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A new species of true morel from Switzerland: *Morchella helvetica*, sp. nov

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ABSTRACT

Morchella helvetica, sp. nov. (*Morchella* sect. *Distantes*) is a new species of true morels discovered in Switzerland. It is formally described in the present study using an integrative approach based on micro- and macromorphological characteristics, multilocus phylogenetics, and a brief description of its habitat. Molecular analyses clearly indicated that *Morchella helvetica* is a sister species to *M. eximioides, M. angusticeps*, and *M. confusa*. It can be distinguished by the two phylogenetic markers RNA polymerase II subunit 2 (*RPB2*) and translation elongation factor-1 alpha *(TEF1-α)*. In addition, *M. helvetica* exhibits particular morphological features, notably the presence of pale hairs on the pileus, a mealy stipe, and darkening ridges when aging.

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KEYWORDS
Integrative taxonomy; *Morchella* spp.; morphology; phylogenetics; 1 new taxon

INTRODUCTION

True morels (Ascomycota, Pezizales, *Morchella*) are edible and prized mushroom-forming fungi that are distributed worldwide, particularly in the Northern Hemisphere (O'Donnell et al. 2011). Recent studies show that *Morchella* is divided into three main sections: *Morchella* sect. *Rufobrunnea* (Rufobrunnea clade; gray or blushing morels), *Morchella* sect. *Morchella* (Esculenta clade; yellow morels), and *Morchella* sect. *Distantes* (Elata clade; black morels) (Loizides et al. 2022; Richard et al. 2015). The Esculenta and Elata clades comprise both formally described and undescribed (i.e., phylogenetic species, or phylospecies, denoted with Mes- or Mel- numbers) species-level designations (Loizides et al. 2022). Current literature formally recognizes the existence of 59 valid morel species with a Latin binomial (Clowez et al. 2022; Loizides et al. 2022; Machuca et al. 2021). In addition, multigene phylogenetics estimates that an additional 24 (or more) unnamed phylospecies exist (Loizides et al. 2022).

Before the advent of molecular tools, *Morchella* taxonomy was particularly confusing because multiple names had been applied to different representatives of the same species based on the macromorphology of the fruiting body (Fatton 2016). *Morchella* taxonomy has since been revised multiple times using molecular data (Loizides et al. 2022; Richard et al. 2015). Reliable genetic identification based on multilocus phylogenetic inference (Sa et al. 2022) has identified phylogenetic species that would still require morphological and ecological data for a formal species description (Loizides et al. 2022). Indeed, to reliably describe new morel species, an integrative approach that combines genetic, morphological, and ecological data is necessary (Loizides et al. 2022). In the present study, a new morel species found in the canton of Neuchâtel (Switzerland) is formally described with ecological, macro- and micromorphological characteristic, and multilocus genetic data using nine distinct populations. This is the first *Morchella* species described from Switzerland using this integrative approach.

MATERIALS AND METHODS

Sample collection.—Fruiting bodies were sampled in spring 2019 (prefix M19-), 2021 (prefix M21-), and 2023 (prefix M23-) in the canton of Neuchâtel (Switzerland). In total, 12 specimens from nine distinct populations were phylogenetically analyzed (populations A to I; TABLE 1). From those, six specimens (one holotype and five paratypes; TABLE 1) were morphologically analyzed in detail, as described in the following sections.

Macroscopic analysis.—Macroscopic pictures of fresh ascocarps were taken with a digital camera (Canon PowerShot SX230 hS; Tokyo, Japan). Fruiting bodies

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Table 1. Sequenced collections of *Morchella helvetica* and references used in the phylogenetic analysis (see status).

Species	Specimen	Status	Collection ID	Date of collection	Sampling location	Country	GenBank Accession numbers				Reference
							ITS	*RPB1*	*RPB2*	*TEF1-α*	
Morchella helvetica	**M19-4**	**Topotype of *M. helvetica***	**NCH NEU19-4**	**26 Mar 2019**	**Cressier, population A**	**Switzerland**	**OR482716**	**PP598873**	**PP598874**	**OR667832**	**This study**
Morchella helvetica	**M19-7**	**Topotype of *M. helvetica***	**NCH NEU19-7**	**26 Mar 2019**	**St-Blaise, population B**	**Switzerland**	**OR482719**	**PP598875**	**PP598876**	**OR667835**	**This study**
Morchella helvetica	**M19-9**	**Topotype of *M. helvetica***	**NCH NEU19-9**	**26 Mar 2019**	**St-Blaise, population B**	**Switzerland**	**OR482721**	**PP598877**	**PP598878**	**OR667837**	**This study**
Morchella helvetica	**M19-12**	**Paratype of *M. helvetica***	**NCH NEU19-12**	**26 Mar 2019**	**Neuchâtel, population C**	**Switzerland**	**PP601261**	**OR667968**	**OR668099**	**OR667840**	**This study**
Morchella helvetica	**M21-10**	**Topotype of *M. helvetica***	**NCH NEU21-10**	**31 Mar 2021**	**Enges, population D**	**Switzerland**	**OR482744**	**N.A.**	**OR668128**	**OR667861**	**This study**
Morchella helvetica	**M21-12**	**Paratype of *M. helvetica***	**NCH NEU21-12**	**31 Mar 2021**	**St-Blaise, population E**	**Switzerland**	**OR482745**	**PP598879**	**PP598880**	**OR667862**	**This study**
Morchella helvetica	**M23-1**	**Paratype of *M. helvetica***	**GCH G00576167**	**21 Mar 2023**	**Hauterive, population F**	**Switzerland**	**OR539921**	**PP598881**	**PP598882**	**OR757070**	**This study**
Morchella helvetica	**M23-3**	**Topotype of *M. helvetica***	**NCH NEU23-3**	**21 Mar 2023**	**Hauterive, population F**	**Switzerland**	**OR539923**	**PP598883**	**PP598884**	**OR757072**	**This study**
Morchella helvetica	**M23-4**	**Holotype of *M. helvetica***	**GCH G00576168**	**21 Mar 2023**	**Hauterive, population G**	**Switzerland**	**OR539924**	**PP598885**	**PP598886**	**OR757073**	**This study**
Morchella helvetica	**M23-6**	**Isotype of *M. helvetica***	**NCH NEU23-6**	**21 Mar 2023**	**Hauterive, population G**	**Switzerland**	**OR539925**	**PP598887**	**PP598888**	**OR757074**	**This study**
Morchella helvetica	**M23-10**	**Paratype of *M. helvetica***	**GCH G00576169**	**28 Mar 2023**	**Neuchâtel, population H**	**Switzerland**	**OR539926**	**PP598889**	**PP598890**	**OR757075**	**This study**
Morchella helvetica	**M23-11**	**Paratype of *M. helvetica***	**GCH G00576170**	**07 Apr 2023**	**St-Blaise, population I**	**Switzerland**	**OR539927**	**PP598891**	**PP598892**	**OR757076**	**This study**
Morchella helvetica	IP245	Czech representative of *M. helvetica*	–	–	–	Czech Republic	MH982718	MH982887	MH982943	MH982982	Petrželová and Sochor (2019)
Morchella importuna	YAASM69	Phylogenetic reference	–	–	–	China	MG589675.1	MG598564	MG598606	MG589713.1	Chai et al., unpublished
Morchella sextelata	M120	Phylogenetic reference	–	–	–	USA	JQ723034.1	GU551070	GU551111	GU550988.1	O'Donnell et al. (2011)
Morchella snyderi	M299	Phylogenetic reference	–	–	–	USA	GU551413.1	GU551450	GU551490	GU551376.1	O'Donnell et al. (2011)
Morchella mediterraneensis	HT 25	Phylogenetic reference	–	–	–	Turkey	HM056397.1	HM056455	HM056517	HM056357.1	Hatira et al. (2010)
Morchella sp. Mel-14	M728	Phylogenetic reference	–	–	–	China	JQ723054.1	GU551663	GU551522	GU551565.1	O'Donnell et al. (2011)
Morchella confusa	Mel-42/1028	Phylogenetic reference	–	–	–	China	MK321849.1	MK321855	MK321861	MK321867.1	Du et al. (2019)
Morchella angusticeps	M304	Phylogenetic reference	–	–	–	USA	JQ723055.1	GU551658	GU551509	GU551560.1	O'Donnell et al. (2011)
Morchella angusticeps	M407	Phylogenetic reference	–	–	–	USA	GU551416.1	GU551453	GU551493	GU551379.1	O'Donnell et al. (2011)
Morchella eximioides	M494	Phylogenetic reference	–	–	–	Norway	JQ723056.1	GU551655	GU551498	GU551557.1	O'Donnell et al. (2011)
Morchella eximioides	M231	Phylogenetic reference	–	–	–	Sweden	GU551428.1	GU551465	GU551508	GU551391.1	O'Donnell et al. (2011)

Note. Sequences generated in this study are highlighted in bold. The Swiss fruiting bodies are maintained in different collections, represented by unique numbers (collection ID): NCH = Neuchâtel Cryptogamy Herbarium; GCH = Geneva Cryptogamy Herbarium.

were measured (length, width, ratio cap:stipe). Primary and secondary ridges and alveoli were described (aspect, color), counted, and measured. The color was subjectively determined by comparing the pictures with the color palette from Microsoft PowerPoint and noting the hexadecimal code. The sinus (i.e., the portion of the hymenophore that is attached to the stipe) was described (shape) and measured (depth and width). The fruiting bodies were kept in plastic jars in a dark oven at 22 C for 2–3 days to trigger natural sporulation. The spores were kept at room temperature, and the dry fruiting bodies were frozen at −20 C.

Microscopic analysis.—Microscopic features were observed and photographed under a Leica DM4 B microscope (Leica Microsystems, Wetzlar, Germany). Samples were prepared by cutting thin slices of the hymenium (i.e., fertile part of alveoli), ridge, and external layer of the stipe (i.e., ectal excipulum) and immersing the slices in physiological water (0.9% NaCl) on microscope slides. Asci (i.e., fertile elements of the hymenium), paraphyses (i.e., sterile elements of the hymenium), acroparaphyses (i.e., sterile elements of the ridges), sterile elements of the ectal excipulum, and ascospores (144 spores from free spores or spore prints of three fruiting bodies from three populations) were measured (length and width) from microscopic pictures, using the software ImageJ 1.53q (Schneider et al. 2012). In addition, ascospores were stained with 4 mM DAPI (4′,6-diamidino-2-phenylindole) and observed under a Leica DM4 B fluorescence light microscope (Leica Microsystems) to visualize and count the nuclei.

DNA extraction and sequencing.—DNA was extracted from 2-cm^3 pieces of fresh or dry fruiting bodies (hymenia) using the Quick-DNA Fungal/Bacterial Miniprep kit (Zymo Research, Irvine, California) following the protocol provided by the manufacturer. Eluted DNA was quantified with fluorometry using a Qubit kit (Invitrogen, Waltham, Massachusetts) and the Broad Range buffer and reagent. DNA was then diluted with polymerase chain reaction (PCR)-grade water to a concentration of 2 ng/μL to be used as template in PCRs. Four genetic regions were amplified: nuclear rDNA internal transcribed spacer region ITS1–ITS2, including the 5.8S ribosomal RNA gene (ITS) (Gardes and Bruns 1993; White et al. 1990); RNA polymerase II largest subunit (*RPB1*) (Du et al. 2012); RNA polymerase II second largest subunit (*RPB2*) (Hatira et al. 2010); and the translation elongation factor-1 alpha (*TEF1-α*) gene (Rehner and Buckley 2005). Detailed primer information is available in SUPPLEMENTARY TABLE 1. For each sample, the PCR mix contained PCR-grade water, 2× ALLin Red Taq Mastermix (HighQu, Kraichtal, Germany), 0.2 μM forward and 0.2 μM reverse primer, and 1 μL of 2 ng/μL DNA. Amplifications were performed in a Thermo Scientific Arktik thermal cycler (Thermo Fisher Scientific, Waltham, Massachussets). The following parameters were used: denaturation at 95 C for 1 min, 40 cycles of denaturation (95 C for 15s), annealing (62 C [ITS] or 50 C [*RPB1*] or 55 C [*RPB2*] or 55 C [*TEF1-α*] for 15s), and elongation (72 C for 15s), and final elongation at 72 C for 2 min. PCR products were then loaded on a 1.2% agarose gel that underwent electrophoresis (100 V, 30 min). Amplicons were visualized under ultraviolet (UV) light in a GenoPlex VWR transilluminator (VWR, Radnor, Pennsylvania). Positive PCR products (i.e., single band at the expected size) were then purified with MultiScreen PCRμ96 filter plates (Millipore, Burlington, Massachusetts) as follows: in each well, the PCR product and 50 μL of PCR-grade water was added; a vacuum of 20 bars was applied on the wells until they dry; 20 μL of PCR-grade water was added to each well; after 2 min, DNA contained in the membrane from each well was resuspended by pipetting up and down 20 times. Once purified, the PCR products were quantified by fluorometry as indicated above. Final concentration was adjusted at 2–40 ng/μL and sent to Genesupport (Geneva, Switzerland) for Sanger sequencing.

Phylogenetic analysis.—For the phylogenetic analysis, we used sequences that we generated and sequences of reference sister species downloaded from the public database GenBank (https://www.ncbi.nlm.nih.gov/genbank/) (accession numbers and metadata available in TABLE 1). Forward and reverse sequences were manually trimmed and automatically assembled with Geneious Prime 2022.2 (Biomatters, Auckland, New Zealand). The sequences were aligned with MUSCLE (neighbor-joining cluster method) within the software MEGA 11 (Kumar et al. 2018), and the direction of the sequences was verified and reversed if necessary. Gaps were automatically deleted using the online tool Gblocks 0.91b (Castresana 2000; Dereeper et al. 2008). Four single-locus phylogenies (ITS, *RPB1, RPB2, TEF1-α*) and one concatenated four-locus phylogeny (ITS-*RPB1-RPB2-TEF1-α*) were generated. In the concatenated alignment, gaps situated between the different genes were replaced by missing data ("N") with the software BioEdit 7.2.5. (Dagona 1999). Phylogenies were computed within the IQ-TREE Web server (Nguyen et al. 2015) using the best substitution model for each data set and generating 10,000 ultrafast

bootstrap (UFBoot) values (Hoang et al. 2018). The phylogenies were displayed and annotated in FigTree 1.4.4 (Rambaut 2010) and deposited in TreeBASE (submission 30784).

RESULTS

Phylogenetic analyses.—The four-locus phylogenetic analysis (generated with substitution model Tne+G4) based on concatenated genetic markers (ITS, *RPB1*, *RPB2*, and *TEF1-α*) indicated that the Swiss specimens M19-4, M19-7, M19-9, M19-12, M21-10, M21-12, M23-1, M23-3, M23-4, M23-6, M23-10, and M23-11 (see TABLE 1 for the metadata and FIG. 1 for the sampling location of the populations) belong to a previously undescribed species, which we designate here as *Morchella helvetica*. The multigene ML phylogeny (FIG. 2) supports this taxon statistically with a 96% bootstrap value (UFboot). *Morchella helvetica* is closely related to *M. confusa, M. angusticeps*, and *M. eximioides*. The single-locus phylogenies of the genetic markers indicated that ITS (generated with substitution model K2P+I) and *RPB1* (substitution model K2P) were not reliable to discriminate *M. helvetica* from sister species. In the ITS phylogeny, *M. helvetica* was grouped with *M. angusticeps, M. eximioides*, and *M. confusa* (FIG. 3A). In the *RPB1* phylogeny, similarly, there was only one taxon including the aforementioned species, in addition to *Morchella* sp. Mel-14 (FIG. 3B). In contrast, phylogenies based on the sequences of the *RPB2* and *TEF1-α* genes (both generated with substitution model TNe) distinguished *M. helvetica* from sister species. In both cases, all species were properly delineated from each other (FIGS. 3C–D).

TAXONOMY

Morchella helvetica M. Cravero, G. Bonito, P. Chain, S. Bindschedler & P. Junier, sp. nov. FIG. 4
MycoBank MB855081

Typification: SWITZERLAND. NEUCHÂTEL: Hauterive, 47.0159, 6.9707, 568 m altitude, in a mixed forest under *Fraxinus excelsior* and *Quercus* spp., 21 Mar 2023, *Blaise Hofer, M23-4* (**holotype** G00576168).

Diagnosis: Morchella helvetica belongs to *Morchella* sect. *Distantes* and represents a sister taxon of the subgroup *M. angusticeps*/*M. eximioides*/*M. confusa. Morchella helvetica* is phylogenetically distinct from sister species by *RPB2* and *TEF1-α* sequences. It is similar to *M. eximioides* but differs by its phenology (*M. eximioides* fruits between May and June [Weholt et al. 2020] whereas *M. helvetica* fruits between March and April). *Morchella helvetica* can be also distinguished morphologically by the stipe texture (tomentose and furfurasceous in *M. eximioides* [Jacquetant 1984] and mealy in *M. helvetica*) and by darkening of the pileus ridges when aging (feature also observed in *M. eximioides* [Du et al. 2019]). Furthermore, the presence of pale dense hairs on ridges and alveoli seems to be typical of *M. helvetica* compared with other members of *Morchella* sect. *Distantes*.

Etymology: helvetica, based on "Helvetia," the Latin word for "Switzerland," in reference to the country where the species was found and described.

Description: Ascomata 54–100 mm tall. Pileus ovoid-conical or oblong to sharply conical, 31–59 mm high and 24–36 mm wide (at widest point), attached to the stipe with a distinct, skirting sinus measuring 2–3 mm deep and 3–4 mm wide. Pileus pitted and ridged, with 16–22 vertically elongated primary ridges and numerous transecting, horizontal, sunken secondary ridges. No vertical secondary

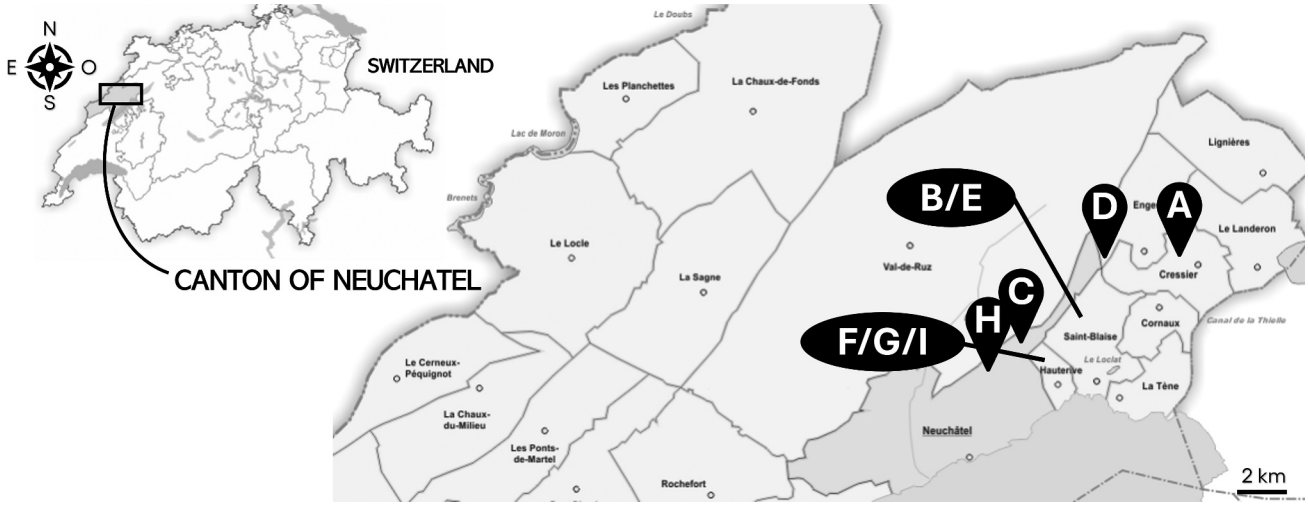

Figure 1. Sampling map. Map of the canton of Neuchâtel (Switzerland) representing the sampling locations of the different populations of *Morchella helvetica* (A–I; referred to in TABLE 1) collected for this study between 2019 and 2023.

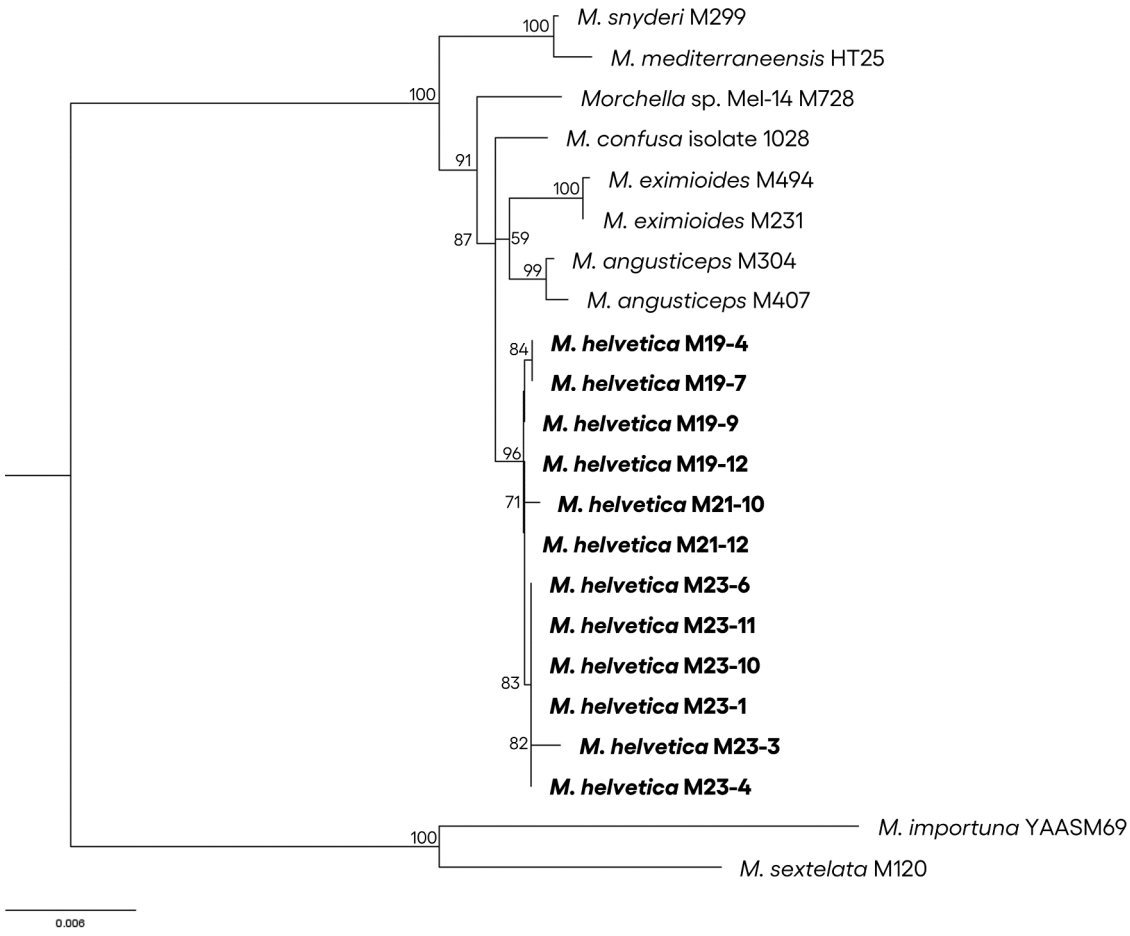

Figure 2. Four-locus phylogeny. Multilocus (ITS-*RPB1-RPB2-TEF1-α*) ML phylogeny of sister clades of *Morchella helvetica* (*Morchella* sect. *Distantes*) with Swiss specimens (bold) and reference sequences. The phylogeny was based on 10 000 ultrafast bootstrap values (UFBoot displayed at branch nodes). The tree was rooted with *M. sextelata* and *M. importuna*.

ridges. Primary ridges 1 mm thick, brown (around 70440F) and slightly rounded when young, blackening from the apex and becoming sharp or eroded with age. Primary ridges finely tomentose with pale hairs. Secondary ridges concolorous with the pits and rarely with the same texture and color as the primary ones. Primary alveoli vertically elongated, slightly irregular to regular with a laddered appearance. Alveoli lighter than the ridges with yellow tones when young, becoming brownish orange (around BB7725) with light reddish tones (around C8864E) with age, tomentose with pale hairs. Usually 0–9 secondary alveoli per pit. Context whitish and layered, about 3 mm thick. Stipe cylindrical, often basally enlarged, slightly to strongly channeled all along the stipe, often folded and perforated basally (even young), whitish (around FCF9F6) and mealy, 20–55 × 9–27 mm (FIG. 4A–C). Ratio cap:stipe usually >1. Sterile inner surface whitish and granulose. Odor and taste unknown. Ascospores elliptical, smooth, hyaline, (17–)20–25(–29) × (13–)14–15(–16) µm (FIG. 4F), with 12–17 nuclei (FIG. 4G). Asci cylindrical, hyaline, 8-spored, operculate, thin-walled (230–)265–325 (–350) × (9–)15–19(–20) µm (FIG. 4E). Paraphyses were scattered, cylindrical, with cylindrical, ophiomorphous, acute, or globular apices, septate, 6–7 µm wide (FIG. 4E). Acroparaphyses cylindrical, clavate, or ophiomorphous, uni- or bifurcate, hyaline, with 1–3 septa, occasionally with a small bud on the terminal cell. Sterile elements of the stipe cylindrical or ophiomorphous, uni- or bifurcate, hyaline, can be septate (FIG. 4D).

Ecology and distribution: In Switzerland, in mixed forests under *Fraxinus excelsior* and *Quercus* spp., on calcareous soils or on mossy limestone rocks. Fruiting between late March to early April, solitary, between 560 and 1050 m altitude in the canton of Neuchâtel, Switzerland.

Other material morphologically examined: SWITZERLAND. NEUCHÂTEL: Neuchâtel, Forêt de Peseux, 47.0245, 6.9549, 1041 m altitude, in mixed forests under *Fraxinus excelsior*, 26 Mar 2019, *Blaise Hofer, M19-12* (NEU19-12); St-Blaise, Forêt du Chuffort, 47.0268, 6.9814, 641 m altitude, in mixed forests under *Fraxinus excelsior*, 31 Mar 2023, *Blaise Hofer, M21-12*

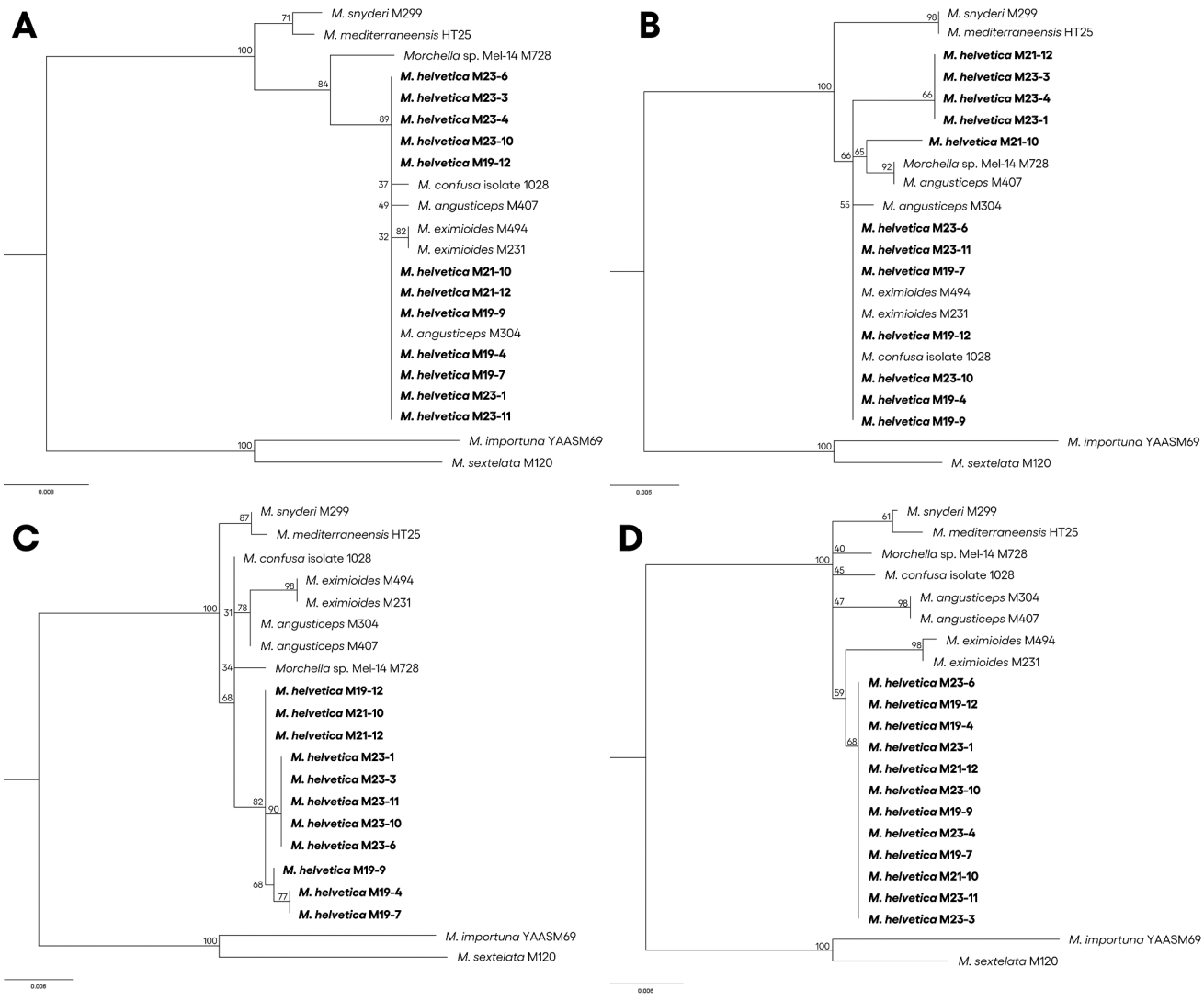

Figure 3. Single-locus phylogenies. Single-locus (ITS) ML phylogeny of sister clades of *Morchella helvetica* (*Morchella* sect. *Distantes*) with Swiss specimens (bold) and reference sequences for the single-locus genetic regions ITS (A), *RPB1* (B), *RPB2* (C), and *TEF1-α*. (D) Phylogenies were based on 10 000 ultrafast bootstrap values (UFBoot displayed at branch nodes). Trees were rooted with *M. sextelata* and *M. importuna*.

(NEU21-12); Hauterive, 47.0156, 6.9686, 600 m altitude, in mixed forests under *Fraxinus excelsior*, 21 Mar 2023, *Blaise Hofer, M23-1* (G00576167); Neuchâtel, Forêt de Peseux, 47.0145, 6.9587, 662 m altitude, in mixed forests under *Fraxinus excelsior*, 28 Mar 2023, *Blaise Hofer, M23-10* (G00576169); St-Blaise, Forêt du Chuffort, 47.0243, 6.9825, 586 m altitude, in mixed forests under *Fraxinus excelsior*, 7 Apr 2023, *Blaise Hofer, M23-11* (G00576170).

Comments: Morchella helvetica is distinct from *M. angusticeps* and *M. confusa* by the absence of vertical secondary ridges, the orange to reddish tones of the aging primary alveoli, and the presence of hairs (i.e., tomentose) on the primary alveoli (Kuo et al. 2012; Du et al. 2019). Additionally, *M. helvetica* differs from *M. confusa* by the presence of folds and channels on the stipe, and by darkening edges with aging (Xi-Hui et al. 2019). Concerning the maturation process, young specimens of *M. helvetica* have yellowish brown alveoli. They often turn reddish with age but remain lighter than the ridges, which darken from the apex until becoming completely dark brown to black. The pileus becomes narrower with age, and alveoli become more regular. The secondary ridges are more apparent with aging. Genetically, the single ITS or *RPB1* markers do not distinguish it from *M. eximioides, M. angusticeps*, or *M. confusa* (FIGS. 3A–B); however, *RPB2* and *TEF1-α* distinguish these species (FIGS. 3C–D). In a BLAST search in GenBank, a 99.88% identity match was discovered between the *RPB2* sequence of *M. helvetica* M23-3 and the sequence of the Czech specimen "*Morchella* sp. voucher IP245" (Petrželová and Sochor

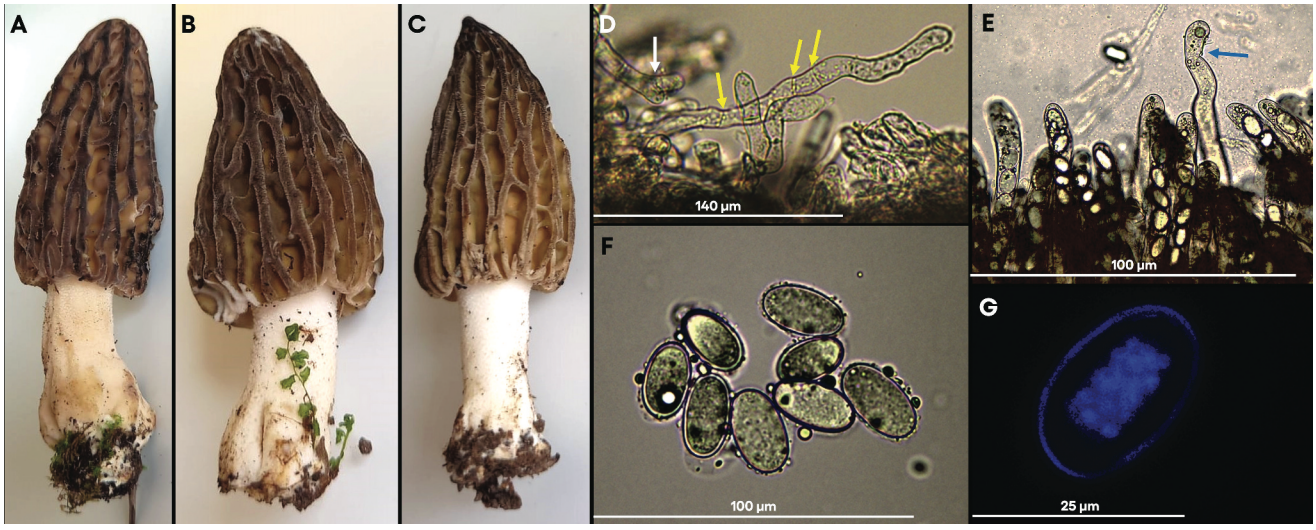

Figure 4. Macroscopic and microscopic pictures of *Morchella helvetica*. (A) Mature fruiting body of *Morchella helvetica* M23-4 (holotype). (B) Young fruiting body of *M. helvetica* M23-2. (C) Mature fruiting body of *M. helvetica* M23-3. (D) Ectal excipulum (outer layer) of the stipe of *M. helvetica* M19-5, highlighting bipartite cylindrical elements (white arrow) and septa (yellow arrows) (light microscope, physiological water, 20×). (E) Asci and paraphyse (blue arrow) of *M. helvetica* M21-13 (light microscope, φw, 20×). (F) Ascospores of *M. helvetica* M19-43 (light microscope, physiological water, φw, 40×). (G) Multinucleate ascospore of *M. helvetica* M19-43 (light microscope, DAPI, 40×).

2019). ITS, *RPB1*, *RPB2*, and *TEF1-α* sequences of specimen IP245 were concatenated and included in the initial *M. helvetica* phylogenetic data set. The resulting phylogeny indicated that IP254 belonged to *M. helvetica* (SUPPLEMENTARY FIGURE 2).

DISCUSSION

Our data show that *Morchella helvetica* is a new species of black morels. We formally describe the species based on material sampled from nine populations in the canton of Neuchâtel, Switzerland (FIG. 1), and one Czech specimen (TABLE 1). In the description of this new species, the criterion of monophyly (i.e., a species can be recognized if the monophyly from a multilocus tree is not contradicted by any of the single-locus trees) and the genealogical concordance criterion (i.e., genealogical exclusivity must be supported by bootstrap analysis in both the multilocus tree and in at least one of the single-locus trees) are fulfilled (FIGS. 2–3). This supports the designation of *M. helvetica* as a phylogenetic species (O'Donnell et al. 2011). This species is closely related to a species subgroup in the Holarctic comprising the *M. angusticeps* from North America and *M. eximioides* from Europe and China (Loizides et al. 2022).

The Czech representative of *M. helvetica* (IP245) was initially attributed by its authors as an undescribed lineage within the *M. eximioides*/*M. angusticeps* complex (Petrželová and Sochor 2019). They indicated that the lineage fulfilled the criterion of monophyly and the genealogical criterion and could therefore be considered a new phylospecies. However, the authors did not formally assign it to a new species, because it was only discriminated by *RPB2* and *TEF1-α*, and not by ITS and *RPB1*, as confirmed here with a broader sampling (see FIG. 3). In addition, due to limited sampling and phenotypic data, the authors could not exclude that the genetic variation of the lineage of IP245 represented a geographic divergence of the species *M. eximioides*/*M. angusticeps* (Petrželová and Sochor 2019). In the present study, we demonstrate that *Morchella* sp. IP245 belongs to *M. helvetica* (SUPPLEMENTARY FIGURE 2). Our combined morphological and genetic analysis led us to exclude that IP245 and our Swiss collections are geographic variants of *M. eximioides*/*M. angusticeps*.

Furthermore, to investigate whether *M. helvetica* could instead correspond to another already described species with an unresolved status and/or that has been abandoned in recent literature due to lack of species representatives or genetic data, species that have been described from the canton of Neuchâtel were searched for in literature. We found four *Morchella* species described in 1890 by Fritz Leuba, a mycologist from Neuchâtel, that are absent from the current morel taxonomy: *M. autumnalis, M. canina, M. pratensis*, and *M. radicosa* (Fatton 2016; Leuba 1890). The morphological and ecological descriptions of these species were compared, but none corresponded to *M. helvetica: M. autumnalis* fruited in autumn (September to November); *M. canina* had a rufescent stipe when

aging and fruited in mountains under *Abies* spp.; *M. pratensis* had a brown red pileus and a rufescent stipe, fruited in June in meadows; and *M. radicosa* was described as a yellow morel (Leuba 1890).

Phenotypically, the maturation process is an important feature in *Morchella* taxonomy (Loizides et al. 2016) that allows us here to distinguish *M. helvetica* from *M. confusa* and *M. eximioides*, the two latter lacking dark ridges when aging (Du et al. 2019). The presence of pale hairs on both alveoli and ridges of the pileus found on all specimens of the Swiss *M. helvetica* is apparently a unique feature of *Morchella* sect. *Distantes* that was used as one of the principal diagnosis characteristics. Ascospores of *M. helvetica* contained 12–17 nuclei. Nuclei are usually not considered in *Morchella* phylogenetics (Loizides et al. 2022), as their number can vary intraspecifically. For instance, in *Morchella galilaea*, between 0 and 20 nuclei were found per ascospore and that number was related to spore size (Du et al. 2023). In members of the species complex *M. angusticeps*/*M. eximioides*/*M. confusa*, spore number was only assessed for *M. eximioides* and corresponded to ≥6 per nucleus (Du and Yang 2021).

The sister species of *M. helvetica*, namely, *M. angusticeps* from North America, *M. eximioides* (found in Europe and Asia), and *M. confusa* from China, have never been reported in Switzerland (Loizides et al. 2022; Du et al. 2019). In Switzerland, *M. helvetica* has so far only been found in the canton of Neuchâtel, in mixed forests under ash and oak trees. The species should be searched in other areas of the country to investigate the extent of its range of distribution. The phenology was used as a diagnosis criterion to differentiate *M. helvetica* from *M. eximioides*, as they fruit in March–April and May–June, respectively, but this variation could also represent climatic divergences between Switzerland and countries further North (Norway, Sweden) where *M. eximioides* is found (Weholt et al. 2020). Finally, *M. helvetica* does not appear to be a fire-adapted morel, as no forest fires have been reported in its habitat.

To conclude, this study presents the new species *M. helvetica*, a sister taxon within the subgroup *M. angusticeps*/*M. eximioides*/*M. confusa* in *Morchella* sect. *Distantes*, from which it differs by phylogenetic, morphological, and ecological data. This true morel has so far only been found in Switzerland, fruiting near ash and oak trees in early spring, but several sequenced collections were also reported in the Czech Republic (Petrželová and Sochor 2019), indicating that it potentially has a broader range.

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DISCLOSURE STATEMENT

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