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# Neohygrocybe pseudoingrata, a new grassland species from Slovakia and the Czech Republic

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Key words:	Abstract: Neohygrocybe pseudoingrata, a new waxcap species known from Slovakia and the Czech Republic, is
Agaricomycetes	characterised by its pale greyish coloured and often robust basidiomata (or sporocarps), nitrous smell, context without
grasslands	colour changes, hollow, contorted and compressed stipe and smooth or slightly fibrillose pileus surface. Based on
Hygrophoraceae	morphology and DNA analysis of ITS and LSU sequences of the collected specimens, N. pseudoingrata belongs to
meadows	Neohygrocybe sect. Neohygrocybe together with N. ovina, N. nitrata and N. ingrata. Collections of N. pseudoingrata
new taxon	form a well-supported clade in phylogenetic trees.
Waxcaps	

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#### **INTRODUCTION**

The genus *Hygrocybe* as delimited in Boertman (2010) has been split into a number of genera (*Chromosera, Cuphophyllus, Gliophorus, Gloioxanthomyces, Hygrocybe, Neohygrocybe* and *Porpolomopsis*) by Lodge *et al.* (2013). *Neohygrocybe* differs from most *Humidicutis, Porpolomopsis* and *Gliophorus* species in lacking bright pigments. Lodge *et al.* (2013) described *Neohygrocybe* as having swollen, and stuffed stipes that become hollow; pileus 2–6 cm, hemispherical, becoming umbonate, smooth to scaly, margin becoming fissured, brick colour to fuscous cinereous; lamellae few, sublunate, uncinate, broad, venose, white at first, becoming cinereous.

Members of the genus Hygrocybe s. l. (Hygrocybe, Neohygrocybe, Gliophorus, Porpolomopsis) and Cuphophyllus fall into distinct clades but they usually occur together and are often treated as one group for conservation purposes (e.g., Boertmann 2010). Most of these genera occur in "unimproved", mowed or grazed grasslands in Europe, where they figure as good indicators of conservation value of seminatural and natural grasslands (Adamčík & Kautmanová 2005, Boertmann 2010, Fuljer et al. 2020). These grasslands are usually characterised by very low levels of dissolved nitrate and phosphate (Ejrnæs & Brunn 1995). Hygrocybe s. l. species, together with a Clavariaceae, Entoloma and Geoglossaceae, form a so called "CHEG" group, by reason of sharing ecological similarities (Rotheroe 2001). However, waxcaps can also produce basidiomata in habitats such as peat bogs, sand dunes and woodlands (Cantrell & Lodge 2000, Griffith et al. 2004, Boertmann 2010) and in North America and the tropics they

are mainly found in forests (*e.g.* Hesler & Smith 1963, Pegler & Fiard 1978, Læssøe & Boertmann 2008).

The ecological role of waxcaps is still unclear, despite intensive research in this field. Griffith *et al.* (2004) referred to the fact that some of the waxcaps can occur in the grasslands together with mosses and this connection was also noticed by Boertmann (2010). However, their biology remains a mystery since isotopic signatures indicate that they are neither mycorrhizal nor saprotrophic (Seitzman *et al.* 2011, Halbwachs *et al.* 2013). Recent studies revealed that some of the waxcaps can be associated with plant roots and they probably have a biotrophic lifestyle with plants (Halbwachs *et al.* 2013, 2018). Tello *et al.* (2013) proved that at least one species, *Hygrocybe virginea*, is a maternally transmitted endophytic fungus associated with *Plantago lanceolata*.

In this report we describe a taxon new to science found in central European grasslands. It is also likely to have a wider distribution.

#### MATERIAL AND METHODS

#### **Collections and morphological analyses**

Waxcaps were collected in Slovakia and the Czech Republic during 2014–2020, from July to October, at 23 localities by F. Fuljer, M. Zajac and M. Mička. Most of the collections were from the Javorníky Mts. (northwestern part of Slovakia) and the rest were from Biele Karpaty, Jablunkovské medzihorie, Kysucká vrchovina, Turzovská vrchovina (Slovakia) and Českotřebovská

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vrchovina (Czech Republic) mountains. Soil type has been determined based on the geological map of Slovakia (http://apl. geology.sk/gm50js).

Descriptions of macro-morphological features were based on fresh material. Colours were coded according to the Pantone colour chart (Pantone Colour Finder 2021). Twenty basidiomata were studied and measured from the holotype collection.

The micromorphology of the studied specimens was investigated by F. Fuljer, D. Boertmann and I. Kautmanová using a Kapa Mic D117 with integrated camera, a Leica SM-Lux, a DIC microscope Nikon Eclipse Ni-U and microphotography were captured by a Nikon DS-Ri2 camera. NIS-Elements Basic Research and MiCam v. 2.4 imaging software were used to measure and examine microscopic features. Tissues, spores and other micromorphological structures were examined fresh or rehydrated in H<sub>2</sub>O or in Congo Red ammonia solution. Altogether 575 spores from 14 basidiomata were studied and measured; spores were measured mainly from spore deposits in H<sub>2</sub>O. Fifty basidia, 50 sterigmata and 50 basidioles from five basidiomata were investigated from the rehydrated material in ammonial Congo Red solution. Other microscopic structures, such as gill trama, pileipellis and stipitipellis, were observed in three basidiomata from the holotype. Q value refers to the division of length and width of microscopic structures. Qav refers the average value of Q and av. refers the average length and width of microscopic features.

Type material was deposited in the herbarium of the Slovak National Museum-Natural History Museum, Bratislava (BRA). Nomenclature follows Lodge *et al.* (2013) and Index Fungorum (indexfungorum.org).

### DNA extraction, amplification, sequencing

Total genomic DNA was extracted from dried tissue using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol, but with prolonged incubation time of up to 3 h after addition of the RNA-lytic enzyme. PCR was performed using a BioRad C1000 Touch™ Thermal Cycler. Target region of the internal transcribed spacer regions of ribosomal DNA (ITS) was amplified using primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'; White et al. 1990). The large ribosomal subunit of ribosomal DNA (LSU) was amplified using primers LROR (5'-ACCCGCTGAACTTAAGC-3') and LR5 (5'-TCCTGAGGGAAACTTCG-3'; Vilgalys & Hester 1990). The amplification reactions were conducted in 25 µL total volume using a GoTaq Flexi PCR kit (Promega), the reaction mixture containing 20–25 ng total DNA template, 1  $\mu$ L of both primers (10  $\mu$ M), 5  $\mu$ L of Buffer (5×), 2.5  $\mu$ L of dNTP (2 mM), 2  $\mu$ L of MgCl<sub>2</sub> (25 mM), 0.2 µL GoTaq Flexi polymerase (5 U) and the final volume was added with ultra pure water. The amplification reaction for ITS and LSU regions was set up as follows: 3 min initial denaturation at 95 °C, 32 cycles (95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min + increasing time 2 s per cycle), 10 min final elongation at 72 °C. The PCR products were analysed on 2 % agarose gel. PCR products were purified using a Thermosensitive Alkaline Phosphatase (FastAP) and Exonuclease 1 (Exo 1) (Thermo Fisher Scientific Inc., USA) according to manufacturer's instructions. The partial gene was sequenced in a commercial laboratory (Eurofins Genomics GmbH, Cologne, Germany). Sequences were visualised, edited and aligned in MEGA-X (Kumar et al. 2018). Sequence similarity searches were performed using GenBank BLASTn (http://www.ncbi.nlm.nih.gov/BLAST/) and BOLD Identification System (https://www.boldsystems.org/).

# **Phylogenetic analysis**

DNA sequences of Neohygrocybe species and selected outgroup of Cuphophyllus fornicatus were downloaded from NCBI on 21 Jan. 2021. All sequences retrieved in this study were sent to BOLD database and transferred to GenBank and accession numbers are listed in Table 1. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018) by using the Maximum Likelihood method and Tamura-Nei model (Tamura & Nei 1993). The tree with the highest log likelihood (-3667.62) is shown (Fig. 1). The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pair wise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 26 ITS sequences. There were a total of 782 positions in the final dataset. In the tree, Neohygrocybe species were positioned on a separate branch close to the clade of N. nitrata, which is consistent with the results from macro- and microcharacters observations.

# RESULTS

# Taxonomy

*Neohygrocybe pseudoingrata* Fuljer, Zajac, Boertm. & Kautmanova, *sp. nov.* MycoBank MB 842316. Figs 2, 3.

*Etymology*: Name refers to *Neohygrocybe ingrata*, a species with similar morphology.

*Typus*: **Slovakia**, Javorníky Mts., Melocík, Veľké Rovné, *ca*. 300 m E from the main road, N49°20′28.14″ E18°30′37.65″, alt. 798 m, cow grazed and mowed grassland, 21 Jul. 2020, *F. Fuljer* (**holotype** BRA CR33023, ITS GenBank MZ479356, LSU GenBank MZ479363, ITS BOLD NEOHY001-21).

Habitat & Distribution: Known from Slovakia and the Czech Republic, probably more widespread but possibly misidentified as *N. ingrata* or *N. nitrata*. Growing gregarious and very often caespitose and may also sporulate in half rings, sometimes solitary or scattered. It has been recorded in different vegetation types, but always in unimproved semi-natural mesic meadows and pastures, from July to October, on acidic, neutral and calcareous soils.

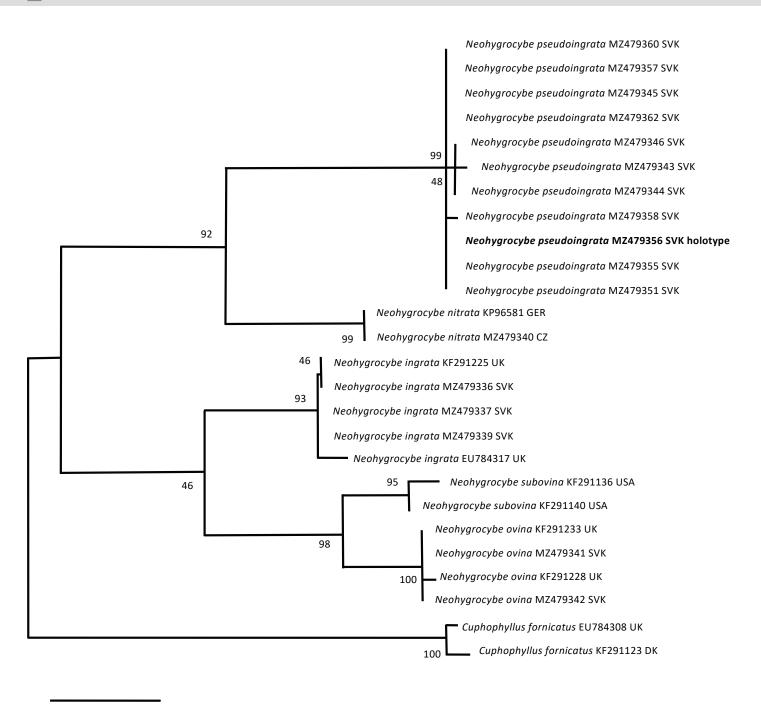
*Pileus* 20–80 mm, at first hemispherical, later convex to applanate, irregular, often irregularly contorted, sometimes umbonate, or centrally compressed and with splitting margin; surface smooth, or radially fibrillose, dry, when old very often uneven, buff brown, pale brownish, greyish brown, dark brownish grey (Pantone 463C to Pantone 466C). *Stipe* 35–100 × 8–32 mm, fusiform, clavate; irregularly furrowed, compressed, often contorted and tawn; hollow; surface smooth, dry, white with slightly greyish or brownish tinges (Pantone 4246C to

**Table 1.** Collections studied and analysed in this study by molecular methods with collection numbers, country of origin, GenBank and BOLD accession numbers (some collections of *N. pseudoingrata* were not sequenced, for all collections check Additional materials examined).

Species	Herbarium number	Origin	ITS GenBank Accession No.	ITS BOLD Accession No
N. ingrata	BRA CR34493	Slovakia	MZ479336	NEOHY 008-21
	BRA CR34490	Slovakia	MZ479339	NEOHY 019-21
	BRA CR34489	Slovakia	MZ479337	NEOHY 025-21
	BRA CR34488	Slovakia	MZ479338	NEOHY 026-21
N. nitrata	BRA CR34492	Czechia	MZ479340	NEOHY 009-21
N. ovina	BRA CR34491	Slovakia	MZ479341	NEOHY 010-21
	BRA CR34487	Slovakia	MZ479342	NEOHY 027-21
N. pseudoingrata sp. nov.	BRA CR33023 holotype	Slovakia	MZ479356	NEOHY 001-21
	BRA CR34363	Slovakia	MZ479355	NEOHY 002-21
	BRA CR34377	Slovakia	MZ479354	NEOHY 003-21
	BRA CR34369	Slovakia	MZ479353	NEOHY 004-21
	BRA CR34368	Slovakia	MZ479352	NEOHY 005-21
	BRA CR34367	Slovakia	MZ479351	NEOHY 006-21
	BRA CR34364	Slovakia	MZ479350	NEOHY 007-21
	BRA CR34374	Slovakia	MZ479349	NEOHY 011-21
	BRA CR34373	Slovakia	MZ479348	NEOHY 012-21
	BRA CR34362	Slovakia	MZ479347	NEOHY 013-21
	BRA CR34371	Slovakia	MZ479346	NEOHY 014-21
	BRA CR34511	Slovakia	MZ479345	NEOHY 015-21
	BRA CR34382	Slovakia	MZ479344	NEOHY 016-21
	BRA CR34365	Slovakia	MZ479343	NEOHY 017-21
	BRA CR34372	Slovakia	MZ479362	NEOHY 018-21
	BRA CR34502	Slovakia	MZ479361	NEOHY 020-21
	BRA CR34378	Slovakia	MZ479360	NEOHY 021-21
	BRA CR34384	Slovakia	MZ479359	NEOHY 022-21
	BRA CR34383	Slovakia	MZ479358	NEOHY 023-21
	BRA CR34370	Slovakia	MZ479357	NEOHY 024-21

Pantone 4247C). Lamellae adnexed, often very broad and thick, ventricose, brittle, white with brownish or greyish hue, much paler than pileus, slightly paler than stipe (Pantone P 1-9 C, Pantone 7527C), sometimes with paler edges. Context not reddening (without any colour changes), white, white with brownish hue, especially in cap (in stipe Pantone 7527C, in pileus Pantone 4645C, Pantone 4655C or Pantone 4665C); rather fragile, fibrillose. Smell unpleasant, significantly nitrous. Taste neutral, sometimes farinaceous. Spore deposit white. Basidiospores broadly ellipsoid, ellipsoid to ellipsoid-oblong, thin-walled, smooth, hyaline, non-amyloid, sometimes with one big vacuole, (6.5–)7.2–10.2(–11.8) × (4.4–)4.7–6.4(–7.5)  $\mu$ m, av. = 8.4 × 5.5 μm, Q = (1.1–)1.3–1.8(–2.1), Qav. = 1.56 (575 spores from 14 basidiomata measured from the type collections). Basidia (33.5-)35-51(-55) × (5.5-)6.8-9.5(-11.3) μm, av. =  $42 \times 8 \ \mu m$  (50 basidia from five basidiomata measured from the holotype), predominantly 4-spored, narrowly clavate to clavate, sterigmata (2.5–)2.7–6.6(–6.9) μm (50 sterigmata from five basidiomata measured from the holotype), awl-shaped. Basidioles  $(30.5-)33-46(-49) \times (5.4-)5.9-8.7(-10.1) \mu m$  (50 basidioles from five basidiomata measured from the holotype), clavate to broadly clavate. Cystidia absent. Pileipellis a cutis with cells 28–146  $\times$  3.5–15  $\mu$ m. Stipitipellis a cutis with some free hyphal ends (resembling a thrichoderm) with cells 25–160 × 3.9–17 µm, cells below pileipellis with brownish content. *Gill trama* subregular with cells 30–155 × 4–26.5 µm (some up to 400 µm), ± cylindrical, vermiform and sometimes with slightly inflated ends, long slender cells in centre and shorter cells to the sides. *Clamps* abundant in all tissues.

Additional materials examined: Czech Republic, Českotřebovská vrchovina Mts., Česká Třebová, alt. 475 m, mesic mowed meadow, 27 Jul. 2020, M. Mička (BRA CR34358). Slovakia, Javorníky Mts., Tomborov Salaš, Pšurnovice (Bytča), N49°14'2.85" E18°31'59.94", alt. 384 m, cow grazed and mowed meadow, 4 Oct. 2014, F. Fuljer (BRA CR34502); Melocík, Veľké Rovné, N49°20'33.42" E18°30'31.18", alt. 791 m, small overgrown meadow hidden in the forest, 29 Aug. 2019, F. Fuljer (BRA CR34375); Dučkov, Vysoká nad Kysucou, N49°21'38.56" E18°31'51.30", alt. 722 m, mesic mowed meadow, 31 Aug. 2018, F. Fuljer (BRA CR34374); Škápová, Petrovice, N49°14'54.65" E18°31'47.48", alt. 458 m, mesic mowed meadow, 1 September 2019, F. Fuljer (BRA CR34370); under the Holý vrch, Hvozdnica, N49°12'46.52" E18°27'0.67", alt. 547 m, mesic mowed meadow on calcareous soils, 19 Sep. 2019, F. Fuljer (BRA CR34377); Škápová, Petrovice, N49°15'2.01" E18°31'52.58", alt. 426 m, mesic mowed meadow, 2 Oct. 2019, F. Fuljer & M. Zajac (BRA CR34376);



0.10

Fig. 1. Maximum likelihood tree obtained from the analysis of ITS sequences of *Neohygrocybe* and *Cuphophyllus fornicatus* as outgroup. Bootstrap support values are indicated at the nodes.

under the Medvedie hill, Petrovice, N49°15'46.82" E18°31'1.80", alt. 422 m, mesic overgrown meadow, 2 Oct. 2019, *F. Fuljer & M. Zajac* (BRA CR34372); Benková, Petrovice, N49°16'4.53" E18°30'52.90", alt. 451 m, mesic mowed meadow, 2 Oct. 2019, *F. Fuljer & M. Zajac* (BRA CR34371); Medvedie, Petrovice, N49°15'53.80" E18°30'57.08", alt. 444 m, overgrown part of mesic meadow, 27 Oct. 2019, *F. Fuljer* (BRA CR34370); Baránkovci, Štiavnik, N49°16'50.71" E18°25'12.50", alt. 692 m, cow grazed pasture, 8 Jul. 2020, *F. Fuljer* (BRA CR34363); Benková, Petrovice, N49°16'2.26" E18°30'47.03", alt. 477 m, mesic mowed meadow, 13 Jul. 2020, *F. Fuljer* (BRA CR34369); Setechov, Petrovice, N49°16'7.00" E18°29'46.72", alt. 560 m, overgrown meadow, 16 Jul. 2020, *F. Fuljer* (BRA CR34368); Vrchrieka, Vysoká nad Kysucou, N49°21'41.23" E18°33'3.74", alt. 790 m, mesic mowed meadow, 22 Jul. 2020, *F. Fuljer* (BRA CR34367); Kržeľ, Papradno, N49°17'40.49"

E18°20'15.30", alt. 772 m, overgrown complex of meadows, 24 Jul. 2020, *F. Fuljer* (BRA CR34366); Čiakov, Kolárovice, N49°19'27.38" E18°31'25.04", alt. 674 m, overgrown meadow, 25 Jul. 2020, *F. Fuljer* (BRA CR34365); Tomborov Salaš, Pšurnovice (Bytča), N49°14'0.45" E18°31'57.01", alt. 373 m, cow grazed meadow, 25 Jul. 2020, *F. Fuljer* (BRA CR34364); Brezie, Petrovice, N49°15'41.07" E18°30'57.63", alt. 456 m, mowed meadow, 12 Sep. 2020, *F. Fuljer* (BRA CR34356); Zákysučie, Krásno nad Kysucou, N49°22'44.18" E18°48'59.35", alt. 559 m, overgrowing mesic heathland, 10 Oct. 2020, *F. Fuljer* (BRA CR34356); Medvedie 2, Petrovice, N49°15'46.45" E18°30'51.77", alt. 466 m, small sized overgrown meadow, 26 Oct. 2020, *F. Fuljer* (BRA CR34357); Jablunkovské medzihorie Mts., Poľana, Skalité, N49°30'16.3" E18°55'32.9"alt. 730 m, mesophilic mowed meadow, 25 Jul. 2020, *M. Zajac* (BRA CR34381); Turzovská vrchovina Mts.,



Fig. 2. Macromorphological characters of *Neohygrocybe pseudoingrata*. A. Basidiomata in the natural habitat, where the holotype was collected (BRA CR33023, holotype). B. Different shapes of basidiomata (PHFF1143, paratype). C. The robust stature of *N. pseudoingrata* in the natural habitat (PHFF11554, paratype). D. Basidiomata in the natural habitat (PHFF10723, paratype). E. Basidioma with brownish pileus, in the natural habitat (PMZ554, paratype). F. Basidioma with greyish pileus, in the natural habitat (PHFF11080, paratype). G. Cross-section of the well-grown basidioma, hollow stipe and adnexed lamellae visible (BRA CR33023, holotype). H. Closer, ventral view on the compressed stipes and lamellae (BRA CR33023, holotype). I. Closer view showing the colour, shape and smooth surface of the pileus (BRA CR33023, holotype). Scale bars = 20 mm.



Fig. 3. Neohygrocybe pseudoingrata (BRA CR33023, holotype). A. Basidiospores. B. Pileipellis. Scale bars: A = 10 µm; B = 20 µm.

Boháčovci, Korňa, N49°26'33.33" E18°31'49.19", alt. 714 m, mesic mowed meadow, 8 Sep. 2019, F. Fuljer (BRA CR34362); Ďurajčíkovci, Korňa, N49°25'46.13" E18°31'4.40", alt. 698 m, mesic mowed meadow, 8 Sep. 2019, F. Fuljer (BRA CR34379); Hlavice - Flintovci, Klokočov, N49°27'52.4" E18°36'33.2", alt. 720 m, mesic mowed meadow, 10 Sep. 2019, M. Zajac (BRA CR34382); Kysucká vrchovina Mts., Tatarovci - Senkov, Povina, N49°18'08.7" E18°43'52.7", alt. 633 m, mesic mowed meadow, 11 Sep. 2019, Z. Václavová (BRA CR34384); Harvelka, Nová Bystrica, N49°21'27.10" E19°8'50.17", alt. 808 m, sheep grazed pasture, 25 Sep. 2019, F. Fuljer & M. Zajac (BRA CR34378); Harvelka, Nová Bystrica, N49°21'25.21" E19°8'4.39", alt. 783 m, sheep grazed pasture, 17 Sep. 2020, F. Fuljer (BRA CR34359); Brodenec, Snežnica, N49°15'42.87" E18°47'4.41", alt. 459 m, mesic mowed meadow, 7 Oct. 2020, F. Fuljer (BRA CR34360); Kysucké Beskydy Mts., Serafinov vlek, Skalité, N49°29'39.1" E18°57'48.8", alt. 725 m, mesic mowed meadow, 28 Sep. 2019, M. Zajac (BRA CR34383); Biele Karpaty Mts., Kopánka, Horné Orechové, N48°55'31.31" E18°1'59.62", alt. 261 m, cow grazed pasture, 20 Oct. 2020, F. Fuljer (BRA CR34361).

### DISCUSSION

Due to the dull colouration of the basidiomata and dry surfaces of stipe and pileus, this new waxcap clearly belongs to the genus Neohygrocybe, as has been confirmed also by the phylogenetic analysis. It is a well recognisable species, characterised by robust dull coloured basidiomata, nitrous smell, non-reddening context, pale brownish and grevish, smooth or finely fibrillose pileus, slightly greyish or brownish, contorted, compressed and hollow stipe and broadly ellipsoid to ellipsoid spores (Figs 2, 3). Closely related species are N. ingrata, N. nitrata and N. ovina. The most similar species is N. ingrata, in which the context stains reddish. Young basiomata of N. pseudoingrata and N. ingrata can be very similar, distinguished only by the reddening context of N. ingrata. Neohygrocybe nitrata also has a nitrous smell and also lacks the reddish reaction of the context, but it is usually smaller (up to 60-70 mm high), with a more or less squamulose dark brown pileus and thinner stipe (up to 6 mm diam) which is also dark brown. Neohygrocybe oving is much darker, with dark brown, dark grey or almost black stipe, pileus and lamellae and the context is strongly reddening, and the cap may be squamulose. Several other Neohygrocybe-taxa have

been described from North and Central America, Australia, New Zealand and China (many not yet combined into the genus) such as Hygrocybe lepidopellis, H. cinerascens, H. mellita, H. albomarginata, H. caespitosa, H. melleofusca, H. ovinoides, H. fuligineosquamosa, H. waolipo, Neohygrocybe griseonigra, N. innata, N. subovina, and N. squarrosa (Hesler & Smith 1963, Pegler 1983, Horak 1990, Desjardin & Hemmes 1997, Cantrell & Lodge 2004, Young 2005, Bessette et al. 2012, Wang et al. 2018). None of these have been sequenced, but all differ from N. pseudoingrata in darker colouration, spore morphology, structure of pileus surface or colour changes. Cuphophyllus species differ by deeply decurrent lamellae; C. fornicatus is the single species of the genus that lacks decurrent lamellae and strongly resembles *N. pseudoingrata* but for the nitrous smell. Dull coloured Gliophorus species differ by lubricous cap and stipe surfaces. Pseudotricholoma metapodium is characterised by amyloid spores, solid, non-compressed stipe and lamellae that are not veined.

The indicator value for valuable grasslands of *N. pseudoingrata* is uncertain. Recorded collections from Slovakia and the Czech Republic are from mowed meadows or extensively grazed pastures and were accompanied by various CHEG fungi. Further research will reveal whether the species is rare or only overlooked and misinterpreted. Based on the numerous collections from NW Slovakia it can be assumed that *N. pseudoingrata* is probably common in Slovakia and should be searched for in neighbouring countries.

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**Conflict of interest:** The authors declare that there is no conflict of interest.



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