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What are the most typical leaf mines of Nepticulidae? Identified diagnostic characters and their detection frequency

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This study identifies major diagnostic morphological and some biological characters of leaf mines of Nepticulidae and provides a pictorial guide to the morphological characters with detection frequencies for each character. Drawing on detection frequencies, it establishes that the most typical leaf mine of Nepticulidae belongs to one of the two morphological types of (1) serpentine, gradually slightly widening and (2) serpentine, strongly widening, and that it can be characterised by black or brownish black frass accumulated in a solid line or band with wide clear margins of the gallery. Such a typical leaf mine is sinuous or contorted and, as a rule, found on Rosaceae or Betulaceae; the feeding larva is yellow.

Keywords: leaf mine morphology, Lepidoptera, pygmy moths, species diagnostics

INTRODUCTION

Morphologically and biologically, leaf mines of lepidopterans are very diverse: depending on the larval mode of feeding, from epidermal to parenchymal, upper or lower sided or bilateral, or capturing both layers of the parenchyma. Leaf mines can also be diverse in their morphological type: star-shaped, folded, swollen (Gerasimov, 1952), or of the morphological types described and illustrated in our paper. The mining can occur in all stages of the larval development or only in the early instars. Sometimes pupation also occurs in the mine.

Plant miners from the order Lepidoptera belong to various and sometime phylogenetically distant families. The diversity of mines is associated both with the diversity of the families of Lepidoptera themselves, and, often, with the diversity within the same family of mining moths. For example, in the family Bucculatricidae, in which species from the genus *Bucculatrix* predominate, leaf mines are usually slender, thread-like, or spiral, not convoluted, and relatively short (Baryshnikova, 2013). In the taxonomically diverse family Gracillariidae, leaf mines are very diverse in their shape and arrangement (Ellis, 2020).

In some groups of lepidopterans, the morphological type of mines makes it possible to almost unmistakably identify the genus of the miners and, sometimes, with a high degree of probability, to determine their species affiliation. For example, predominantly lower-sided and folded mines in leaves

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are produced by representatives of the genus *Phyllonorycter*, and long, tortuous mines inside the epidermis on the upper side of the leaves are produced by larvae of the genus *Phyllocnistis*, both from the family Gracillariidae.

Some examples showing the diversity of leaf mines of Tischeriidae were provided in the recent papers by Stonis et al. (2019, 2020, 2021).

The family of pygmy moths (Nepticulidae) deserves special consideration. Mines of Nepticulidae are very diverse (Johansson et al., 1990; Puplesis, 1994; Puplesis, Diškus, 2003; Diškus, Stonis, 2012) and, in some cases, can be used for species identification. Meanwhile, there is lack of diagnostic tools that could be used not only by biotaxonomists but also by other users.

The goal of this study is to present a short morphological review with identification of diagnostic characters and their detection frequencies in the studied sample and characterisation of the most typical leaf mine of Nepticulidae from the temperate forest biome.

MATERIALS AND METHODS

The analysis encompassed all 77 species of Nepticulidae (Stonis et al., 2022) currently known in the Lithuanian fauna. Lithuania, with the area of 65,200 km^2, is located in the Baltic region of Europe and lies at the edge of the North European Plain, between latitudes 53° and 57°N and mostly between longitudes 21° and 27°E. Therefore, the studied leaf mines belong to the fauna of the temperate forest biome.

For each species of the sample, a character-coding datasheet was compiled. In this datasheet, morphological (as well as some biological) characters were marked (coded). Although most species of Nepticulidae were characterised by only one character in each group of characters, there were species where two or three characters were selected in the same character group. For example, if a species was characterised by a wide line of frass leaving wide clear margins but the same species was also detected with a wide band of frass leaving slender clear margins, both characters were marked in the datasheet and counted later in our analysis. The detection frequency revealing the variability of the leaf mines of each individual species was ignored and not counted.

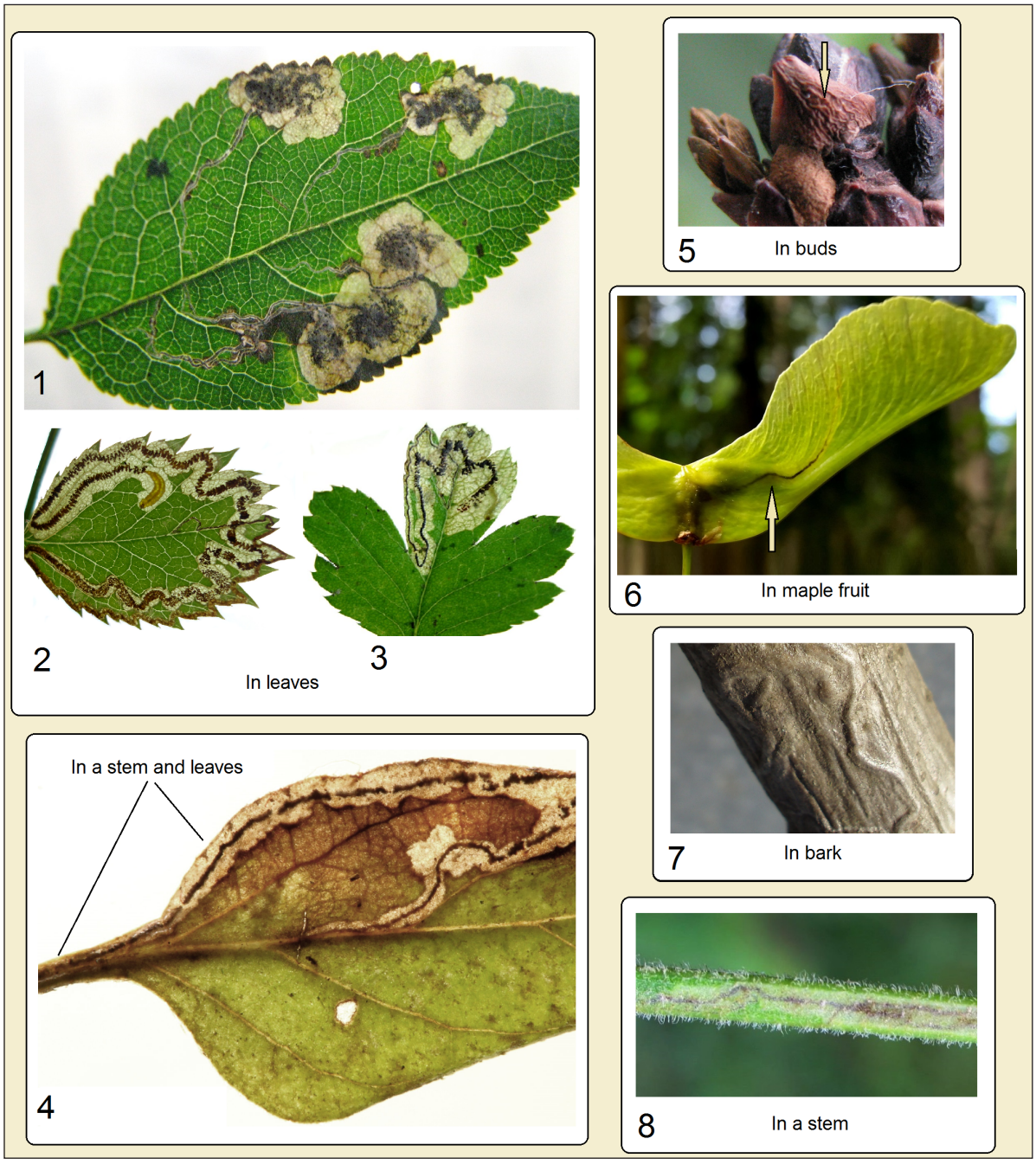
Since some species of Nepticulidae are characterised by variability of leaf mines, we aimed to check as many leaf mines or digital photographs of each species as possible. The lowest (minimal) number of mines inspected (3–4) was in the case of very few species that were poorly collected or understudied in Lithuania. Mostly 10–150 or more leaf mines of each species were examined (with $n = 15$).

In the case of this particular study, the detection frequency is the number of cases that a character was detected in the given number of the examined sample, i.e., 77 species of the Lithuanian fauna. The detection frequency was expressed in percentage (Frequency Index). The concept of frequency refers to the uniformity of a character in the sample. No further counting was involved, only the record of character presence.

GROUPING AND IDENTIFICATION OF MORPHOLOGICAL CHARACTERS OF LEAF MINES AND THEIR DETECTION FREQUENCY

Because of trophical association of monophagous or oligophagous Nepticulidae species to certain plants, a hostplant species, genus or hostplant family are very important diagnostic characters that can be used in the identification of Nepticulidae species (Stonis et al., 2022). However, both morphological and biological characters of leaf mines (including the colour of feeding larva and season of mining) can be used to characterise and identify leaf mines of pygmy moths.

The list of characters below applies only to those species of Nepticulidae larvae which produce mines in plant leaves (96% of the examined sample). Nepticulidae mines can also be found in green plant stems, bark, buds, or maple fruit (samara) (Figs 1–8). The morphology of such mines is not discussed in the article because they are rare and difficult for morphological study and because the current article confines itself to leaf mines.



Figs 1–8. Nepticulidae mines can be found in green plant stems, bark, buds, or maple fruit (samara)

On the basis of the examined sample, the following most important diagnostic features and their groups were distinguished, and detection frequency for each character was indicated. In order to help the user to navigate the diversity of leaf mines, a pictorial guide to these characters is provided in this paper (Figs 9–93).

Morphological types of leaf mines:

spiral (detection frequency 5.2%);

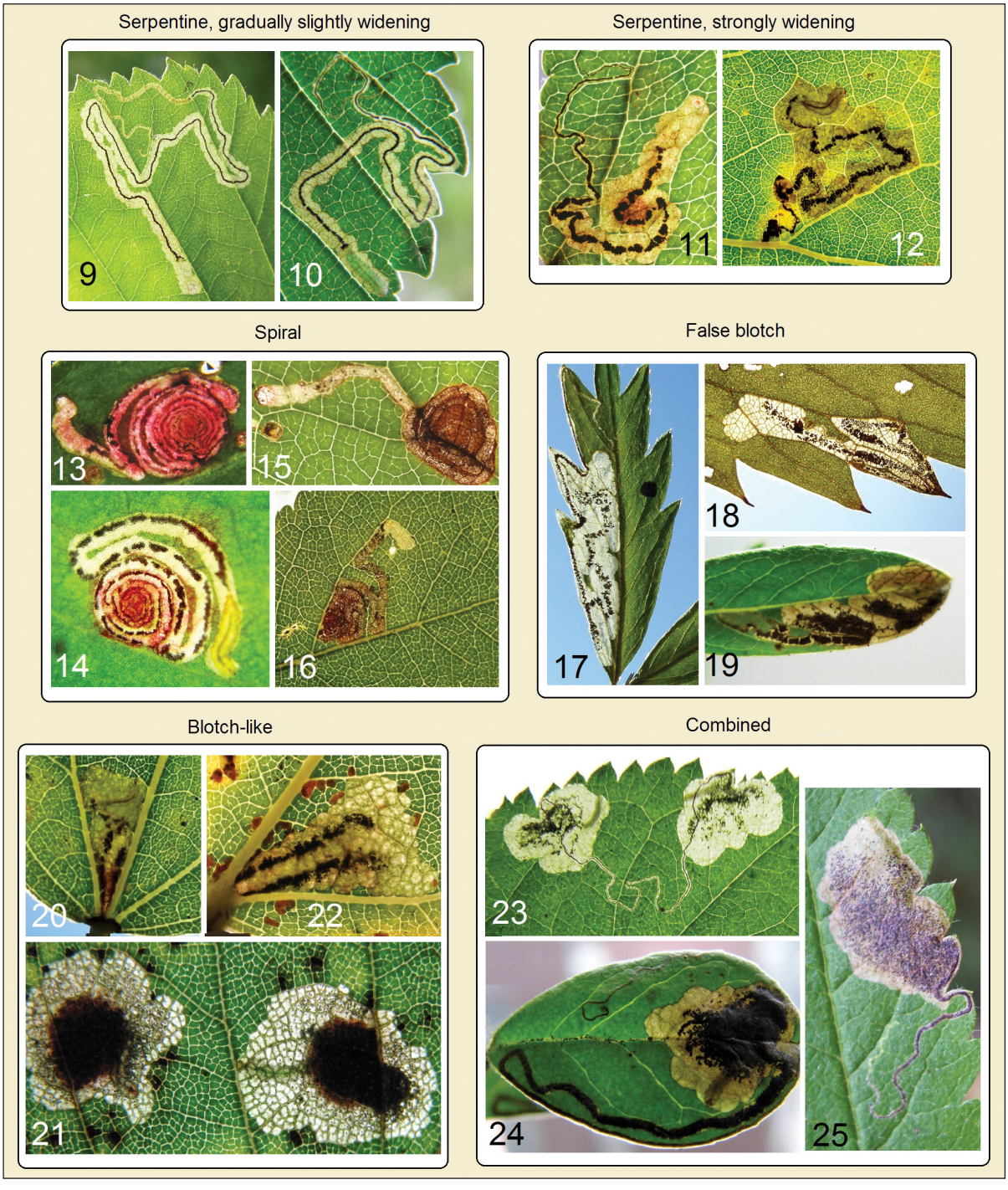
serpentine, gradually slightly widening (50.7%);

serpentine, strongly widening (45.5%);

false blotch (22.1%);

combined (a slender gallery abruptly widening into a blotch) (15.6%);

blotch-like (12.9%).



Figs 9–25. Morphological types of leaf mines

Winding of leaf mines:

sinuous (detection frequency 58.5%);

contorted (59.8%);

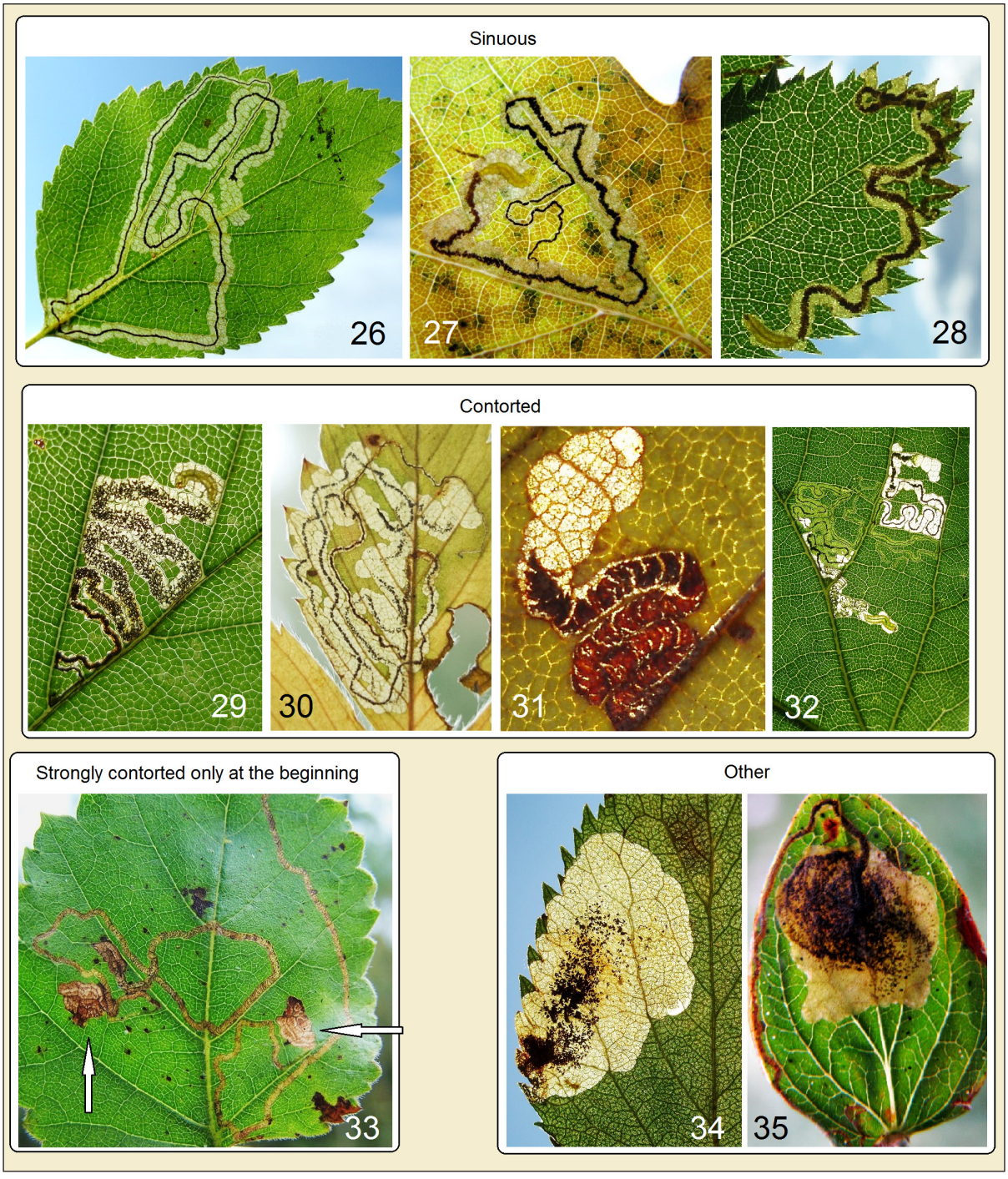
strongly contorted only at the beginning (3.9%);

other (24.7%).

Filling of leaf mines with frass:

a slender line of frass (leaving wide clear margins of the gallery) (detection frequency 38.9%);

a wide line of frass (leaving wide clear margins) (53.3%);



Figs 26–35. Winding of Nepticulidae leaf mine

a wide band of frass (leaving slender clear margins) (37.7%);

the entire width of the gallery filled with frass (18.2%);

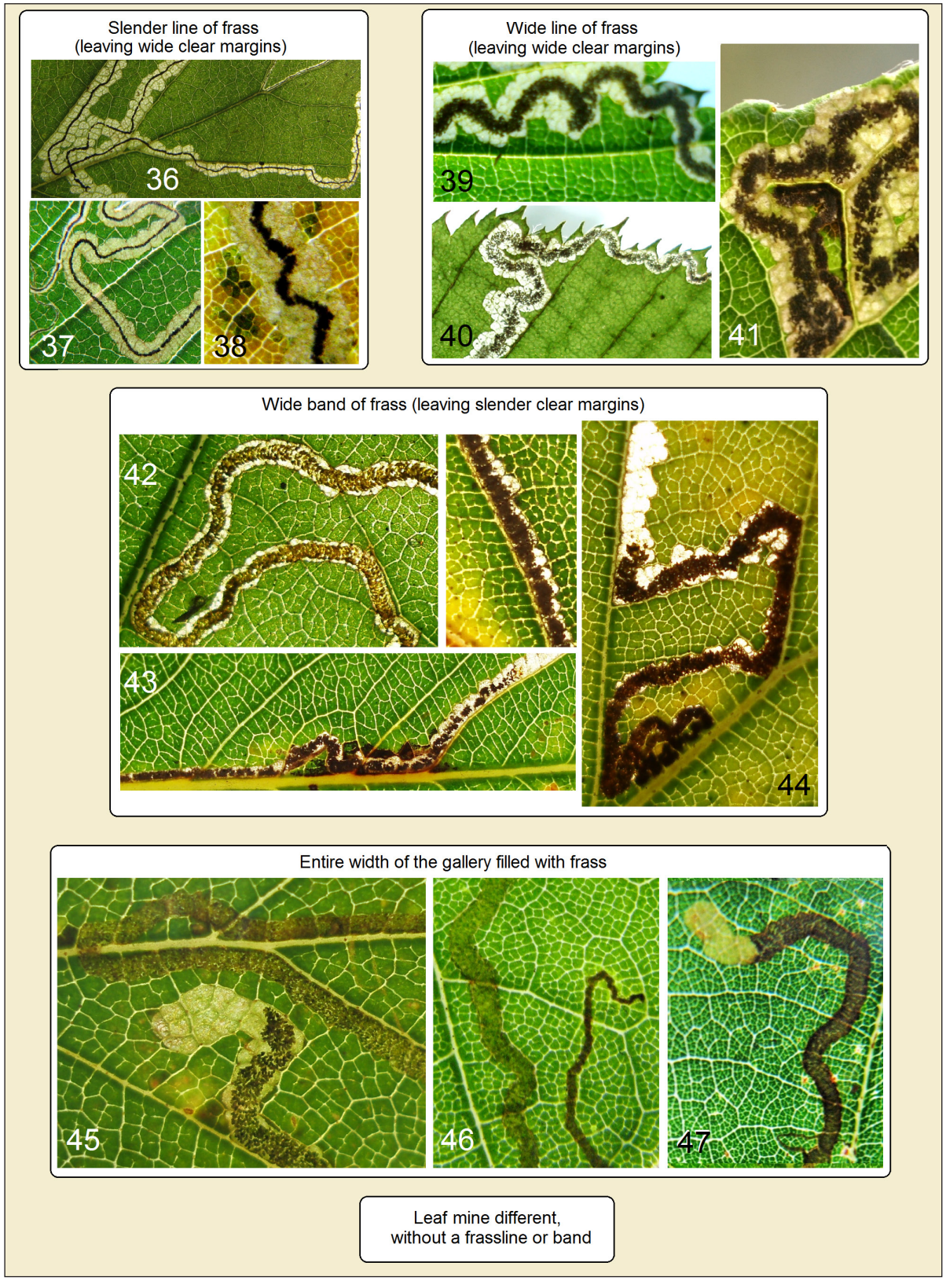
a different leaf mine, without a frassline or a band (23.4%).

Leaf mines at the beginning:

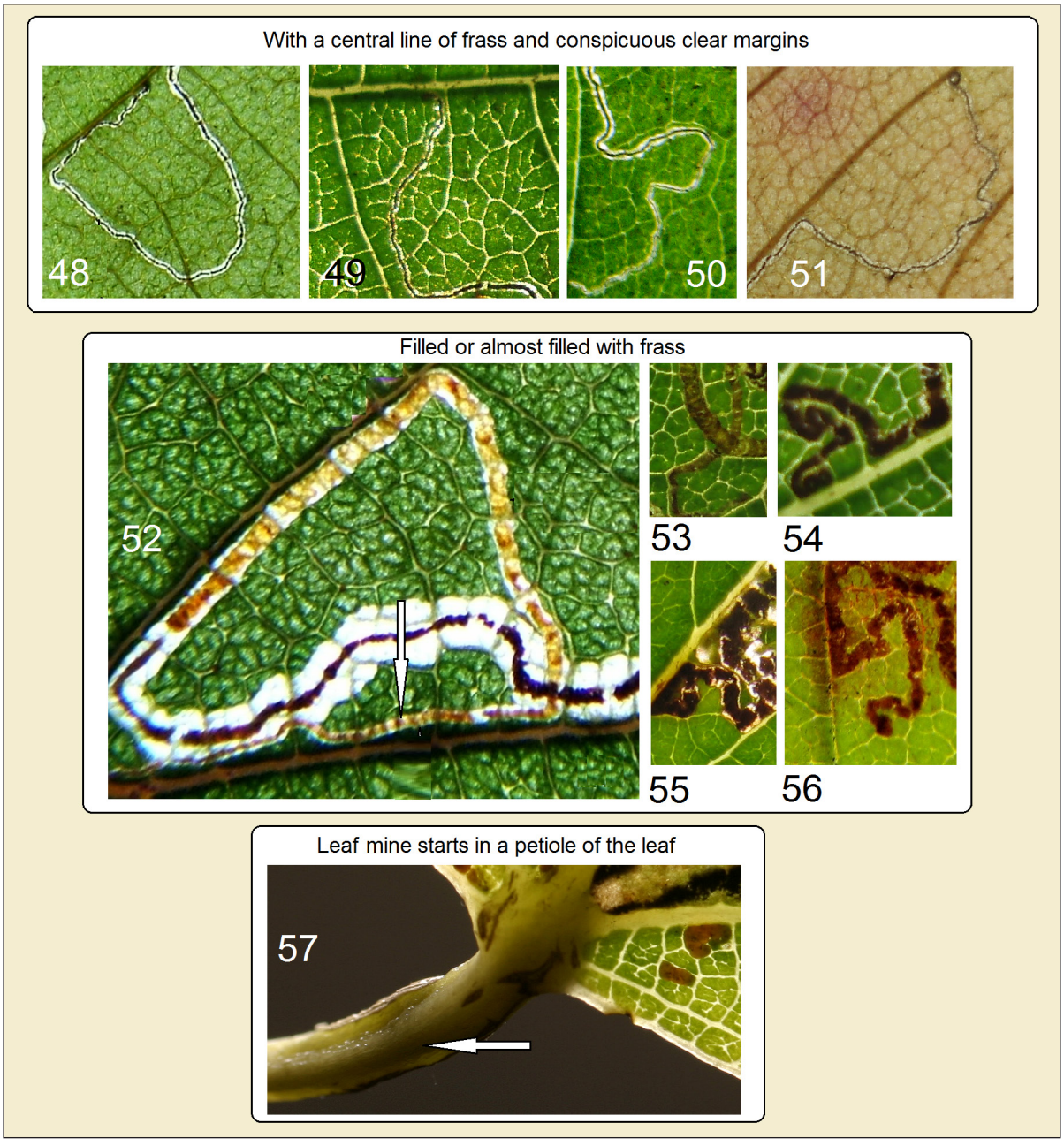
with a central line of frass and conspicuous clear margins (detection frequency 57.2%)

filled or almost filled with frass (62.4%);

leaf mine starts in a petiole of the leaf (6.5%).



Figs 36–47. Filling of Nepticulidae leaf mine with frass



Figs 48–57. Nepticulidae leaf mine at the beginning

Frass deposition:

accumulated in a solid line or band (detection frequency 72.7%);

frassline interrupted ('a dotted line') (27.3%);

frass is coiled (at least at some parts) (33.8%);

frass accumulated in a large blotch or oblong clumps (28.6%);

most of the frass irregularly scattered (35.1%).

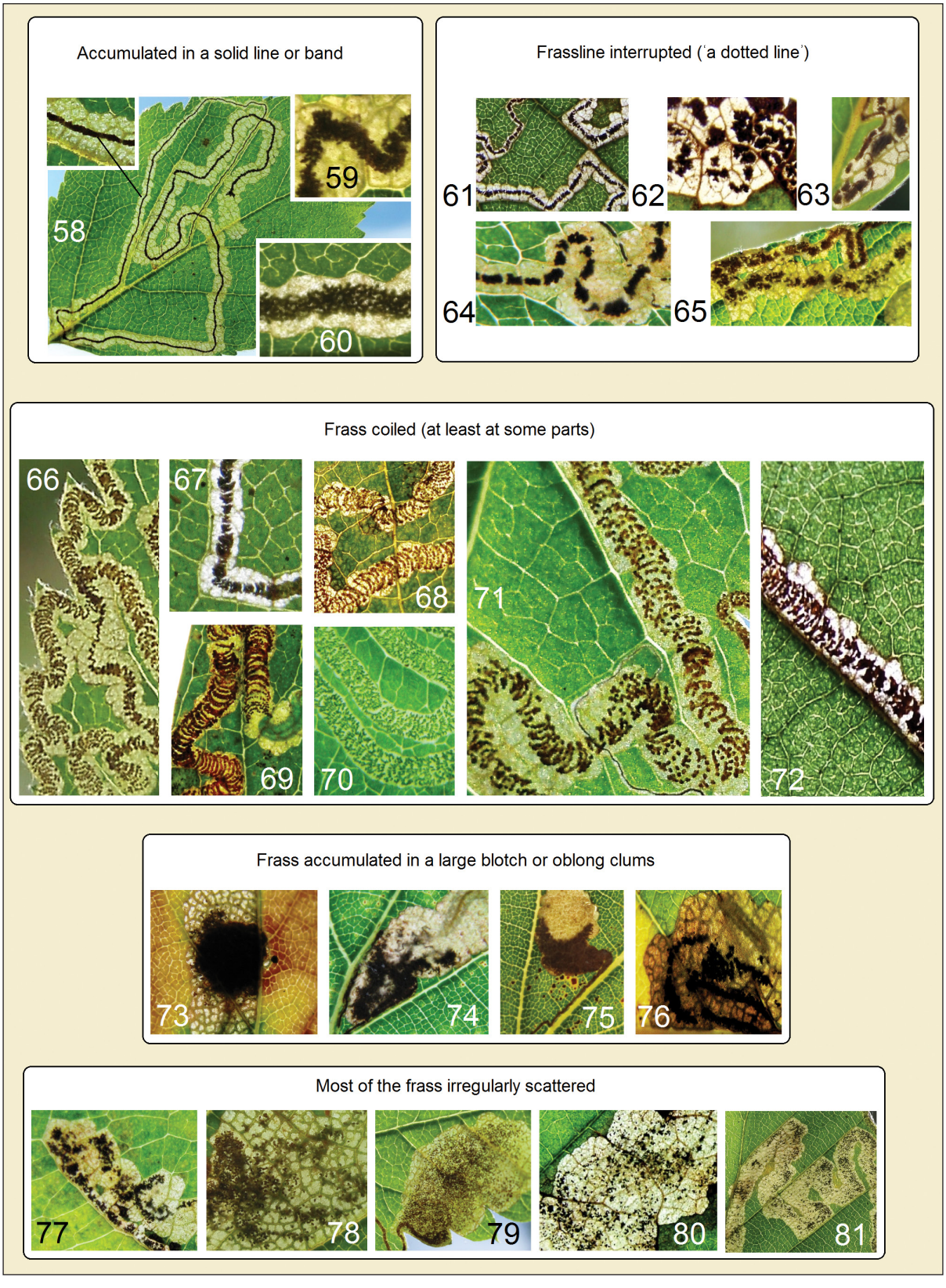
Frass colour:

black or brownish black (detection frequency 88.3%);

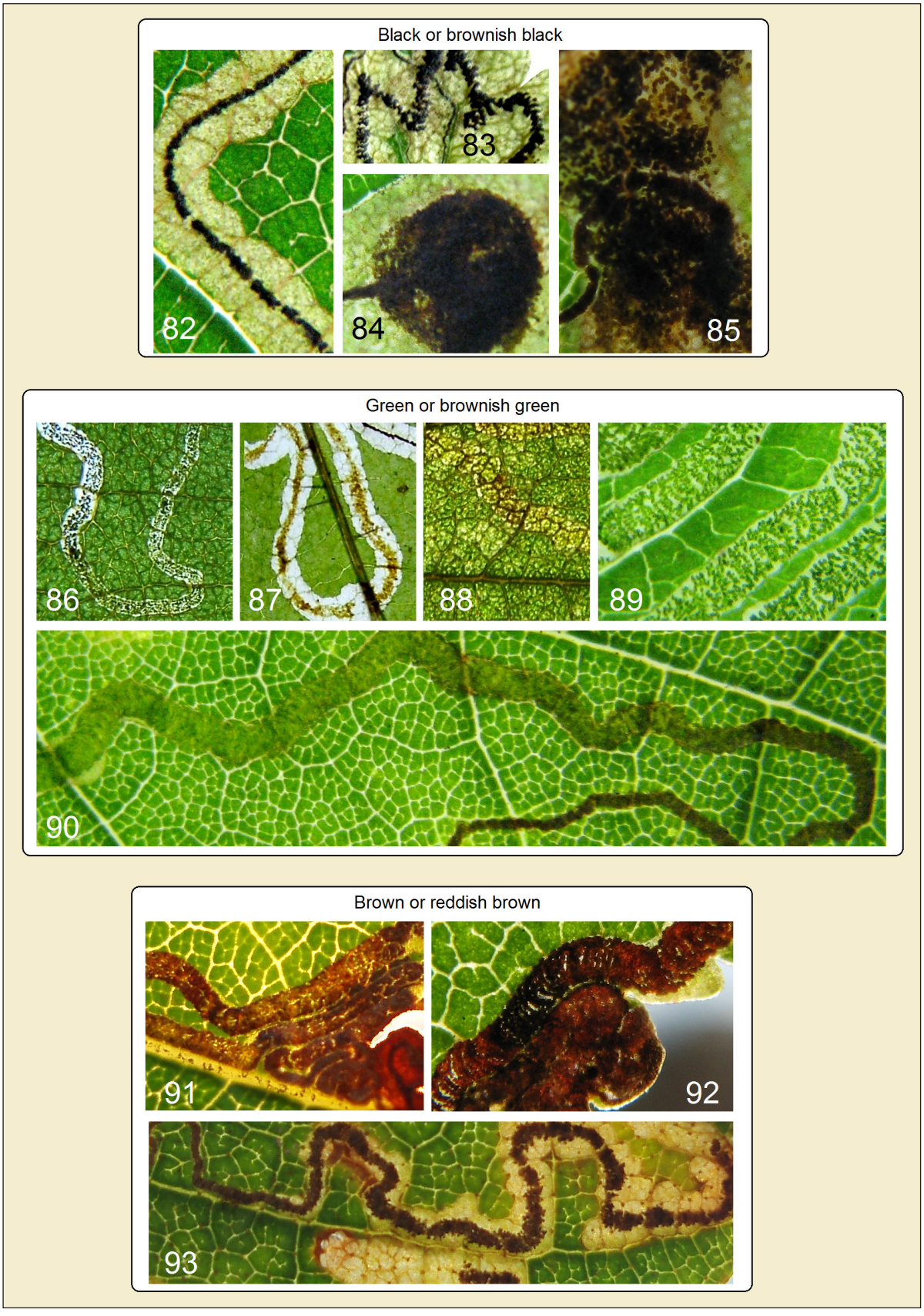
brown or reddish brown (32.5%);

green or brownish green (all or part of it) (19.5%).

Some other, mostly biological, characters were additionally examined in the studied



Figs 58–81. Frass deposition in Nepticulidae leaf mines



Figs 82–93. Frass colour in Nepticulidae leaf mines

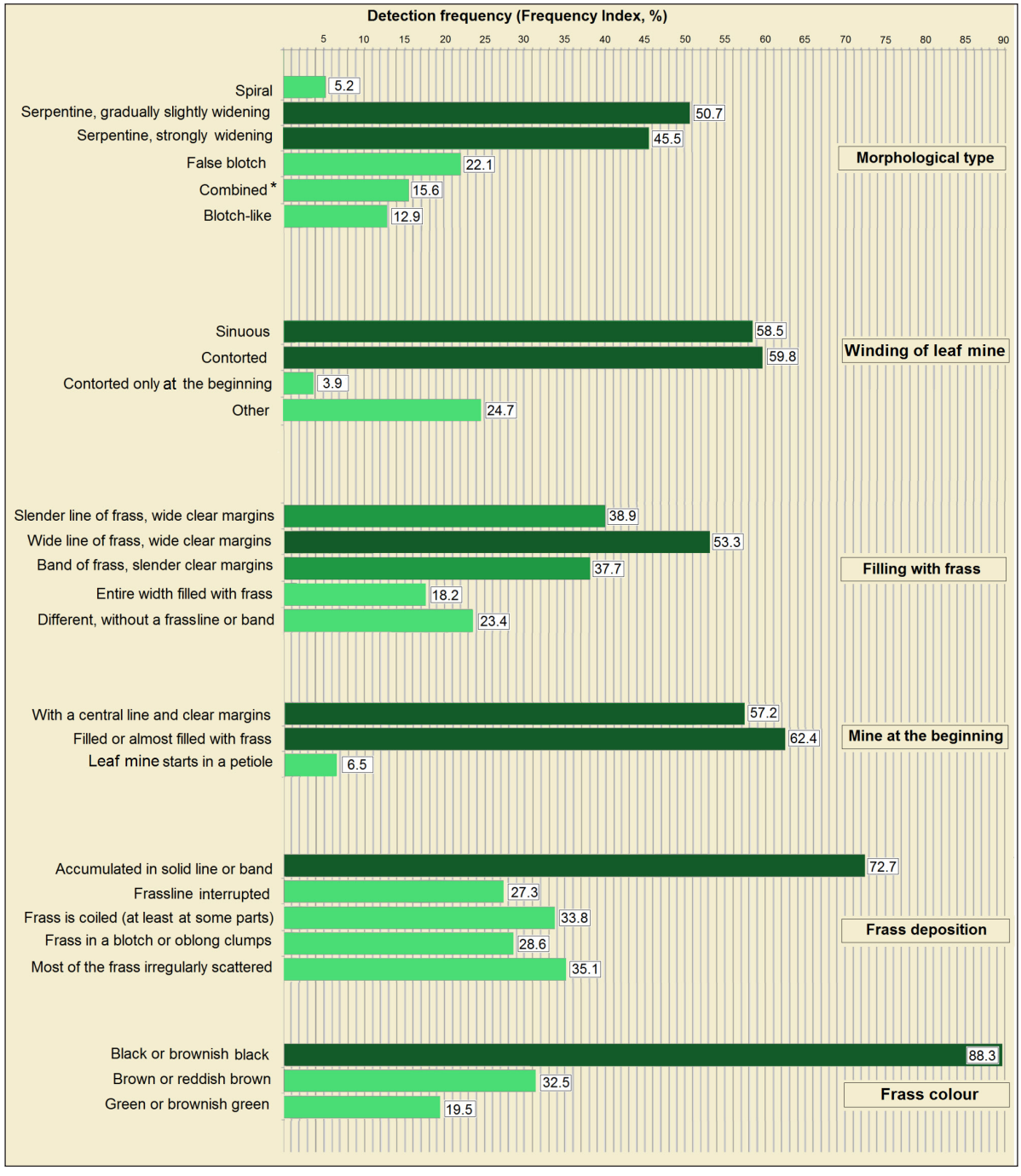


Fig. 94. Detection frequencies of Nepticulidae leaf mine characters based on the analysis of the sample from the temperate forest biome

sample: the colour of the feeding larva is often yellow (detection frequency 75.3%); the majority of the host plants belong to Rosaceae (detection frequency 34%), Betulaceae (17%), Salicaceae (16%), and Fagaceae (13%).

The diversity of the Nepticulidae leaf mines is not limited to the characters listed above. For the identification of leaf mines, some other characters can be also helpful, e.g., the side of the leaf (underside or upper side) on which the leaf mine occurs and the egg is attached (close to the leaf veins or beside it, the underside or the upper side of the leaf); whether or not the larger veins of the leaf of the plant are

cut through by larva during the formation of the mine; whether the mining caused any discolouration of the damaged leaf, etc. Sometimes the season of mining itself can be important, i.e., the time of the year when the mines contain feeding larvae (Stonis et al., 2022).

DISCUSSION

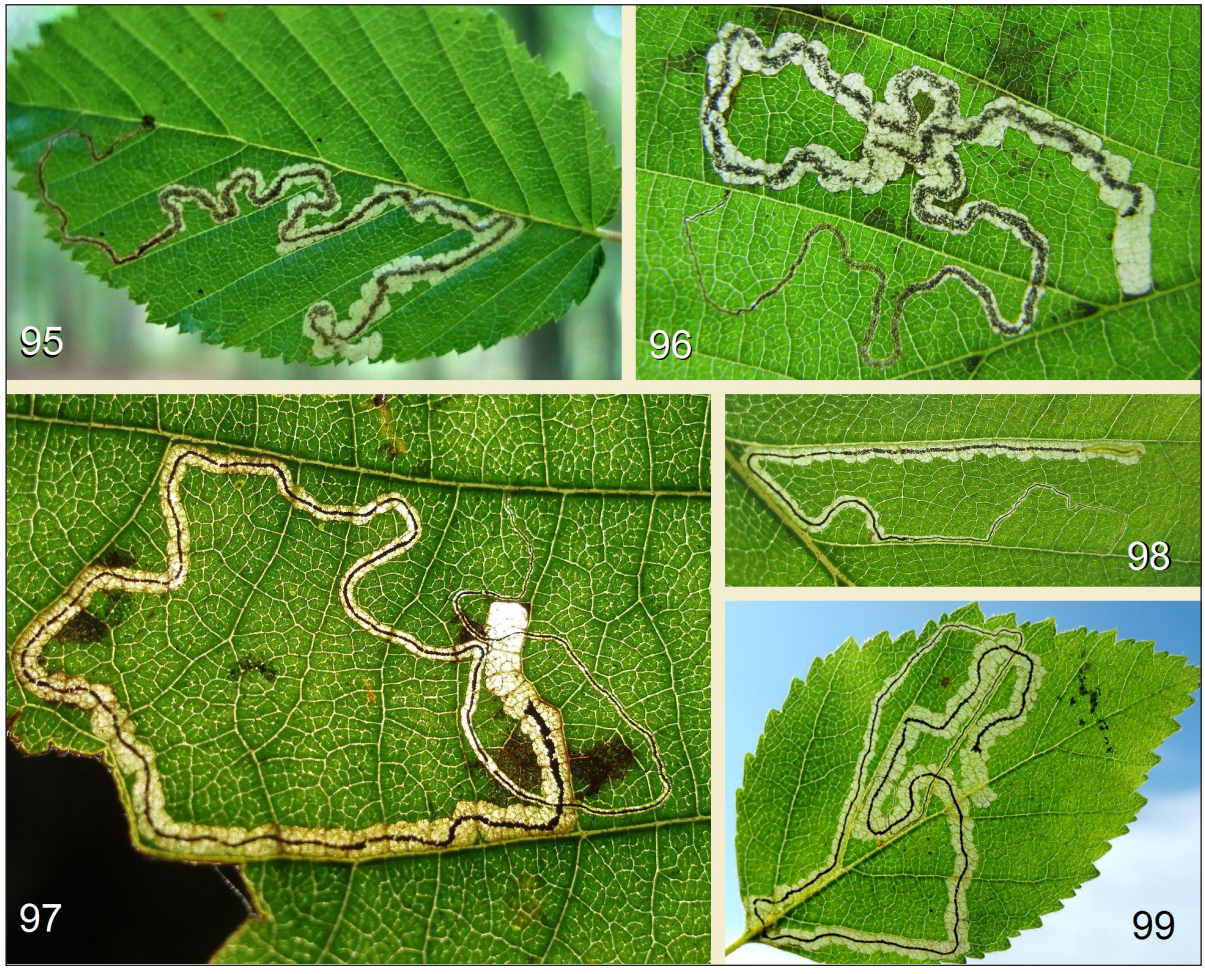
Building on the detection frequencies (Fig. 95), the most typical Nepticulidae leaf mine can be described as belonging to one of the two morphological types of (1) serpentine, gradually slightly widening, and (2) serpentine, strongly widening, with black or brownish black frass accumulated in a solid wide line but leaving wide clear margins. The leaf mine of such a typical species is sinuous or contorted and, according to the indicated detection frequencies, is found on Rosaceae or Betulaceae. The feeding larvae were yellow in two-thirds of the studied sample; therefore, it can be speculated that a typical leaf mine has to be produced by a yellow (not green or whitish) larva.

It was interesting to check which Nepticulidae species fully match the description of the most typical leaf mine. It was found that many European species conformed to the characteristics of the most typical leaf mine. However, a full match was found only in the case of *S. carpinella*, *S. floslactella*, *S. alnetella*, and *S. minusculella* (Table, Figs 95–96). The leaf mines of these species have 'accumulated' all morphological characters that are the most widespread among the leaf mines of Nepticulidae; moreover, their larvae feed on Rosaceae or Betulaceae and they are yellow.

Having found out that the most commonly detected characters (or the states of characters) have a high Frequency Index (>50%), the fact that only three species fully coincided with the description of the typical species, i.e., had a complete set of the commonest characters, is rather surprising. However, the cases when species showed an overlap with the most common characters by 70–90% occurred in about one-third of the analyzed sample. Since our sample included only 77 species of the geographically limited Lithuanian fauna, we did not attempt to model the evolution of characters and, therefore, we did not use packages such as Phytools (version 1.0-1) contMap: Map continuous trait evolution

Table. **Nepticulidae species matching the description of the typical leaf mine**

	Species	Leaf mine morphology	Other, biological, characters	
		Matching morphological of the typical leaf mine	Larva yellow	Leaf mine on the most characteristic host-plant families: Rosaceae or Betulaceae
1.	*Stigmella carpinella* (Heinemann) (Fig. 95)	**Full match**	**Yes**	**Yes** (Betulaceae)
2.	*Stigmella floslactella* (Haworth) (Fig. 96)	**Full match**	**Yes**	**Yes** (Betulaceae)
3.	*Stigmella alnetella* (Stainton) (Fig. 97)	**Full match**	**Yes**	**Yes** (Betulaceae)
4.	*Stigmella confusella* (Wood & Walsingham) (Figs 98, 99)	**Full match**	No, other	**Yes** (Betulaceae)
5.	*Stigmella minusculel*la (Herrich-Schäffer)	**Full match**	No, other	**Yes** (Rosaceae)
6.	*Stigmella lediella* (Schleich)	Similar	**Yes**	No, other
7.	*Stigmella pretiosa* (Heinemann)	Similar	**Yes**	**Yes** (Rosaceae)
8.	*Stigmella malella* (Stainton)	Similar	**Yes**	**Yes** (Rosaceae)
9.	*Stigmella roborella* (Johansson)	Similar	**Yes**	No, other



Figs 95–99. The most typical leaf mines of Nepticulidae. 95 – *Stigmella carpinella*; 96 – *S. floslactella*; 97 – *S. alnetella*; 98, 99 – *S. confusella*

on the tree. However, in the future, this could be done with a sample from a large region.

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TIPIŠKIAUSIŲ MAŽŲJŲ GAUBTAGALVIŲ MINŲ NUSTATYMAS: IDENTIFIKUOTI NEPTICULIDAE MINŲ DIAGNOSTINIAI POŽYMIAI IR JŲ APTIKIMO DAŽNIS

Santrauka

Buvo sugrupuoti ir įvardyti pagrindiniai diagnostiniai morfologiniai ir biologiniai mažųjų gaubtagalvių (Nepticulidae) minų požymiai. Straipsnyje taip pat pateikiamas iliustratyvus minų diagnostinių požymių ir jų aptikimo dažnio vadovas. Nustatyti šie tipiškų mažųjų gaubtagalvių minų požymiai: priklauso gyvatiškųjų minų morfologiniams tipams su šiek tiek tolygiai platėjančiu ir labai platėjančiu minos taku; juodi ekskrementai, sutelkti į ištisinę liniją arba juostą su plačiais neužpildytais minos tako kraštais; mina vingiuota arba susiraizgiusi, o lerva – geltona, minuoja Rosaceae bei Betulaceae augalus. Tokie požymiai būdingi šioms rūšims: *Stigmella carpinella*, *S. floslactella*, *S. alnetella* ir *S. confusella*.

Raktažodžiai: lapų minos, Lepidoptera, mažieji gaubtagalviai, Nepticulidae, rūšių diagnostika