

## Variability of the composition of orcinol depsidones in *Hypogymnia vittata* and *H. subduplicata*

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**Abstract.** The variability of the composition of secondary metabolites in *Hypogymnia vittata* and *H. subduplicata* was studied. Three chemotypes have been identified for *H. vittata* differing in the composition of the orcinol depsidones of the medulla: I – contains vittatolic acid, 3-hydroxyphysodic and physodic acids; II – contains 3-hydroxyphysodic and physodic acids; III – contains physodic and 2'-O-methylphysodic acids. The chemotype III was shown to be associated with a specific substrate. The composition of secondary metabolites was determined for the first time for *H. subduplicata*. The species is represented by two chemotypes: I – contains vittatolic acid, 3-hydroxyphysodic and physodic acids; II – contains 3-hydroxyphysodic, physodic acids and lividic acid. The latter was not previously reported for the genus *Hypogymnia*.

**Keywords:** HPLC-MS/MS, lichen substances, secondary metabolites, orcinol depsidones.

## Изменчивость состава орциноловых депсидонов у *Hypogymnia vittata* и *H. subduplicata*

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**Резюме.** Проведено исследование изменчивости состава вторичных метаболитов у *Hypogymnia vittata* и *H. subduplicata*. Для *H. vittata* выявлено три хемотипа, отличающихся по составу орциноловых депсидонов медулярного слоя: I – содержит виттатоловую, 3-гидроксифизиодовую и физиодовую кислоты; II – 3-гидроксифизиодовую и физиодовую кислоты; III – физиодовую и 2'-О-метилфизиодовую кислоты. Показано, что хемотип III связан с определенным субстратом. Впервые определен состав вторичных метаболитов для *H. subduplicata*. Вид представлен двумя хемотипами: I – содержит виттатоловую, 3-гидроксифизиодовую и физиодовую кислоты; II – содержит 3-гидроксифизиодовую и физиодовую кислоты, а также ливидовую кислоту, которая ранее для рода *Hypogymnia* не была известна.

**Ключевые слова:** ВЭЖХ-МС/МС, лишайниковые вещества, вторичные метаболиты, орциноловые депсидоны.

Initially, the genus *Hypogymnia* (Nyl.) Nyl. was introduced by Nylander as a subgenus of *Parmelia* Ach. (Nylander, 1881). He assigned *Hypogymnia* the status of a genus in 1896 (Nylander, 1896). Despite this, many researchers continued to include these lichens in *Parmelia* until the genus *Hypogymnia* was finally accepted due to the works of Santesson (1943) and Krog (1951) in the middle of the last century (Goward, 1986).

Most of representatives of *Hypogymnia* are common worldwide, but mainly distributed in the Northern Hemisphere. At present time, *Hypogymnia* includes about 126 species (Hyde *et al.*, 2024), 26 of which are known in Russia (Zhdanov, 2023). The morphological features of the genus are the inflated, hollow lobes characteristic for most

species. Upper surface grey to dark brown, often soredate, rarely isidiate. The lower surface is dark and without rhizines (Wetsberg *et al.*, 2011). Although a number of specialists assert that rhizines and hapters are found on the lower surface of some species (Wei *et al.*, 2015).

*Hypogymnia vittata* (Ach.) Parrique has narrow elongated lobes with a distinct black edge, small secondary lobules along the margins and numerous, well-developed soralia (Wetsberg *et al.*, 2011). The species is widespread in the Northern Hemisphere, but has large gaps in its range (McCune, Wang, 2014). *Hypogymnia subduplicata* (Rass.) Rass. has wider lobes without a distinct black margin, and also few and underdeveloped soralia (Wetmore, 2003). The species is

mainly found in the Russian Far East (Zhdanov, 2023).

*Hypogymnia physodes* (L.) Nyl. has great variability and may be similar to both mentioned species in the shape of the soralia lining splayed open lobe tips. *Hypogymnia physodes* differs by producing physodalic acid (PD<sup>+</sup> red medulla), having white medullary ceiling and lacking lobules (Brodo et al., 2001).

*Hypogymnia vittata*, along with six other Asian species, forms the *Hypogymnia vittata* group. The characteristic features of this group include a perforated lower surface, dark walls of the cavity, the absence of physodalic acid, and the development of small secondary lobes. Although *H. subduplicata* is morphologically similar to *H. vittata*, it is not included in this group due to being an understudied species (McCune, 2011; Zhdanov, 2023).

The first studies of the composition of secondary metabolites of *Hypogymnia vittata* revealed the presence of only physodic acid and atranorin (Asahina, 1951), as well as three unidentified substances reacting differently with FeCl<sub>3</sub> (Nuno, 1964). More recent studies showed that the thallus of *H. vittata* contains atranorin, chloroatranorin in the upper cortex and physodic, 3-hydroxyphysodic, and vitatolic acids in the medulla (Hirayama et al., 1975, 1976). At the same time, information about the composition of secondary metabolites of *H. subduplicata* was not found in the literature.

The purpose of this work is to study the variability of the composition of orcinol depsidones in specimens of *Hypogymnia vittata* and *H. subduplicata* stored in herbarium of the Komarov Botanical Institute of the Russian Academy of Sciences (LE).

## Material and methods

A total of 77 herbarium specimens collected in 1866–2024 and stored in the lichenological herbarium of the Komarov Botanical Institute of the Russian Academy of Sciences (LE) were analyzed. Some specimens did not match the morphological and chemical characteristics of *Hypogymnia vittata* or *H. subduplicata*. These specimens were either reidentified or excluded from the analysis. Two specimens with atypical morphology and secondary metabolite composition for the genus were also excluded from the analysis. The geographic coordinates are presented in the WGS 84 system (Table 2, [electronic supplement](#)<sup>1</sup>). The coordinates

of old specimens were determined based on the label data, and therefore can be considered to be approximate. The maps were drawn using the QGIS software package v. 3.42.0.

For the analysis, air-dried lichen thalli were grounded in a vibration ball mill M-0.05 Techno-Center (Russia). Then, 1 ml of acetone was added to 10 mg of the resulting powder. Extraction was carried out with constant stirring for 12 hours at 20–25 °C in a vortex RM-1L ELMI (Latvia). The resulting extracts were centrifuged for 5 minutes at 15.000 g and then kept at 4 °C until analysis.

LC-ESI-MS analysis was performed with a Shimadzu LC-30 Nexera chromatograph (Japan). For chromatographic separation, a Thermo Hypersil-Keystone C18, column (150 × 2.1 mm × 5 μm) was used. The mobile phase consisted of (A) water: acetonitrile: formic acid (95 : 5 : 0.1 v/v), and (B) acetonitrile: water: formic acid (90 : 10 : 0.1 v/v). Analyses were performed at 40 °C with a flow rate of 0.3 ml/min in the gradient elution mode, the percentage of B was programmed as follows: 5% (2 min) – 50% (5 min) – 70% (15 min) – 100% (25 min) – 100% (30 min). The volume of injected sample was 2 μL. Spectra of eluting substances were recorded in UV at 254 nm.

After chromatographic separation, the samples were analyzed using a triple quadrupole mass-selective detector LCMS-8030 Shimadzu (Japan) with electrospray ionization (ESI) in the negative mode. The voltage on the capillary was 3.5 kV, nebulizing gas flow rate 3 L/min, drying gas (nitrogen) temperature 250 °C, drying gas flow rate 15 L/min. Mass spectra were recorded in the range 100–800 m/z. MS/MS spectra were obtained by collision-induced dissociation of precursor ions with an energy of 30 eV. The resulting chromatograms were processed using the MZmine 4 v. 4.1.0 software. To identify lichen substances, we compared of their polarity related to their retention time (Rt), obtained m/z values of pseudo-molecular and fragment ions with the authentic standards from the BIN RAS collection.

To estimate the relative quantitative content of secondary metabolites in the samples, the peak areas of the substances in the chromatograms obtained at a wavelength of 250 nm (the absorption maximum for β-orcinol depsides and orcinol depsidones) were compared. This method is reliable because closely related biosynthetic compounds with similar chemical structures exhibit comparable molar extinction coefficients at certain wavelengths (Huneck, Yoshimura, 1996). The area of

<sup>1</sup> <sup>1</sup> Electronic supplement is available at the end of the article page on the journal website (<https://doi.org/10.31111/nsnr/2025.59.1.L43>).

the largest peak in the chromatogram was set as 100% and designated as the main substance (M), while peaks with areas less than 50% of the main substance's area were classified as minor compounds (m) (Table 2, [electronic supplement](#)).

## Discussion

We studied 61 herbarium specimens of *Hypogymnia vittata* and *H. subduplicata*. Eight secondary metabolites were identified (Table 1). The obtained retention times and mass spectra of substances from the extracts corresponded to authentic standards of lichen substances from the BIN RAS collection. In addition, the mass spectra of atranorin, chloroatranorin,  $\alpha$ -alectoronic acid, physodic acid, 2'-O-methylphysodic acid, and 3-hydroxyphysodic acid were consistent with previously published data (Latkowska *et al.*, 2015). Atranorin and chloroatranorin ( $\beta$ -orcinol depsides) were present in the cortex of all specimens. At the same time, the composition of the substances in the medulla, which are orcinol depsidones, exhibited some variations.

Three chemotypes were identified for *Hypogymnia vittata*: I – contains vittatolic, 3-hydroxyphysodic, and physodic acids; II – contains 3-hydroxyphysodic and physodic acids; III – contains physodic and 2'-O-methylphysodic acids (Fig. 1).

Chemotypes I (41 specimens) and II (10 specimens) are more widespread in Russia and Europe compared to chemotype III and do not exhibit substrate specificity being most commonly found on tree species with acidic bark, sometimes on lignum or rocks. The chemotype III specimens (6 specimens) was found in the Nenets Autonomous Area, southern and eastern Siberia and the Far East (Chukotka Autonomous Area) (Fig. 2).

*Hypogymnia vittata* has not previously been reported to contain 2'-O-methylphysodic acid. However, in specimens of chemotype III, this

substance is present as one of the main metabolites, whereas in most other representatives of the genus, it occurs as a minor component. Based on the label data, it was discovered that specimens of *H. vittata* chemotype III was most frequently found on soil (Table 2, [electronic supplement](#)).

The composition of secondary metabolites has not been previously identified for *Hypogymnia subduplicata*. The cortex contained atranorin and chloroatranorin. The medulla contained, in addition to 3-hydroxyphysodic and physodic acids, also lividic acid (4-O-methyl ether of 3-hydroxyphysodic acid), which has not been previously reported for the genus *Hypogymnia*.

The holotype of *Hypogymnia subduplicata* contained vittatolic acid instead of lividic acid. This specimen is from Primorye Territory (in the southern Russian Far East). According to the literature data (McCune, Wang, 2014), vittatolic acid is predominantly characteristic for the Asian *Hypogymnia* species. For example, in Russia, vittatolic acid was found in only two species, *H. vittata* and *H. papilliformis*, the latter of which was found in Primorye Territory (Zhdanov, 2023). In North America, vittatolic acid was found only in *H. vittata* (McFarlin, 1991). At the same time, in southwest China, it was detected even in *H. physodes* as a minor compound, and in 15 other species it is one of the major secondary metabolites (McCune, Wang, 2014).

Vittatolic acid is known to be exclusively characteristic of the genus *Hypogymnia* (Hirayama *et al.*, 1975). Although the exact biosynthetic mechanism of vittatolic acid remains unclear, it is hypothesized to be a derivative of physodic acid, and its presence in the lichen thallus is modulated by environmental conditions.

Thus, it can be assumed that *Hypogymnia subduplicata* has two chemotypes, Chemotype I with

Table 1

The lichen substances in *Hypogymnia vittata* and *H. subduplicata* identified by HPLC-ESI-MS/MS

Peak no.	t <sub>r</sub> (min)	Molecular formula	[M-H] <sup>-</sup> m/z	MS/MS fragments	Identification
1	23.0	C <sub>19</sub> H <sub>17</sub> ClO <sub>8</sub>	407	211, 167, 163, 139, 119	Chloroatranorin
2	21.5	C <sub>19</sub> H <sub>18</sub> O <sub>8</sub>	373	177, 163, 133, 119, 105	Atranorin
3	18.1	C <sub>28</sub> H <sub>32</sub> O <sub>9</sub>	511	467, 449, 425, 423, 369	$\alpha$ -alectoronic acid
4	16.9	C <sub>26</sub> H <sub>30</sub> O <sub>8</sub>	469	425, 407, 383, 381, 357	Physodic acid
5	16.4	C <sub>27</sub> H <sub>32</sub> O <sub>8</sub>	483	439, 424, 407, 395, 383	2'-O-methylphysodic acid
6	14.4	C <sub>26</sub> H <sub>30</sub> O <sub>9</sub>	485	441, 423, 399, 397, 373	3-Hydroxyphysodic acid
7	12.7	C <sub>26</sub> H <sub>30</sub> O <sub>9</sub>	485	441, 423, 399, 379, 369	Vittatolic acid
8	11.5	C <sub>27</sub> H <sub>32</sub> O <sub>9</sub>	499	455, 437, 413, 411, 387	Lividic acid

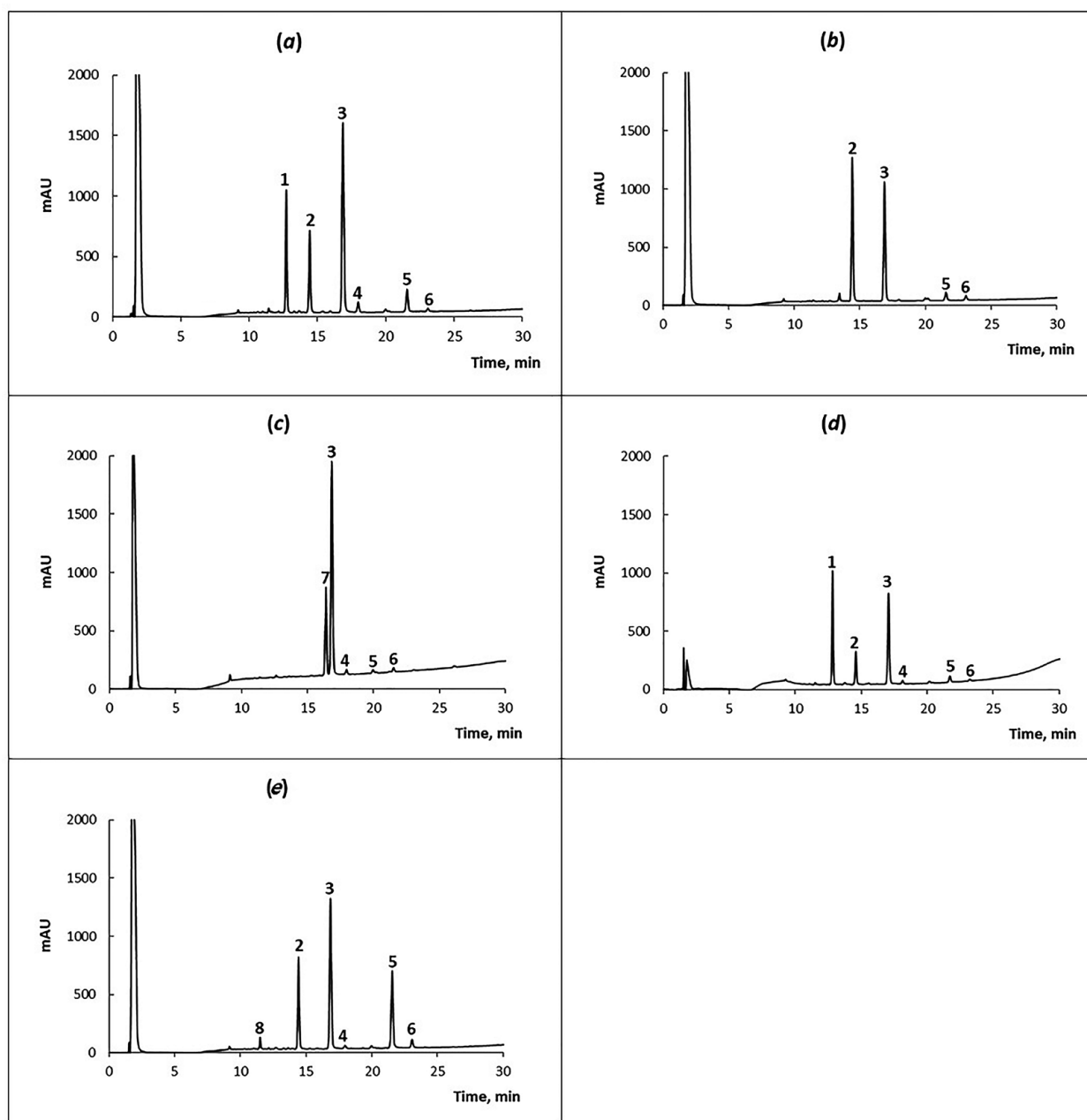


Fig. 1. HPLC chromatograms (250 nm) of acetone extracts of lichens *Hypogymnia vittata* – (a) chemotype I, (b) chemotype II, (c) chemotype III and *H. subduplicata* – (d) chemotype I, (e) chemotype II.

1 – vittatolic acid, 2 – 3-hydroxyphysodic acid, 3 – physodic acid, 4 –  $\alpha$ -alectoronic acid, 5 – atranorin, 6 – chloroatranorin, 7 – 2'-O-methylphysodic acid, 8 – lividic acid.

vittatolic acid (holotype) is confined to the south of the Russian Far East and a more humid climate; Chemotype II with lividic acid (3 specimens) is associated with the territory of southern Siberia and eastern Yakutia and is confined to more continental conditions.

The results of the study showed that both species, *Hypogymnia vittata* and *H. subduplicata*, exhibit chemical variability. Three previously unknown chemotypes were identified for *H. vittata*, one of which (chemotype III) revealed the most interesting combination of secondary metabolites and could probably be related to the

soil substrate. The composition of secondary metabolites of *H. subduplicata* was determined for the first time. This species is represented by two chemotypes, one of which contains lividic acid, previously not identified for the genus. Due to the limited amount of material, it can only be assumed that the differences in secondary metabolite composition between chemotypes depend on different geographical and environmental conditions. Further studies using molecular approaches may help us to better understand the nature of chemical variability in the genus *Hypogymnia*.

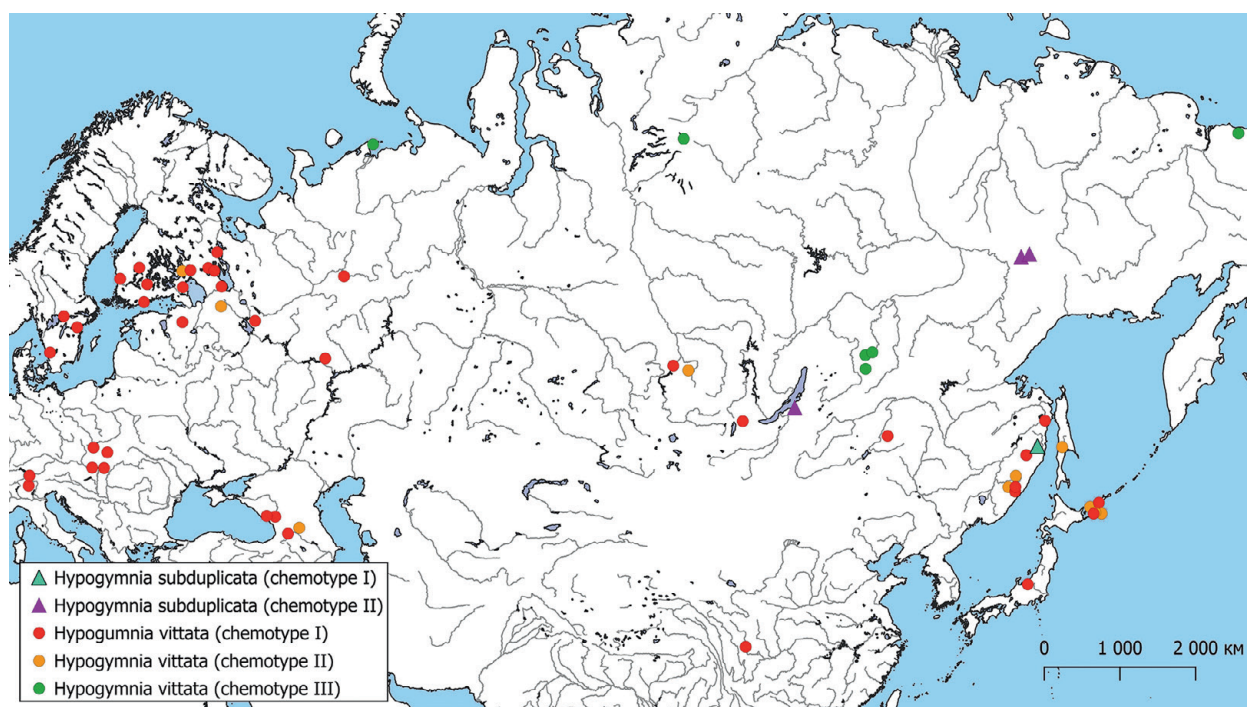


Fig. 2. Distribution of studied specimens of *Hypogymnia subduplicata* and *H. vittata*.

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**Conflicts of Interest.** The authors declare no conflict of interest.

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