RESEARCH ARTICLE



A new species of *Mesoneura* (Hymenoptera, Tenthredinidae) associated with a xerothermic oak forest in the Western Carpathians, Slovakia

Ladislav Roller¹, Ján Kočišek¹

I Institute of Zoology, Slovak Academy of Sciences, Dúbravská cesta 9, 84506, Bratislava, Slovakia

Corresponding author: Ladislav Roller (ladislav.roller@savba.sk)

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Abstract

A new species of tenthredinid sawfly, *Mesoneura tematinensis* Roller, **sp. nov.**, was discovered in the Tematinske kopce Mountains in the Western Carpathians in Slovakia. Adults of both sexes and larvae of different stages are described and illustrated. Based on morphology and DNA barcoding, the new species is closely related to *Mesoneura opaca* (Fabricius), a widespread oak sawfly in Europe, with which it occurs in the same locality and shares a common host plant. Larvae of the new species are part of a rich assemblage of a total of 13 Symphyta species that feed on leaves of the pubescent oak *Quercus pubescens* in a thermophilic supra-Mediterranean forest. A key to the European species of *Mesoneura* Hartig is provided.

Keywords

assemblage, biology, key, larva, Quercus pubescens, Symphyta, taxonomy

Introduction

Thermophilic forests dominated by the pubescent oak (*Quercus pubescens*), especially in southern Europe, provide a suitable habitat for a unique assemblage of sawflies (Lacourt 2020). In Central Europe, this habitat is rare, threatened and often protected. As part of the ongoing research on the Symphyta fauna of Slovakia, we surveyed

several sites with pubescent oaks, which provided new data on the occurrence of rare species, including some first records for the territory of Slovakia (Roller 2004; Macek et al. 2020; Smetana et al. 2020). The surveys in the Tematínske kopce (Tematín Mountains) with Malaise traps also brought the discovery of the rare sawfly *Periclista lenta* Konow, 1903 (= *vernalis* Lacourt, 1985), whose biology is unknown (Roller 2004; Macek et al. 2020). While searching for larvae of *P. lenta*, we found for the first time the larvae of an unknown sawfly species belonging to the genus *Mesoneura* Hartig, 1837.

Mesoneura is a small genus of the family Tenthredinidae and the subfamily Nematinae with nine extant species distributed in the Palaearctic region (Wei et al. 2013; Taeger et al. 2018). Only two species, *Mesoneura opaca* (Fabricius, 1775) and *M. lanigera* Benson, 1954, occur in Europe (Liston 2012). The larvae of both species feed on fresh leaves of various oaks (*Quercus* spp). *Mesoneura opaca* is associated with most oak species throughout Europe, while *M. lanigera* is known only from the Transcarpathian region, Crimea, the North Caucasus (Stavropol region) and Cyprus, where its hosts are *Q. pubescens* and *Q. infectoria* (Ermolenko 1967; Liston and Späth 2008). In this article we describe the third European species of *Mesoneura* and provide information on its biology and the assemblage of sawflies it is involved in.

Materials and methods

Sampling of Symphyta was carried out in several stands of thermophilic supra-Mediterranean forests in two neighbouring localities in western Slovakia. The pubescent oak stands were sampled on several days in April and May 2018, 2019, 2020 and 2021. Larvae were collected by tree beating and adults were caught with an entomological net (RNDr. Ondrej Šauša-Entomologické pomôcky). In addition, samples from the Malaise trap, which had been set up in 1999 at the site where the newly described species was found (Roller 2004), were re-examined. The species were identified using the keys of Macek et al. (2020) and Lacourt (2020).

Representatives of each captured larval instar were preserved in 96% ethanol with isopropanol and other larvae were reared on the oak leaves. After feeding was completed, larvae were transferred to glass jars with soil substrate and stored outdoors until the following spring. Adults were mounted dry or stored in ethanol at -20 °C until DNA analysis and then mounted.

Morphological terminology and measurement conventions were adopted from Viitasaari (2002). Genitalia were separated from fresh insects without maceration or digestion of soft tissues and temporarily mounted on slides in ethanol for examination and photography. The detached parts were then glued onto a card and pinned together with the specimen. Adults and larvae were examined and photographed using a Leica M205C stereomicroscope and a Leica Flexacam C1 camera, including operating software with a Z-stack projection tool. Images of the penis valve and ovipositor lancet were taken with a Nikon Coolpix P7700 camera connected to a Nikon Eclipse 600 microscope with Nomarski DIC optics.

Total DNA extracted from an adult insect leg or the abdominal wall of a larva was used for barcoding. DNA sequences of approximately 1,078 bp of the cytochrome c oxidase subunit I (COI) gene region were obtained using primers SymF1 and A2590-R (Normark et al. 1999; Prous et al. 2016) and kits and reagents were described previously (Semelbauer et al. 2021). PCR amplification conditions were as follows: initial denaturation step at 95 °C for 5 min, 36 cycles of 30 sec denaturation at 95 °C, 30 sec annealing at 53 °C, 1 min 20 sec extension at 72 °C, followed by a final extension of 10 min at 72 °C. The data of the analysed sawflies are stored in the GenBank database.

Abbreviations for collections

IZ SAS Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia;SNM Slovak National Museum, Nature Science Museum, Bratislava, Slovakia.

Links to genetic data

ON226738 https://www.ncbi.nlm.nih.gov/nuccore/ON226738.1/; ON227048 https://www.ncbi.nlm.nih.gov/nuccore/ON227048; ON228194 https://www.ncbi.nlm.nih.gov/nuccore/ON228194; ON231583 https://www.ncbi.nlm.nih.gov/nuccore/ON231583; ON231584 https://www.ncbi.nlm.nih.gov/nuccore/ON231584.

Results and discussion

Taxonomy

Mesoneura tematinensis Roller, sp. nov.

https://zoobank.org/C8A61A3D-87AC-48DB-ABD8-4B6B72C27A06

Type material. *Holotype* \bigcirc . **Type locality.** SLOVAKIA SW, Považský Inovec Mts, Tematínske kopce, Lúka env, Kňaží vrch nature reserve., 450 m a.s.l. Labelled [white and printed]: "SLOVAKIA SW, Tematín. kopce, Lúka env., Kňaží vrch, 48°39'49.29"N, 17°55'37.04"E, L. Roller leg." "Larva on *Quercus pubescens*, Larva: 21.V.–7.VI.2021, Adult: 27.–30.III.2022, L. Roller leg." "LR73" (tissue sample ID for genetic data) "Holotypus, *Mesoneura tematinensis* Roller, 2023, des. L. Roller [red and printed]. Good condition: right mid and hind legs removed as tissue samples. SNM. *Paratypes.* 1 \bigcirc , the same data as the holotype, IZ SAS; 1 \bigcirc , the same locality data, but "ex larva on *Quercus pubescens* coll. 11.V.2019, adult 7.IV.2020, L. Roller leg." [white label, printed], IZ SAS; 1 \bigcirc 2 \bigcirc \bigcirc "SLOVAKIA SW, Tematín. kopce, Lúka env., Hradlová nivka, 48°40'3.37"N, 17°54'53.09"E, (330 m a.s.l.)", "Larva on *Quercus pubescens*, Larva: 21.V.–7.VI.2021, Adult: 27.–30.III.2022, L. Roller leg.", \bigcirc bears white label "LR72" and one \bigcirc "LR74" (tissue sample ID for genetic data) [white labels, printed], "Paratypus, *Mesoneura tematinensis* Roller, 2023, des. L. Roller [red and printed]. SNM.

Larvae from *Quercus pubecens*: 1 mature feeding larva, Kňaží vrch, 8.V.2020, "LR2l" (tissue sample ID for genetic data); 6 small feeding larvae, Kňaží vrch, 8.V.2021 and 21.V.2021; 4 small feeding larvae, Hradlová nivka, 21.V.2021; 2 prepupae, one from each type locality, 4.VI.2021. IZ SAS and SNM.

Description. Female (Figs 1, 4; Holotype Fig. 1C) (variability in specimens other than the holotype in brackets). Body length 8.7 (7.7–8.7) mm, wing length 8.0 (7.2–8.1) mm.

Colour (Fig. 1A–C). Body black; following parts ochre to reddish-brown: apical half of clypeus, labrum, bases of mandibles; apical 2/3 of forewing costa and basal 2/3 of pterostigma; apical 1/3 and dorsal margin of profemur, apical 1/4 of mesofemur and apex of metafemur; apices of tibiae and tarsomeres; posterior margin of pronotum; tegula; flecks on mesonotum: anterolaterally on medial lobe (nearly whole lobe), medially anterior to scutellum, medially on scutellum (absent); postscutellum and metascutellum (absent to extensively); central mesepisternum (absent); ventral margins of lateral terga; dorsal parts of terga 1–3 and 8–9 extensively, terga 4–7 slightly (absent); sterna 2–7 extensively; and cerci; following parts pale to whitish-yellow: palps; trochanters and trochantelli; basal 2/3 to almost entire tibiae, tarsomeres extensively; cenchri; and posterior margins of terga and sterna slightly. Wings translucent; basal 1/3 of costa and anterior face of subcosta on forewing, hindwing costa, longitudinal veins in basal 1/3 of wings whitish-yellow; remaining veins and apical 1/3 of pterostigma dark to black.

Head (Figs 1B, C, 4C). Shiny and punctate, with most dense punctures on face, frons and lower gena; pale dense and long pubescence, hairs on postocellar area distinctly longer than diameter of ocellus; narrowed behind eyes; frontal area poorly-defined, shallowly depressed; frontal pit deep, narrowly elliptical; malar space almost half as long as diameter of lateral ocellus; postocellar area about 2.5 times as long as diameter of lateral ocellus; postocellar area about 2.5 times as long as diameter of lateral ocellus; postocellar area about 2.5 times as long as diameter of lateral ocellus; postocellar area about 2.5 times as long as diameter of lateral ocellus; postocellar area about 2.5 times as long as diameter of lateral ocellus, with shallow median furrow; OOL : POL : OOCL = 1 : 1.1 : 1; clypeus emarginated; antenna reaching postscutellum; antennomeres 3 and 4 about same length, following segments gradually becoming slightly shorter; and antennomere 9 about 3.5 times longer than its width.

Thorax (Fig. 1B–D). Shiny and punctate, interspaces weekly alutaceous, sunken lateral meso- and metanotum densely punctate; pale dense and long pubescence, longest hairs on mesepisternum twice as long as ocellar diameter; distance between cenchri as long as or slightly longer than cenchrus; metafemur slightly longer than 1/2 of metatibia, metatarsus slightly longer than metafemur, metatarsomere 1 slightly shorter than three following tarsomeres together; and claws with subapical tooth slightly shorter er than apical tooth.

Abdomen (Fig. 1B, C, E–G). Cylindrical, slightly tapering from segment 6 to blunt apex; terga and sterna with microsculpture, alutaceous on most surfaces and finely strigulate on posterior margins of terga; hypopygium sinuous posteriorly with broadly incised median lobe (Fig. 1G); sawsheath broadly emarginated posteriorly, trifid and 3.5 times as wide as cercus in dorsal view; cerci longer than sawsheath in dorsal view; lancet of ovopositor with 14 annuli, annular sutures extending from serrulae to 1/2–2/3 of height of lamnium, serrulae flat and lacking denticles, and basal 5–6 annuli with strongly sclerotised and finely serrated ctenidial ridges (Figs 1F, 4C).



Figure 1. *Mesoneura tematinensis* sp. nov., female **A** dorsolateral habitus **B** ventral habitus **C** dorsal habitus, holotype **D** meso and metathorax and 1st abdominal segment dorsal **E** abdominal segments 5–10 dorsal **F** apex of abdomen with retracted saw lateral **G** hypopygium and sawsheath ventral. Scale bars: 1 mm (**C**, **E**); 0.1 mm (**F**).

Male (Fig. 2). Body length 7.8 mm, wing length 6.7 mm.

Colour similar to female with the following differences: anterior half of clypeus, labrum and palps whitish-yellow; thorax largely black, only outer margins of tegulae and posterior margins of pronotum reddish-brown; metatibia with tips and metatarsomeres largely blackish-brown; pro- and mesotrochanters and trochantelli largely black; terga and sterna black with pale outer margins; penis valves and harpes largely reddish-brown; and posterior margin of tergum 8 and sternum 9 slightly reddish-brown.



Figure 2. *Mesoneura tematinensis* sp. nov., male **A** lateral habitus **B** abdomen dorsal **C** abdomen and hind leg lateral **D** genital capsule dorsal **E** genital capsule ventral **F** penis valve. Scale bars: 1 mm.

Morphology similar to female with the following differences: antenna as long as head and thorax; malar space very short, almost indistinct; metafemur swollen and short, about 0.7 length of metatibia (Fig. 2C); posterior half of tergum 8 with glabrous, shallow median depression (Fig. 2B); sternum 9 obtusely produced medially; genital capsule and penis valve (Fig. 2D–F).

Larva (Figs 3, 4).

Mature feeding larva (Figs 3A, C–F, 4E). Body length (maximum of larva preserved in etanol) 18.5 mm. Ground colour green, head grey-green; body dusted with whitish wax (Fig. 3A); ocularia, mandibles apically and claws of thoracic legs black; basal margin of coxa and adjacent anterior venter narrow black (Fig. 3D); cuticle of trunk with dense dark granules (Fig. 3F, in wax deprived larva); thoracic segments with sparse, fine, short setae with distinct pinacula arranged as in Fig. 3C, D; thoracic legs with longer setae; abdominal segments with six annulets, annulets 1, 2, 3, 4 and 5, subspiracular and surpedal lobes, and hypopleurite with fine setae distributed as in Fig. 3E.



Figure 3. Larva of *Mesoneura tematinensis* sp. nov. **A** live mature feeding larva **B** live prepupa **C–F** mature larva in ethanol, freed from wax, head and prothorax (**C**), meso and metathorax (**D**), abdominal segments 2 and 3 (**E**), granular surface on prothorax tergum (**F**) **G** live smaller feeding larva **H** smaller larvae in ethanol. Note the dark markings on the head and thoracic legs and the dark pinacula on the body of larvae preserved in ethanol. Scale bars: 1 mm.

Prepupa (Fig. 3B). Length 12–13 mm. Trunk green to yellowish green, glossy, without whitish dust; head turquoise.

Smaller larva, probably second to fourth instar (Fig. 3G–H). Trunk yellowish green without whitish dust, head turquoise; black markings on head and thoracic segments more distinct than in mature feeding larva, additionally with black parietal and weak facial flecks; fine setae with distinct pinacula distributed as in Fig. 3H.

Genetic data. One female, one male and one larva were DNA barcoded and their approximately 1,050 bp sequences are available (Accessions ON231583, ON231584

and ON226738). All three sequences are identical and differ significantly from the available barcodes of the related taxa *Mesoneura opaca*, *M. lanigera* and *M. truncat-atheca*, including *M. opaca* from the locality of the new species (accession for larva ON228194 and female ON227048). The closest relative of *M. tematinensis* appears to be *M. opaca*, which has a sequence dissimilarity of at least 5% (N = 13).

Etymology. Tematinensis is a latinised adjective referring to the Tematín Mountains, the area of origin of the new species.

Differential diagnosis

The body colouration and morphology of the sawsheath, lancet and cerci in females and the penis valve in males distinguish *M. tematinensis* from the other nine extant species of this genus. The cerci protruding behind the tip of sawsheath and the broadly incised middle lobe of the hypopygium may place *M. tematinensis* in the *M. opaca* group, which consists of the other two species *M. lanigera* and *M. opaca* from Europe (Wei et al. 2013). The European species of *Mesoneura* can be distinguished using the following key.

Key to the species of Mesoneura Hartig in Europe

1	Female
_	Male
2	Abdomen with terga 2–8 predominantly black
_	Abdomen with at least part of terga 2-8 largely reddish yellow
3	Terga 5–7 largely reddish yellow, pterostigma largely pale with darker base
_	Terga 5–7 largely black, pterostigma bicoloured with darker apical third
4	Abdomen with reddish band
_	Abdomen without reddish band5
5	Abdominal terga 5-8 with sharply defined medial depression, pterostigma
	completely dark
_	Abdominal terga 5-8 without distinct medial depression, pterostigma bicol-
	oured, partly pale

In addition, *M. lanigera* differs from *M. tematinensis* in that the female has a lancet with a higher number of annuli (about 20) and the male has a thicker penis valve with a longer valvispina (Benson 1954; Liston 2012).

The newly described species is most similar to *M. opaca*, with which it occurred in the same locality and shared a common host plant. In addition to the features used in the key, females can be distinguished by the pterostigma, which has a distinct dark apical third in *M. tematinensis*, while in *M. opaca* it is monochrome yellowish-brown (material from Slovakia) or has a darker base (some individuals imaged in ECatSym, Taeger et al. 2018). The body hair is also significantly longer in *M. tematinensis*. This is



Figure 4. Differences between *Mesoneura tematinensis* sp. nov. (**A**, **C**, **E** left) and *M. opaca* (**B**, **D**, **E** right). Note the different length of pubescence on the head (**A** versus **B**), the ctenidia of ovipositor lancet (**C** versus **D**) and the habitus of full grown feeding larva (**E** left versus right). Scale bars: 0.1 mm (**A**, **B**); 1 mm (**C**, **D**).

well observed on the head, where the hairs in the postocellar region are almost 2 times as long as the ocellar diameter in *M. tematinensis* (Fig. 4A) and only as long as the ocellar diameter in *M. opaca* (Fig. 4B; N = 15). The lancet of *M. tematinensis* is characterised by strongly sclerotised ctenidial ridges (Fig. 4C) instead of ctenidial teeth, which are largely present in *M. opaca* (Fig. 4D). Males of both species have a distinct penis valve, which is curved and slender in *M. tematinensis* (Fig. 2F), while it is straighter and more robust in *M. opaca* (fig. 4 in Liston 2012). The feeding larvae can also be distinguished. *M. tematinensis* has black horizontal stripes on the bases of the thoracic legs, which are absent in *M. opaca* (Fig. 4E). Furthermore, the mature feeding larva of *M. tematinensis* has a distinct whitish dusting. Finally, the body size of adults and larvae



Figure 5. Localities of *Mesoneura tematinensis* sp. nov. photographed when the larvae appeared **A** Hradlová nivka **B** Kňaží vrch, holotype locality **C** solitary oaks in Hradlová nivka **D** edge of the oak stand in Kňaží vrch.

of *M. tematinensis* is larger than that of *M. opaca*. In females it is 7.66–8.8 mm (N = 5, mean 8.3 mm, median 8.4 mm) for *M. tematinensis* and 6.2–7.45 mm (N = 18, mean and median 6.9 mm) for *M. opaca*.

Material of *Mesoneura opaca* examined: Total 18 $\bigcirc \bigcirc$ S and SW SLOVAKIA [leg. L. Roller and in IZ SAS]: 5 $\bigcirc \bigcirc$, Tematínske kopce, Lúka env., Kňaží vrch, 23.IV.2000, 30.IV.2021, 10.V.2021; 4 $\bigcirc \bigcirc$, Malé Karpaty Mts., Devínska Kobyla – Sandberg, 24.IV. and 26.IV.1994, 14.IV.2017; 1 \bigcirc , Podunajská nížina, Rusovce – park, 19.IV.2020; 1 \bigcirc , Burda, Kamenica nad Hronom, 21.IV.2019; 3 $\bigcirc \bigcirc$, Borská nížina, Šaštínsky les, 24.IV.2006; 2 $\bigcirc \bigcirc$, Borská nížina, Rohožník, 10.V.2010, 20.IV.2011; 1 \bigcirc , Borská nížina, Jakubov, 1.V.1994; 1 \bigcirc Borská nížina, Sološnica, 25.IV.1994.

Biology

The species inhabits pubescent oaks in a thermophilic supra-Mediterranean forest (Fig. 5A, B). Despite continuous sampling with the Malaise trap and with an entomological net at approximately one-week intervals during oak leaf sprouting in spring, no adults were collected. All adults examined were reared from the larvae of the previous year and appeared from late March to early April. The flight period can only be predicted to the time of early sprouting of the oak leaves. The finding of the male specimen is particularly noteworthy. The related *M. opaca* is considered to be predominantly parthenogenetic, with few males reported (Liston 2012). Parthenogenesis also seems to be the preferred reproductive strategy in *M. tematinensis*, as only one male was reared out of a total of six adults.

The larvae feed externally on fresh leaves of the pubescent oak. Depending on the weather conditions in the respective season, they were found on fresh oak leaves for about 15 days from the end of April to the beginning of June. They occurred singly on branches of solitary trees and trees at the edge of open areas (Fig. 5C, D). Larvae about 1 cm in size (probably second to third instars) were found on about half-grown leaves, and mature larvae (probably fourth or fifth instar) were feeding on fully grown but still light green leaves. The mature larvae remained on the leaves for a few days longer than most other phyllophagous oak sawflies (*M. opaca, Periclista* and *Apethymus* species), of which only *Pristiphora fausta* was still present. Shortly after ecdysis to prepupa, the larvae burrowed into the soil where they overwintered. It is noteworthy that after several failed attempts with different soil substrates we reared the adults only with the soil we had taken from near the oaks where the larvae were found. Our rearing shows that *M. tematinensis* has only one brood per year.

Accompanying sawfly species

When sampling stands where *M. tematinensis* occurs, 13 other Symphyta species were found associated with the pubescent oak (Table 1). With the exception of *Harpiphorus lepidus*, *Periclista lenta* and *Janus cynosbati*, this association was confirmed by collecting feeding larvae and their growth into adults. *Harpiphorus lepidus* is known to feed on oak leaves (Enslin 1914) and its larvae probably hatch after our sampling period

Species	Malasie trap	Individual sampling
Apethymus cereus (Klug, 1818)		6 L; 3 A ex L
Apethymus serotinus (Müller, 1776)	15 A	4 L
Harpiphorus lepidus (Klug, 1818)	1 A	1 A
Periclista albida (Klug, 1816)	1 A	4 L; 1 A; 1 A ex L
Periclista albipennis (Zaddach, 1859)		10 L; 2 A ex L
Periclista lenta Konow, 1903	3 A	
Periclista lineolata (Klug, 1816)	1 A	7 L; 1 A; 2 A ex L
Periclista pubescens (Zaddach, 1859)	1 A	8 L; 3 A ex L
Periclista pilosa Chevin, 1971		12 L; 2 A; 4 A ex L
Mesoneura opaca (Fabricius, 1775)	7 A	18 L; 3 A; 2 A ex L
Pristiphora fausta (Hartig, 1837)		6 L
Pamphilius sylvarum (Stephens, 1835)		2 L
Ianus cynosbati (Linné, 1758)	1 A	

Table 1. Numbers and stages of Symphyta species collected from pubescent oaks at *Mesoneura tematinensis* sp. nov. type localities.

Sampling with a Malaise trap in 1999; individual sampling in 2018–2021; L = larva, A = adult, A ex L = adult reared from larva.

(April–May). *Periclista lenta* is a very rare species with unknown larvae (Macek et al. 2020). However, it is very likely that it develops on oak like all other members of the genus *Periclista*, and the pubescent oak is the only oak species found in the sampled stand. The larvae of *Janus cynosbati* feed inside oak shoots and the larvae of the other ten species were found on fresh oak leaves at the same time as *M. tematinensis*. This indicates that the stands studied harbour a very rich assemblage of phyllophagous Symphyta. Particularly noteworthy is the occurrence of all *Periclista* species found in Central Europe, with the exception of *P. albiventris* (Klug, 1816). However, the species generally occurred in very low numbers, with *Mesoneura opaca* being the most common. Most of the oaks examined were free of Symphyta larvae, and a maximum of five larvae of two to three species were found on a single tree.

Remarks

The discovery of a new species of the genus *Mesoneura* on pubescent oak in Slovakia can be considered surprising, as the sawfly fauna of oaks (*Quercus* spp.) is relatively well known in Central Europe and its study has a long tradition in Slovakia (Patočka et al. 1962; Roller 2004; Smetana et al. 2020). Pubescent oak has a large distribution range in Southern and Central Europe and reaches the northern limit of its range in Slovakia. It was therefore unlikely that a previously unknown taxon associated with this tree would appear for the first time in the Tematín Mountains. However, the supra-Mediterranean oak stands in the Tematín Mountains are very well preserved, as evidenced by the occurrence of thermophilic insect species rare in Central Europe, such as the large predatory bush cricket *Saga pedo* and the true cicadas *Tibicen plebejus* and *Cicada orni* (Vidlička et al. 2002; Májsky and Janský 2006). *Mesoneura tematin-ensis* is thought to have a wider range and is likely to be overlooked due to its secretive lifestyle in relatively extreme habitats. In order to monitor its occurrence at suitable sites, we recommend collecting the larvae at the time of fresh, almost fully developed oak leaves.

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