the Scandinavian one. As for the Rovno amber, its source area is located even further to the South, i.e. at the southern coast of Subparathetis (now Volyn Upland) [Perkovsky et al., 2010b].

The hypothesis of climatic differentiation between the above mentioned types of amber allows to understand the enigmatic distribution of the easily distinguishable myrmicine genus *Fallomyrma* Dlussky et Radchenko, 2006, which was never found in the best explored ant fauna of the Baltic amber, and comprises 1.1% of Rovno ants as the most abundant member of its subfamily and 6.9% of the Scandinavian ants (the third most abundant ant in that assemblage). This justifies an assumption that *Fallomyrma* represents another thermophilous (“tropical”) ant genus that is absent from the Baltic amber due to climatic differences. Adding *Fallomyrma* to the list of the “tropical” ant genera increases their share up to 17.7, 13.1, 12 and only 3.8–9.4% of ant specimens in the Scandinavian and Rovno ambers as well as in the representative collection from Bitterfeld at Humboldt Museum and in the representative collections of the Baltic amber from Sambia and Gdańsk respectively. The number of ant specimens belonging to those 18 genera is 1.1 times more than that of the Holarctic ones (excluding *Lasius*) in the Bitterfeldian amber. However, their numbers are equal in the Rovno and Scandinavian ambers, and “tropical” ants are 2.0–3.9 times less abundant than “Holarctic” ones in the Baltic amber [Dlussky, pers. comm.].

Certain features of the amber fossil assemblages are based only on their supposed geographic and climatic differences. One of the most striking facts among those features is the high abundance of the inclusions of stellate hairs from scales of the oak buds (that are shed off in spring) in place of the needles of the amber trees. Nevertheless, this situation can be explained if we suppose that the amber trees were represented by deciduous conifers (e.g. by species of *Pseudolarix* Gordon [Anderson, LePage, 1996]), and that those trees had the strictly time-limited season of the massive resin output which coincided with the flowering time of the

oaks and predated that of the appearance of foliage of the amber trees. Moreover, this hypothesis allows to explain and reconcile a number of other observations. Specifically, the relative scarcity of workers and abundance of alate *Prenolepis* in the Rovno and Scandinavian ambers as compared to the Baltic and Bitterfeldian ones might depend on the different timing of the resin-producing period in respect to seasonal events of the ant life cycles. In the Scandinavian amber, the males of *P. henschei* comprise more than a quarter of all male ants (they are as numerous as the males of *Nylanderia pygmaea*), and this is also quite unusual for the Late Eocene ambers. The proportion of the workers of *P. henschei* in the Scandinavian and Rovno ambers, i.e. 2.8 [Dlussky, pers. comm.] and 3.5% of all workers identified to the genus level respectively is significantly less than those characteristic of the large representative collections of the Baltic amber (6.5–6.9%). It is important that the number of alates of *P. henschei* in the Scandinavian amber equals that of the worker ants. On the other hand, alates comprise 17.5% of all individuals of *P. henschei* in the Rovno amber and only 3.7% in the Baltic amber [La Polla, Dlussky, 2010]. For the latter case, the above figures are corrected according to the results of the last revision [La Polla, Dlussky, 2010]. These results show that only two gynes and a single male have been correctly identified as *P. henschei* by Wheeler [1915], whereas fifteen other gynes and males belong in fact either to *N. pygmaea* [La Polla, Dlussky, 2010] or even to *Tapinoma aberrans* Dlussky, 2002 [Dlussky, Rasnitsyn, 2009]. Consequently, G.M. Dlussky have found only eight alates (six males and two gynes) among 217 specimens of *P. henschei* in the Baltic amber including a single male in the two representative collections and two males (out of three specimens of *P. henschei*) in the carefully selected Carsten Gröhn collection [Dlussky, Rasnitsyn, 2009]. On the other hand, seven alates (six males and one gyne) and nine alates (only males) were found among 40 specimens of *P. henschei* in the Rovno amber and among 18 *Prenolepis* specimens in the Scandinavian amber respectively. The proportion of *Prenolepis* spp. among worker ants in the Scandinavian and Rovno ambers (decreasing by 1.9 times, as compared to the Baltic amber) is therefore accompanied by the increase of the share of their alates by 3.2 to 6.6 times.

We hypothesize that the abundance of the male burials in the Scandinavian and Rovno ambers could have been caused by the coincidence between the time of emergence of the males of *P. henschei* and the seasonal activity of resin traps. Since the only amber syninclusion of the gyne and male found in the Rovno amber appears to belong to *P. henschei* (among all other collections of the Late Eocene ambers, we are aware of the only other probable syninclusion of the ponerine gyne and male in the Scandinavian amber), it can be assumed that the abundance of sexuals of this species is really associated with swarming. In addition, the only syninclusion with the two alates of *P. henschei*



is known among all other specimens of the Late Eocene ambers. In contrast, workers of *P. henschei* greatly outnumber their sexuals in the Baltic amber. This could be explained by the situation when the resin traps in the Baltic amber forest were active at lower temperatures, which was probably favourable for foraging (but not for swarming) of *P. henschei*.

Wheeler [1915] paid a considerable attention to the findings of the tropical and temperate ants as syninclusions in the same pieces of amber. However, all his examples of those syninclusions involved either individuals of *Ctenobethylus* Brues, 1939 as a tropical component (although its tropical nature is questioned now due to its similarity to or even synonymy with *Liometopum*), or the cases of co-occurrence of two tropical or two Holarctic genera. Nevertheless, findings of the tropical and temperate ants in the same syninclusions were confirmed by Dlussky [pers. comm.]. Specifically, there are four syninclusions of *Iridomyrmex geinitzi* (Mayr, 1868) in the Rovno amber, i.e. two with *Formica* spp., one with *L. schiefferdeckeri* and another one with *Dolichoderus polessus* Dlussky, 2002 from the *Dolichoderus quadripunctatus* group. The Bitterfeldian amber provides a syninclusion of *Oecophylla* Smith, 1860 with *Lasius*; the Baltic amber shows syninclusion of *Gesomyrmex* Mayr, 1868 with *Formica* (the same piece of amber also contains many *Germaraphis* aphids and coccids), two syninclusions of *I. geinitzi* together with *Formica*, and another one of *I. geinitzi* together with a species of the *Plagiolepis pygmaea* group. In addition, there are syninclusions of the extinct genera from the tropical tribes with the Holarctic ant genera, specifically *Bradoponera* Mayr, 1868 with *Formica* in the Bitterfeldian amber and with *Lasius* in the Baltic one as well as a syninclusion of *Procerapachys* Wheeler, 1915 with *Lasius* in the Baltic amber [Dlussky, pers. comm.]. However, *Prenolepis* was never found in syninclusions with any of the mentioned thermophilous ants either by Wheeler [1915] or Dlussky [pers. comm.]. In particular, the thermophilous ant *Iridomyrmex geinitzi* which is known by more than a thousand specimens, is the only one out of the five most common amber ants that has never been found in syninclusions with *P. henschei*. These observations imply effective thermal (most probably seasonal) divergence between the least and the most thermophilous ants in the Late Eocene amber forests. The cases of the two “Holarctic” genera are particularly instructive. The amber inclusions of *Formica* spp. are more numerous by one-third than those of *Prenolepis*, although they are recorded in five syninclusions with thermophilous ants (which constitutes one-third of all *Formica* syninclusions with other ants), while *Prenolepis* was never found in syninclusions of that kind [Dlussky, pers. comm.].

In addition to its cryophily, or negative thermotaxis, there is another feature of the life history of *P. imparis*, i.e. its failure to keep root aphids or coccids in ant nests, unlike species of the genus *Lasius* and many

other groups [Wheeler, 1930]. Nevertheless, Wheeler [1930] found that *P. imparis* can effectively utilize honeydew obtained from outside the nest due to its ability to develop “repletes”. It is therefore not surprising that foragers of *P. imparis* from three different colonies gained an average of 130 to 200% of their original body weight while feeding with syrup [Lynch et al., 1980], and corpulent individuals converted carbohydrates to other reserve substances stored in their fat bodies.

Another unusual feature of *Prenolepis* is that syninclusions with more than one worker of *P. henschei* are very rare if compared with the abundance of that species in amber. Although more than 600 specimens of *P. henschei* were found up to now [Dlussky, Rasnitsyn, 2009], only ten syninclusions of two workers of *P. henschei* are known, specifically: two in the Kalandyk collection (see Table 1); three in the Museum of Earth, Warsaw; three in the Paleontological Institute, Moscow; one in the Zoological Museum of the University of Copenhagen (all in the Baltic amber), and another one in the Rovno amber (SIZK). Moreover, findings of more than two workers in the same piece of amber have never been recorded.

Interestingly, in the whole representative collection by Tadeusz Giecwicz (amber from the Western part of the Bay of Gdańsk in the collection of the Museum of Earth) there were only two pieces of amber (out of 24 pieces with *P. henschei*), each containing two workers. The Kalandyk collection with only six pieces of amber with this species (Table 1) also includes two pieces, each containing a pair of workers of *P. henschei*. We believe that this could be explained by the larger size of the pieces of amber in the Kalandyk collection.

At the same time, six syninclusions of *P. henschei* with other ant species are known only in the Rovno amber, i.e. two pieces of amber with *C. goepperti* (one of them, a large piece with a headless worker of *Prenolepis*, also contains *Germaraphis*), two with *L. schiefferdeckeri*, one with *Formica* sp. and another one with an undetermined ant. The collection of the Baltic amber of the Museum of Earth also has two syninclusions of the workers of *P. henschei* with *C. goepperti* and another one with *Camponotus mengei* Mayr, 1868 [Dlussky, pers. comm.].

Other ants of the Rovno and Baltic ambers are more common in syninclusions with the workers of their own species [Perkovsky, 2009]. This is probably explained by the fact that *P. henschei*, like *P. imparis*, and unlike other common ants of the Late Eocene ambers, neither kept aphids in their nests (which might result from the specific seasonal activity of *Prenolepis*), nor intensively exploited aphid colonies that are tended by ants having large families. Solitary foraging could therefore result in the individual burial of *P. imparis* in the resin traps. This also explains the situation when only one out of six ant syninclusions with the workers of *P. henschei* in the Rovno amber contains two workers of

that particular species. Moreover, *P. imparis*, the extant counterpart of *P. henschei*, does not provide tree aphids with an effective protection. Specifically, tending by *P. imparis* does not affect the abundance of the aphids, in contrast to that by the Argentine ant *L. humile* when the aphids double their amount under protection by the ants [Nygard et al., 2008]. Nygard et al. [2008] demonstrated that if aphid colonies were tended only by *P. imparis*, they disappeared in late spring due to destruction of the unprotected colonies in the absence of the ants.

A few solitary foragers are able to collect honeydew from small aphid colonies, especially because the foragers of *P. imparis* can keep large amounts of carbohydrates in their proventriculi (see above). On the other hand, large aphid colonies have to be protected by dominant ants living in large colonies themselves (see [Petráková, Schläghamerský, 2011]). In the only one Rovno amber piece with *Prenolepis* containing three ants, two of them (*P. henschei* and an undetermined ant) are represented by isolated head capsules. Moreover, the head capsule of *P. henschei* has only remained in a particular syninclusion with *L. schiefferdeckeri*. This could result from an antagonism between *P. henschei* and other amber ants. The antagonism between *P. henschei* and *L. schiefferdeckeri* could occur due to the defense of aphid colonies by the latter species against the foragers of the former one.

## Conclusions

We assume that the ants *Prenolepis henschei* were not dendrobiotic and they foraged on amber trees mainly looking for homopterans (particularly aphids) which they did not keep in their nests. The ant *P. henschei*, similarly to *P. imparis*, could supported large aphid colonies nor recruited many workers for a small colony, because a single forager was able to collect sufficient volumes of honeydew from a small colony. The relationship between *P. henschei* and *Germaraphis* was not obligatory since it depended on the abundance of the aphids. Foragers of *P. henschei* could interfere with other common tree-visiting amber ants (including those keeping aphids in their nests), but generally managed to avoid their aggression due to alternative seasonal and circadian activities. This conclusion is supported by the rarity of syninclusions of *P. henschei* with other ant species in the Baltic amber as well as by the total absence of those with the thermophilous ants recorded from any Late Eocene European amber. Since their syninclusions with the conspecific workers are rare, this could indicate predominantly solitary foraging on the amber trees. The activity of the resin traps is supposed to be time-limited, and we hypothesize that they were active in the Baltic amber forest at the relatively lower temperatures as compared to those of the Scandinavian and Rovno amber forests. This hypothesis explains the low share of workers and the high numbers of males of *P. henschei* in the Scandinavian and

Rovno ambers compared to the Baltic one. In addition, we suppose that *Prenolepis* ants started to develop corpulent workers (“repletes”) by the Late Eocene as the key adaptation to their unusual type of seasonal activity also known for modern *P. imparis* and that this allowed them to compete successfully with aggressive thermophilous ants.

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