Disentangling identity of species of the genus *Taphrina* parasitizing herbaceous *Rosaceae*, with proposal of *Taphrina gei-montani* sp. nov.

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Five strains (CCY 058-007-001^T, CCY 058-007-002, CCY 058-007-003, CCY 058-007-004 and CCY 058-007-005) of a novel parasitic yeast belonging to the genus *Taphrina* were isolated from leaf tissues of *Geum montanum* L. (*Rosaceae*), collected from the Vysoké Tatry Mountains, Slovakia. Genetic analyses revealed that these isolates differ by 15 unique substitutions in the ITS region and by six substitutions in the *rns* gene from all other species of the genus *Taphrina* analysed hitherto. The novel strains are also distinguished from all other species of the genus *Taphrina* by their morphology, biochemical properties and ecology. These strains represent a novel species, for which the name *Taphrina gei-montani* sp. nov. is proposed. The type strain is CCY 058-007-001^T (=CBS 14159=BU001). The MycoBank number is MB815677. The present study also demonstrates that two distinct species of the genus *Taphrina* parasitize the herbaceous *Rosaceae: Taphrina gei-montani* sp. nov. on *Geum montanum* and *Taphrina tormentillae* on *Potentilla* species.

Introduction

Members of the genus *Taphrina* are parasites pathogenic to various vascular plants, such as economically important fruit trees, shrubs, herbs and some ferns (Mix, 1949; Gjaerum, 1964; Salata, 1974; Sadebeck, 1893). All species of the genus *Taphrina* are characterized by a dimorphic lifestyle. A sexual (teleomorph) state is filamentous, strictly biotrophic and forms asci in the infected tissues. A range of extraordinary symptoms, manifested as hypertrophies or morphological changes of the host plant tissues, accompanies the sexual state (Mix, 1949; Rodrigues & Fonseca, 2003; Bacigálová, 2010). An asexual (anamorph) state is yeast-like, saprobic, and colonizes organic and inorganic substrates, often irrespective of the presence of a potential host. In contrast to the parasitic state, the saprophytic state can be cultivated on artificial yeast media *in vitro* (Mix, 1949; Rodrigues & Fonseca, 2003; Fonseca & Rodrigues, 2011; Petrýdesová *et al.*, 2013; Selbmannn *et al.*, 2014).

Species of the genus *Taphrina* have been recognized especially by symptoms caused to their host plants, anatomical and morphological features of their sexual stage in the infected tissues and by their geographic distribution (Mix, 1949; Gjaerum, 1964; Salata, 1974; Sadebeck, 1893). They are also distinguished by a unique combination of physiological and biochemical characteristics as well as by the carbohydrate composition of their cell walls (Prillinger *et al.*, 2000; Bacigálová *et al.*, 2003). However, molecular biological

Abbreviations: BI, Bayesian inference; ITS, internal transcribed spacer; MP, maximum-parsimony; rns, mitochondrial small subunit rRNA gene.

The GenBank/EMBL/DDBJ accession numbers of the sequences of the ITS region and the *rns* gene of the holotype of *T. gei-montani* CCY 058-007-001^T determined in this study are KU134800 and KU134828, respectively. Other sequence data determined in this study are detailed in Table 1.

Five supplementary figures and a supplementary table are available in the online Supplementary Material.

techniques involved in yeast identification have led to a revaluation of validity of the species described hitherto (Nishida & Sugiyama, 1994; Sugiyama, 1998; Prillinger *et al.*, 2000; Bacigálová *et al.*, 2003; Rodrigues & Fonseca, 2003).

Species parasitizing the herbaceous *Rosaceae* represent an ambiguous *Taphrina* group with an unresolved taxonomy and evolutionary relationships. These species, together with *Taphrina westergrenii* isolated from ferns of the family *Aspidiaceae*, are the only species parasitizing the herbaceous host plants. Other species of the genus *Taphrina* are confined to arboreous host species (Rodrigues & Fonseca, 2003; Bacigálová, 2010; Fonseca & Rodrigues, 2011). Two species parasitizing the herbaceous *Rosaceae* were described during the 19th century: *Exoascus deformans* (Berk.) Fuckel var. *potentillae* (Farlow, 1883) and *Taphrina tormentillae* (Rostrup, 1885) parasitizing both *Potentilla canadensis* L. and *Tormentilla erecta* L. (*Potentilla erecta* (L.) Raeusch.). The former taxon was elevated to the species level as *Taphrina potentillae* (Farl.) Johanson (Johanson, 1885). Mix (1949) recognized only the species *T. potentillae* parasitizing the herbaceous *Rosaceae* (*Geum montanum* and *Potentilla* spp.), but later he assumed that the correct name for this species should be *Taphrina tormentillae* (Mix, 1954). Nowadays taxonomists consider *T. tormentillae* as the only species parasitizing the herbaceous *Rosaceae* (Rodrigues & Fonseca, 2003; Fonseca & Rodrigues, 2011; Petrýdesová *et al.*, 2013; Selbmann *et al.*, 2014). However, our previous bio-systematic investigations (Bacigálová, 1992; Bacigálová *et al.*, 2014) revealed that *Taphrina* strains isolated from *G. montanum* differ morphologically and ecologically from those isolated from *P. erecta* (Bacigálová, 1992; Bacigálová *et al.*, 2014). Thus, the main goal of the present study was to investigate whether the strains isolated from *Potentilla* spp. and *Geum montanum* are genetically distinct from each other and represent two separate biological entities.

Methods

Isolation and characterization of strains. Strains of the genus *Taphrina* parasitizing both *P. erecta* and *G. montanum* were isolated from infected host plant tissues. The infected plants were collected during the vegetation periods of 2013 and 2014 from mountain localities in the Western Carpathians (Vysoké Tatry Mts, Slovakia) (Table 1). The yeasts in their anamorphic stage were isolated following the procedures described by Bacigálová *et al.* (2003). A teleomorphic stage was characterized according to the previous morpho-anatomical investigations (Bacigálová, 1992; Bacigálová *et al.*, 2014). The isolates were stored at –80 °C in a liquid medium containing 25 % (v/v) glycerol, and deposited in the Mycological Herbarium of the Institute of Botany (SAV), Slovak Academy of Sciences.

An ex-type strain (McNeill *et al.*, 2012, Recommendation 8B) of *T. tormentillae*, isolated from *P. canadensis*, North America, was provided by the Centraalbureau voor Schimmmelcultures. The ex-type strain of *T. gei-montani* sp. nov. was selected from our isolates originating from the location Ladové pleso (tarn), Vysoké Tatry Mts, Slovakia. The type strain of *T. gei-montani* sp. nov. is deposited in the Culture Collection of Yeasts, Institute of Chemistry, Slovak Academy of Sciences (CCY) and the ex-type strain in the Centraalbureau voor Schimmmelcultures, Utrecht, the Netherlands (CBS), under accession numbers CCY 058-007-001 and CBS 14159, respectively.

Other strains used in phylogenetic analyses were obtained from the CBS, the Austrian Centre of Biological Resources and the Institute of Applied Microbiology, University of Natural Resources and Life Sciences, Vienna, Austria (HA strains), and from the Mycological Herbarium, SAV (Table 1). The nomenclature of the host plant taxa followed Euro +Med PlantBase (Euro+Med, 2006).

Morphological and physiological methods. Morphological and physiological characteristics of the yeast cultures were examined using the methods described by Kurtzman *et al.* (2011b). Strains were cultivated in L-shaped tubes, with an initial concentration of 10^8 cells ml^{-1}. Yeasts were grown aerobically at their optimal temperature (20 °C) on a shaker (100 r.p.m.). The cell biomass was measured by its absorbance (660 nm) at regular intervals for a period of 21 days. Assimilation of nitrite was tested in a concentration of 0.25 % KNO_2. The absorbance of strains grown in the presence of carbon and nitrogen compounds was compared to that of strains grown in a solution without these substances (control).

DNA isolation and sequencing. The complete sequences of the nuclear ITS1-5.8S-ITS2 (ITS) region and partial sequences of the mitochondrial small subunit rRNA gene (*rns*) were used to analyse phylogenetic relationships.

The yeast cultures were grown for 7 days on GYP medium that consisted of 2 % glucose, 1 % bacto peptone, 0.5 % yeast extract and 2 % agar (Merck). Total genomic DNA was extracted using a DNeasy Plant Mini Kit (Qiagen). DNA extracts were diluted 1 : 10. PCRs were carried out using a Mastercycler ep Gradient S thermal cycler (Eppendorf). The ITS region was amplified using the ITS5 and ITS4 primers (White *et al.*, 1990), with reactions performed in a total volume of 25 µl, consisting of 0.75 U Pfu polymerase (Fermentas), 0.2 mM each dNTP, 0.2 µM each primer, 1 µl DNA template, and a reaction buffer containing 2 mM $MgSO_4$ (Fermentas).

Amplification of the *rns* gene was performed using The Hot Start Mix Ready-To-Go beads (GE Healthcare Life Sciences), and the SSU1 and SSU5 primers. The thermal cycler program was run according to Petrýdesová *et al.* (2013). PCR products were purified using a NucleoSpin Extract II kit (Macherey-Nagel), according to the manufacturer's protocol. Purified PCR products were sequenced using amplification primers at GATC Biotech AG, European Custom Sequencing Centre, Cologne, Germany.

Sequence alignments and phylogenetic analysis. Sequences were edited and aligned manually using the computer program BioEdit ver. 7.0.4.1 (Hall, 1999). The single nucleotide polymorphisms detected were labelled with NC-IUPAC ambiguity codes. Ambiguously aligned positions were removed using Gblocks ver. 0.91b (Castresana, 2000). Indels were considered as missing data.

Genetic divergences among ITS and *rns* sequences of the strains examined were determined using sequences with trimmed ends. Sequences from our strains were also compared with those available for the genus *Taphrina* in the NCBI GenBank database. Ambiguous regions and indels were considered in calculation of genetic divergences.

Three data matrices, each containing 38 taxa, including both newly generated sequences and those retrieved from the GenBank database, were prepared. The sequences of *Saitoella complicata* and *Protomyces inouyei* (Nishida & Sugiyama, 1994; Sugiyama *et al.*, 2006) were a priori considered as outgroup taxa. Three different approaches were applied to evaluate the interrelationships among the taxa analysed: maximum-parsimony, Bayesian inference and neighbour-net analysis.

Table 1. List of analysed strains belonging to the genera *Protomyces, Saitoella* and *Taphrina*, including data on origin of strains and nucleotide sequences accession numbers

Strains used in the present analyses are in bold type. GenBank accession numbers in bold type were obtained during the course of this study.
Abbreviations: CBS, Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands; CCY, The Culture Collection of Yeasts, Institute of Chemistry, Slovak Academy of Sciences, Dúbravska cesta 9, 845 23 Bratislava, Slovakia; HA, Austrian Centre of Biological Resources, Department of Biotechnology, University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Vienna, Austria.
[T], ex type strain; #, Mycological Herbarium of the Institute of Botany, Slovak Academy of Sciences, Dúbravska cesta 9, 845 23 Bratislava, Slovakia.

Strain	Source	Origin	GenBank accession numbers	
			ITS region	*rns* gene
Taphrina alni	**HA 872**=CBS 683.93[T]	*Alnus incana*, Falbeson, Stubai, Tyrol, Austria, det. et leg. H. Prillinger	AF492077	**KU134812**
Taphrina americana	**HA 860**=CBS 331.55[T]	*Betula fontinalis*, El Vado, USA, A. J. Mix	AF492078	**KU134813**
Taphrina betulina	CBS 119536[T]=NRRL T-726	*Betula nana x pubescens,* Sweden, A.J. Mix	AF492080	–
	Taphrina betulina (TBe)#	*Betula carpatica*, Vysoké Tatry Mts., Slovakia, det. et leg. K. Bacigálová & J. Petrýdesová	–	**KU134847**
Taphrina bullata	**CBS 12783[T]=CCY 58-4-1**	*Pyrus communis*, Malé Karpaty Mts., Kuchy a, Slovakia, det. et leg. J. Petrýdesová & K. Bacigálová	KC491200	KC494909
Taphrina caerulescens	**HA 861**	*Quercus coccinea*, Ithaca, South Hill Marsh, New York, USA, A. J. Mix	–	**KU134815**
	CBS 351.35	*Quercus alba*, A. J. Mix, 1935	AF492081	–
Taphrina carpini	**HA 1307**=CBS 102169[T]	*Carpinus betulus*, Bratislava, Devinska Kobyla, Slovakia, det. et leg. K. Bacigálová	AF492085	**KU134814**
Taphrina communis	**HA 837**=CBS 352.35	*Prunus angustifolia*, USA, A. J. Mix	AF492086	**KU134816**
Taphrina deformans	**HA 855**=CBS 356.35[T]	*Prunus persica*, The Netherlands	AF492093	**KU134817**
Taphrina epiphylla	**HA 1439**=CBS 111109[T]	*Alnus incana*, Belianske Tatry Mts, det. et leg. K. Bacigálová	AF 492096	**KU134818**
Taphrina flavorubra	**HA 871**=CBS 377.39[T]	*Prunus pumila L.* var. *susquehanae*, Ithaca, New York, W. W. Ray	AF492098	**KU134819**
Taphrina geimontani	**CBS14159[T]**=CCY 058-007-001[T]	*Geum montanum*, Ladové pleso, Vysoké Tatry Mts, Slovakia, det. et leg. K. Bacigálová & J. Petrýdesová	**KU134800**	**KU134828**
Taphrina geimontani	**CCY 058-007-002**	*Geum montanum*, Ladové pleso, Vysoké Tatry Mts, Slovakia, det. et leg. K. Bacigálová & J. Petrýdesová	**KU134801**	**KU134829**
Taphrina geimontani	**CCY 058-007-003**	*Geum montanum,* Ladové pleso, Vysoké Tatry Mts, Slovakia, det. et leg. K. Bacigálová & J. Petrýdesová	**KU134802**	**KU134830**
Taphrina geimontani	**CCY 058-007-004**	*Geum montanum*, Ladové pleso, Vysoké Tatry Mts, Slovakia, det. et leg. K. Bacigálová & J. Petrýdesová	**KU134803**	**KU134831**
Taphrina geimontani	**CCY 058-007-005**	*Geum montanum*, Litvorovo pleso, Vysoké Tatry Mts, Slovakia, det. et leg. K. Bacigálová & J. Petrýdesová	**KU134804**	**KU134832**
Taphrina insititiae	**CBS 12782 [T] = CCY 58-5-1**	*Prunus insititia*, Banská Štiavnica, Štiavnické vrchy Mts, Slovakia, det. et leg. J. Petrýdesová & K. Bacigálová	KC491202	KC494911
Taphrina johansonii	*Taphrina johansonii* (**TJ 001**)[#]	*Populus tremula*, Štiavnické vrchy Mts, Slovakia, det. et leg. K. Bacigálová & J. Petrýdesová	KC511055	KC494916
Taphrina letifera	**HA 843**=CBS 335.55[T]	*Acer spicatum*, North America, A. J. Mix	AF492099	**KU134820**
Taphrina padi	*Taphrina padi* (**TPa 001**)#	*Prunus padus*, Bratislava, Slovakia, det. et leg. K. Bacigálová & J. Petrýdesová	**KU134843**	KC494912
Taphrina populina	**HA 869**=CBS 337.55[T]	*Populus nigra*, locality unknown, A. J. Mix	AF492106	**KU134821**
Taphrina populi-salicis	**HA 848**=CBS 419.54[T]	*Populus trichocarpa*, USA, A. J. Mix	AF492113	**KU134822**

Table 1. cont.

Strain	Source	Origin	GenBank accession numbers	
			ITS region	*rns* gene
Taphrina pruni	**HA 1306**=CBS 119537^T	*Prunus domestica*, Malé Karpaty Mts, Slovakia, det. et leg. K. Bacigálová	AF492111	**KU134823**
Taphrina robinsoniana	**HA 850**=CBS 382.39^T	*Alnus incana*, A. J. Mix	AF492115	**KU134824**
Taphrina rhizophora	**CBS 12781^T=CCY 58-6-1^T**	*Populus alba*, Malé Karpaty Mts, Bratislava, Slovakia, det. et leg. J. Petrýdesová & K. Bacigálová	KC491201	KC494917
Taphrina sadebeckii	**HA 1308**=CBS 102170^T	*Alnus glutinosa*, Germany, Oberpfalz, Steinsberg, Erlenbruch near Frauenberg, det. et leg. H. Prillinger	AF492118	**KU134825**
Taphrina tormentillae	**CCY 058-008-001**	*Potentilla erecta*, Tatranská Polianka, Vysoké Tatry Mts., Slovakia, K. Bacigálová & J. Petrýdesová	**KU134805**	**KU134833**
Taphrina tormentillae	**CCY 058-008-002**	*Potentilla erecta*, Tatranská Lomnica, Vysoké Tatry Mts., Slovakia, det. et leg. K. Bacigálová & J. Petrýdesová	**KU134806**	**KU134834**
Taphrina tormentillae	**CCY 058-008-003**	*Potentilla erecta*, Tatranská Lomnica, Vysoké Tatry Mts., Slovakia, det. et leg. K. Bacigálová & J. Petrýdesová	**KU134807**	**KU134835**
Taphrina tormentillae	**CCY 058-008-004**	*Potentilla erecta*, Tatranská Polianka, Vysoké Tatry Mts., Slovakia, K. Bacigálová & J. Petrýdesová	**KU134808**	**KU134836**
Taphrina tormentillae	**CCY 058-008-005**	*Potentilla erecta*, Kamenistá dolina valley, Západné Tatry Mts., Slovakia, det. et leg. K. Bacigálová & J. Petrýdesová	**KU134809**	**KU134837**
Taphrina tormentillae	**CCY 058-008-006**	*Potentilla erecta*, Kamenistá dolina valley, Západné Tatry Mts., Slovakia, det. et leg. K. Bacigálová & J. Petrýdesová	**KU134810**	**KU134838**
Taphrina tormentillae	**HA 1316^T**=CBS 339.55^T	*Potentilla canadensis*, A. J. Mix	AF492120	**KU134845**
Taphrina tormentillae	**CBS 311.31**	Host species and locality unknown, E. M. Martin	**KU134811**	**KU134839**
Taphrina tosquinetii	**HA 1314**=CBS 276.28^T	Host species and locality unknown, M. Wieben	AF492121	**KU134826**
Taphrina ulmi	**HA 1507**=CBS 420.54^T	*Ulmus rubra*, Lawrence, C. L. Kramer	AF492123	**KU134827**
Taphrina wiesneri	*Taphrina wiesneri* (**TW 001**) #	*Cerasus avium*, Kuchy a, Slovakia det. et leg. K. Bacigálová & J. Petrýdesová	**KU134842**	KC494915
Saitoella comlicata	**HA 102**=CBS 7301^T	Soil, Bhutan, Laya, det. S. Goto *et al.*	**KU134841**	**KU134846**
Protomyces inouyei	**HA 1353**=NRRL YB 4354	*Crepis japonica*, Japan, leg. et det. K. Tubaki	**KU134840**	**KU134844**

Maximum-parsimony (MP) was performed with the heuristic search option in PAUP* ver. 4.0b10 (Swofford, 2001) under the following settings: accelerated character transformation (ACCTRAN), gaps treated as missing data, single-site polymorphism determined as uncertainty, tree reconstruction with stepwise addition, 1000 bootstrap replicates each with 10 random sequence addition replicates, tree bisection-reconnection (TBR) branch swapping, and the retention of multiple trees found during branch swapping (MULTREES option in effect).

Bayesian inference (BI) was run with MrBayes ver. 3.1.2, using the Markov Chain Monte Carlo algorithm (Ronquist & Huelsenbeck, 2003) on the CIPRES Portal ver. 1.15 (Miller *et al.*, 2010). Akaike Information Criterion, calculated in jMODELTEST ver. 0.0.1, was used to select the most appropriate nucleotide substitution model (Guindon & Gascuel, 2003; Posada, 2008). Evolutionary models of the ITS dataset were estimated separately for each of its three partitions: the TIM1+G model was assigned to ITS1, the TPM2 model to ITS2, and the GTR+G model to the 5.8S rRNA gene. The *rns* dataset was run under the TPM2uf+G model. Each partition in the concatenated dataset was associated with a respective evolutionary model. All BI analyses were run with four independent, Metropolis coupled MCMC chains (three heated and one cold chain) for ten million generations, sampled every thousandth generation. The first 25 % of sampled trees were considered 'burn-in' trees and were discarded prior to a reconstruction of a 50 % majority-rule consensus tree. Stationarity was confirmed by checking the convergence diagnostic parameters, as indicated in the MrBayes manual.

Neighbour-net analysis (NN) was used to identify consistent as well as potentially contradictory signals in the three datasets. Neighbour-net graphs were constructed with SplitsTree ver. 4.10, with uncorrected p-distances and default settings (Huson & Bryant, 2006). The amount and character of the substitutions supporting splits in the neighbour-net graphs were assessed using the computer program SAMS (Wägele & Mayer, 2007). Split-supporting nucleotide positions were classified according to Wägele & Rödding (1998) and consequently mapped onto the edges of the Neighbour-net diagrams.

Results

Altogether, we isolated eight *Taphrina* strains from *Potentilla* spp. (five localities) and five strains from *G. montanum* (two localities) (Table 1). We compared our isolates with twenty-three available members of the genus *Taphrina*, representing all genetic lineages detected within the genus (Rodrigues & Fonseca, 2003; Fonseca & Rodrigues, 2011; Petrýdesová *et al*., 2013; Table 1).

In total, the assimilation profiles of 12 strains isolated from *Potentilla* spp. and *G. montanum* were examined. Thirteen carbon sources and one nitrogen source were assimilated by all strains. The strains isolated from *G. montanum* also utilized melibiose and xylitol, whereas the strains from *Potentilla* spp. assimilated D-arabinose and L-lysine. None of the strains tested utilized inulin and KNO_2 (Table S1, available in the online Supplementary Material).

Taphrina gei-montani Bacigálová & Petrýdesová sp. nov.

MycoBank number: MB815677.

Type. The holotype strain CCY 058-007-001^T was isolated from the infected host plant tissue of *Geum montanum* L. (herbarium specimen: SAV K1789, deposited in the SAV). Locality: Slovakia, Vysoké Tatry Mts, Dolina Zlomísk valley, Ladové pleso tarn, 24 July 2013; collectors: Kamila Bacigálová, Jana Petrýdesová & Marek Slovák.

Etymology. The species epithet consists of two united Latin words in the genitive [*Ge·um*, *-i*, n and *montan·um*, *-i*, n], referring to the host species *Geum montanum*. Syllabification: *ge.i.mon.ta′ni*. N.L. gen. n. *gei-montani*, of *Geum montanum*. According to Article 60.9+Ex.26 of the *International Code of Nomenclature for algae, fungi, and plants*, the proposed species epithet must be hyphenated because it is constructed from two words each declined separately.

Description. Vegetative mycelium grows in the intercellular spaces of the host's parenchyma and forms networks beneath the epidermis and the cuticle. The mycelial cells, separated by layered septa, are variable in their length and shape. In the subcuticular layer of the infected tissue, the mycelial cells enlarge and give rise to ascogenous cells. These cells are ovoid in their early ontogenetic stages, subsequently become broader and form asci. Single-celled asci are developed irregularly inside the subcuticular layer and recline on the upper side of the leaf tissue (above the palisade parenchyma). The asci are 20–58×8–20 μm (most frequently 24–33×10–12 μm) in size, ovoid and with a narrow base attached to a short pedicel. Ascospores are formed in the early stage of ascus maturation. They are ovoid or spherical and 4–5×3–5 μm in size. The ascospores bud directly inside the asci and give rise to ovoid blastoconidia, being 2–3.5×2–3 μm in size. Afterwards, the asci increase in size and penetrate the cuticle.

Symptoms of infection. The yeast causes locally demarcated convex/concave spots on the living leaves, stems and flowers of *G. montanum*. The infected parts are pale green to yellow, and later turn to red or reddish-brown. The upper part of the roughened and convexly-deformed plant tissues is covered with a white coat of asci with ascospores.

Culture characteristics and physiological properties. On yeast peptone dextrose agar (YPD) after 14 days at 20 °C, the colonies are cream to pinkish-cream, and butyrous to dry with a smooth surface. On yeast peptone broth, after 7 days at 20 °C, the cells are globose to ovoid, 3.3–7.4×6.6–10.7 μm, occurring singly or with buds. Fermentation is absent. The following carbon sources are assimilated: D-glucose, sucrose, raffinose, melibiose, D-galactose, α,α-trehalose, soluble starch, cellobiose, D-xylose, L-arabinose, ethanol, glycerol, D-mannitol (two strains positive), D-glucitol, succinate, xylitol, D-ribose (two strains). Maltose (two strains) is assimilated weakly. The other carbon substances: inulin, lactose, melezitose, salicin, L-sorbose, L-rhamnose, D-arabinose, D-ribose, methanol, erythritol, ribitol, *myo*-inositol, DL-lactate and D-glucosamine, are not assimilated. The nitrogen compounds nitrate and ethylamine (two strains) are assimilated. Nitrite (0.25 %), L-lysine, creatinine and N-*acetyl*-D-glucosamine are not assimilated. Urease reaction is positive. Diazonium blue B reaction is negative. Starch-like polysaccharide is produced. Growth is not observed in a vitamin free medium. Growth at 25 °C is weak or positive; growth at 30 °C is negative.

Genetic distance of species of the genus *Taphrina* parasitizing *Potentilla* spp. and *G. montanum*

The ITS sequences of the isolates examined were 580 nucleotides (nt) long, including ambiguous regions and indels. The *Taphrina* strains isolated from *G. montanum* were considerably divergent from the type strain of *T. tormentillae*, reaching only 93 % identity. The sequences of the strains isolated from *G. montanum* differed by 23 indels (1–3 nt long) from those isolated from *Potentilla* spp. The strains of *T. tormentillae*, isolated from *P. erecta* (Western Carpathians, Europe), differed from the type strain CBS 339.55^T, parasitizing *P. canadensis* (North America), only by two to three substitutions (99 % identity). However, they were indistinguishable (100 % identity) from the *T. carnea* strain (CBS 332.55) and showed 99 % identity with an unidentified strain, KAS028, belonging to *Taphrina* sp., isolated from *Tragopogon pratensis* L. (Schäfer, 2010). Furthermore, the ITS sequences from *T. gei-montani* displayed 99 % identity with an unknown *Taphrina* strain (GenBank accession no. EF635841), isolated from the soil adjacent to *Salix herbacea,* Austrian Central Alps (Oberkofler & Peintner, 2008).

Partial sequences of the *rns* gene, including ambiguous regions and indels, were 1771 nt long. Our strains of *T. tormentillae* differed from the type strain CBS 339.55^T only in a single substitution and can be considered almost identical.

The *rns* sequences of the strains from *G. montanum* and *Potentilla* spp. differed from each other in 15 indels (1–165 nt long). When the *rns* sequences were compared with those deposited in the NCBI database (approx. 15 sequences), *T. deformans* (GenBank accession no. KC494910), with 96 % identity, was the closest relative of both groups of strains from the herbaceous *Rosaceae*.

Primary clade-supporting nucleotide homologies

Analyses of the spectrum of nucleotide-supporting positions in the ITS and *rns* sequences (without ambiguous regions) revealed that:

1. ITS sequences of the *Potentilla* spp. strains shared 15 unique asymmetrical substitutions. Likewise, ITS sequences of strains from *G. montanum* were characterized by 15 unique asymmetrical substitutions. A sister relationship of *T. tormentillae* and *T. gei-montani* was supported only by a single asymmetrical position (Fig. S1B).
2. The *rns* sequences of the strains isolated from *Potentilla* spp. did not show any unique phylogenetically informative nucleotide positions, whereas those from *G. montanum* shared six unique substitutions: one binary and five asymmetrical nucleotide positions (Fig. S2B). Nucleotide positions which could support a sister relationship of *T. tormentillae* and *T. gei-montani* were not detected.
3. In the concatenated dataset, strains isolated from *Potentilla* spp. were characterized by 23 unique asymmetrical positions, and those from *G. montanum* by 15 unique asymmetrical positions (Fig. S3B). A sister relationship of *T. tormentillae* and *T. gei-montani* was supported by a single asymmetrical nucleotide position incoming from the ITS partition.

Phylogenetic analyses

The ITS, *rns* and concatenated alignments were 570, 723 and 1293 nt long, respectively. *Saitoella complicata* was revealed to be an outgroup species in all analyses. Although analyses of the *rns* gene displayed *Protomyces inouyei* as a part of the *Taphrina* in-group partition, this position was statistically unsupported (Figs 1, S4 and S5).

Phylogenetic inferences of the ITS region showed that all species of the genus *Taphrina* form a single and statistically well-supported clade. However, the overall resolution, especially in the basal part of the Bayesian and MP trees, was very low. Terminals corresponding to particular species or species pairs were, in general, highly statistically supported. The strains isolated from *G. montanum* were clearly genetically distinct from those isolated from *Potentilla* spp. (Fig. S4) and each group of strains formed a highly supported cluster. All phylogenetic analyses indicated that *T. tormentillae* and *T. gei-montani* are sister taxa (Figs S1A, B and S4).

Phylogenetic analyses of the *rns* gene provided less resolution than those of the ITS region (Figs S2A, B and S5). All clades and subclades of species of the genus *Taphrina* were placed in a basal polytomy, along with *P. inouyei.* Genetic divergences between the strains of *T. tormentillae* and *T. gei-montani* were also clearly shown by the *rns* gene. However, their mutual relationships were different, in comparison to those found in the ITS phylogenetic trees. The strains isolated from *G. montanum* were placed in a separate subclade, whereas those originated from *Potentilla* spp. were co-clustered with *T. ulmi* and *T. letifera* (Figs S2A, B and S5).

Although ITS and *rns* gene trees were not congruent, both datasets were concatenated for the following reasons: (1) the incongruence and main branching patterns were not statistically supported and (2) the synergistic effect of concatenation might prevail over noise in both datasets. Both MP and BI analyses confirmed the monophyly of all analysed species of the genus *Taphrina*, whereas *S. complicata* and *P. inouyei* were classified, with a moderate support, as real outgroup taxa (Fig. 1). Moreover, a genetic distinction between the strains originated from *G. montanum* and *Potentilla* spp. was strongly confirmed (Figs. 1 and S3A, B). However, none of the phylogenetic analyses (MP, BI and NN) provided statistically significant support of their assumed sister relationship (Figs 1, S4 and S5).

Discussion

Symptoms, morphology, physiology and ecology of species of the genus *Taphrina* parasitizing the herbaceous *Rosaceae*

Both recognized species, *T. tormentillae* and *T. gei-montani*, parasitizing the herbaceous *Rosaceae,* cause very similar morphological changes on the infected plant tissues, but they differ from each other in their morphology and ecological preferences (Bacigálová, 1992; Bacigálová *et al.*, 2014). The species are distinguished by the shape and size of the asci and, especially by the location of the asci within the host plant tissue. The mature asci of *T. tormentillae* form a characteristic compact palisade layer in the cuticule and epidermis of the infected plant tissues, as typical for the genus *Taphrina* (Mix, 1949; Rodrigues & Fonseca, 2003; Fonseca & Rodrigues, 2011; Bacigálová *et al.*, 2014). On the other hand, *T. gei-montani* forms asci irregularly in the subcuticular layer on the upper side of the leaf tissue (Bacigálová, 1992).

Strains of *Taphrina* parasitizing the herbaceous *Rosaceae* did not show apparently a distinct assimilation profile in comparison to all the other species analysed (Table S1). *Taphrina gei-montani* together with a recently described *T. antarctica* were the only species which utilized melibiose. An intraspecific variation within *T. tormentillae* was also found. In contrast to the type strain of *T. tormentillae* CBS 339.55^{T}

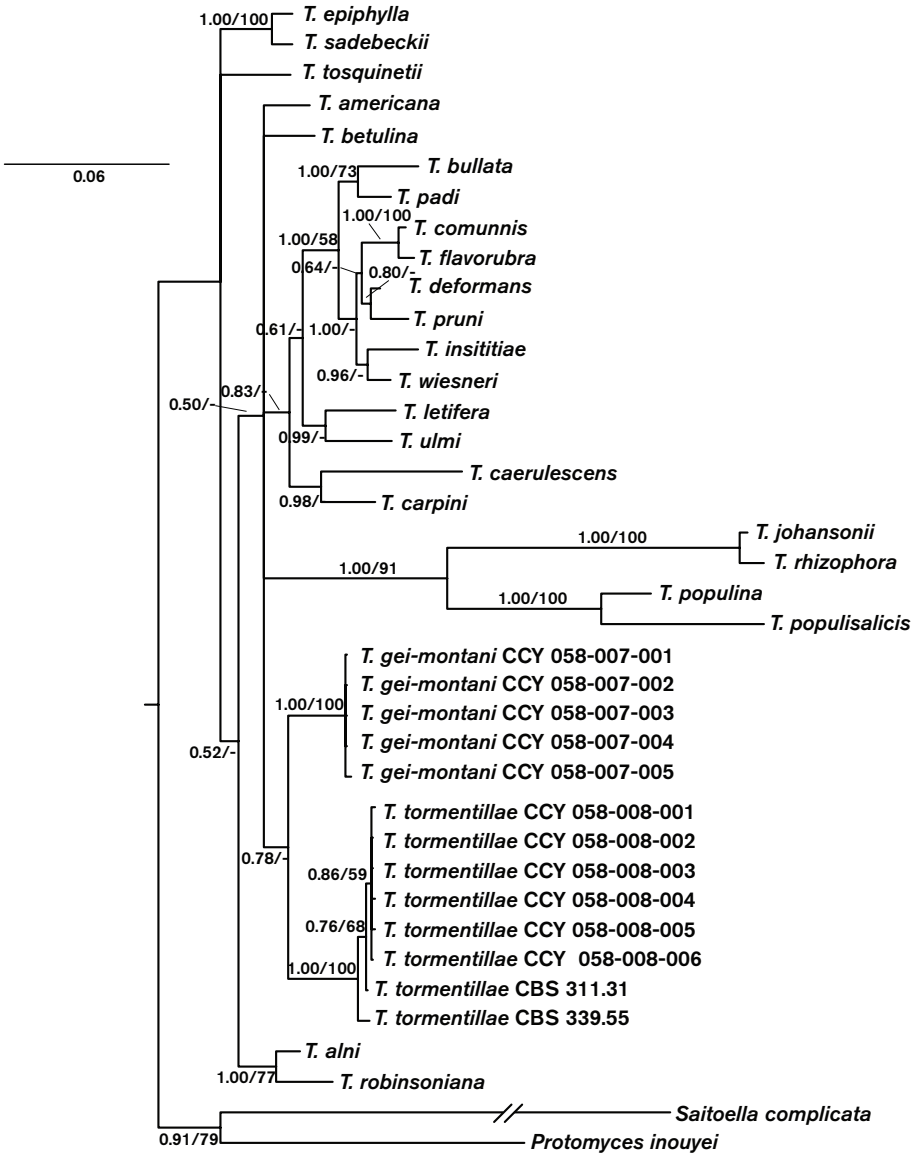

Fig. 1. Majority-rule consensus Bayesian tree inferred from the concatenated dataset (38 concatenated ITS and *rns* sequences). Numbers above the branches refer to posterior probabilities/MP bootstrap support (values <50 % are not shown). Each accession label includes the strain code given in Table 1. Bar represents six substitutions per one hundred nucleotide positions.

(Fonseca & Rodrigues, 2011), our strains of *T. tormentillae* did not assimilate inulin, xylitol and nitrite.

Both species of the genus *Taphrina* parasitizing the herbaceous *Rosaceae* are allopatric, which correlates well with different distribution areas of their host plant species. *Taphrina tormetillae* attacks *Potentilla* species which occur in mesophylous grasslands or forest clearings at mid-altitudes of mountains, while *T. gei-montani* parasitizes *G. montanum* which grows in cold and wet snow bed areas and along springs and mountain lakes, from the subalpine to alpine level of mountains. Although *T. gei-montani* inhabits the cold alpine level in high mountains, it cannot be considered psychrophilic species since it can grow also at temperatures above 20 °C (Table S1). Manifestation of the infection by both species of the genus *Taphrina* is, however, *in situ* detectable only under specific microclimatic conditions, which thus may cause difficulties in their finding (Bacigálová, 1992; Bacigálová *et al.*, 2014).

Taxonomic-evolutionary inferences of *Taphrina* strains parasitizing the herbaceous *Rosaceae*

Intensive field investigations linked to molecular-genetic analyses have recently led to the discoveries or descriptions of several new species of the genus *Taphrina*

(Petrýdesová *et al.*, 2013; Selbmann *et al.*, 2014). The present study brought further evidence of cryptic speciation within the genus *Taphrina*. Analyses of both the ITS region and the *rns* gene revealed that the genetic divergence between the two groups of strains from the herbaceous *Rosaceae* is clearly below the 99 % threshold, a value which delimits the species level within the genus *Taphrina* (Rodrigues & Fonseca, 2003; Fonseca & Rodrigues, 2011, Kurtzman *et al.*, 2011a). In accordance with previous molecular studies (Rodrigues & Fonseca, 2003; Fonseca & Rodrigues, 2011; Petrýdesová *et al.*, 2013; Selbmann *et al.*, 2014), both the nuclear ITS region and the mitochondrial *rns* gene provided a sufficiently high resolution for species delimitation, including the *Taphrina* strains parasitizing the herbaceous *Rosaceae.*

Taphrina strains isolated from host plants of the same genus differed genetically to a lesser extent. Divergences between strains isolated from the North American *Potentilla* spp. and *P. erecta* from the Western Carpathians reached up to three substitutions. This low level of genetic divergence most probably reflects an initial state of the speciation process that has been triggered by a recent adaptation to a new allopatric host species. All strains isolated from *G. montanum* share genetic uniformity in both markers, most probably caused by the close proximity of the sampled sites, which might be colonized by a single genotype (Kurtzman, 2010). Thus, it cannot be ruled out that we isolated only the main ubiquitous genotype and other rare genotypes simply remained undetected.

Host specificity often highly correlates with the species delimitation of particular species of the genus *Taphrina* and has been used as an effective diagnostic character (e.g. Rodrigues & Fonseca, 2003; Fonseca & Rodrigues, 2011; Petrýdesová *et al.*, 2013). Although our results basically corroborate this statement, species parasitizing the herbaceous *Rosaceae* are distinguishable only at the generic level of their host plants.

Genetic similarities and possible errors in identification of *Taphrina* strains

Direct comparison of the genetic divergence of strains isolated from the herbaceous *Rosaceae* with all other known *Taphrina* taxa confirmed their mutual genetic distinction. A few exceptions were detected, particularly with the sequences retrieved from the GenBank database. These sequences displayed a high level of similarity (above the 99 % threshold) with sequences of all strains isolated from the herbaceous *Rosaceae*. The origin and identity of their host species is, however, questionable. The ITS sequence of an unknown fungus (GenBank accession no. EF635841) isolated from a soil sample near a glacier in the Austrian Alps (Oberkofler & Peintner, 2008) showed 99 % identity with the strain we isolated from *G. montanum*. Since no potential host plant was recorded at this locality, this sequence might represent the asexual, saprobic yeast state of *T. gei-montani*. It has been proven that the yeast states of *Taphrina* are adapted for survival outside host plant tissues in free environments and their presence in various organic and inorganic substrates was noted (Babjeva & Reshetova, 1998; Webb *et al.*, 2000; Inácio *et al.*, 2004; Maksimova & Chernov, 2004; Wuczkowski *et al.*, 2005; Yurkov *et al.*, 2008; Jumpponen & Jones, 2009, 2010; Fonseca & Inácio, 2011; Cordier *et al.*, 2012; Selbmann *et al.*, 2014).

In the present study, 99 % identity for ITS sequences of *Taphrina* strains from *P. erecta* with the unidentified *Taphrina* strain KAS028, isolated allegedly from *Tragopogon pratensis*, was also detected. However, species of the genus *Taphrina* parasitizing *Tragopogon* sp., or any other member of the family *Asteraceae*, has not been described yet (Mix, 1949; Rodrigues & Fonseca, 2003; Fonseca & Rodrigues, 2011). It seems that this record is most probably erroneous, at least with respect to the host identity. A similar misidentification or mislabelling was detected and discussed by Rodrigues & Fonseca (2003) and Fonseca & Rodrigues (2011). These authors noted 100 % identity in the ITS sequences of *T. tormetillae* isolated from *P. erecta* with that of the *T. carnea* strain CBS 332.55 obtained from *Betula intermedia* Thomas ex Rchb.

Summarizing all available data, the strains isolated from *Potentilla* spp. and from *G. montanum* represent two separate biological entities and thus deserve a species rank each. With respect to the current version of the *International Code of Nomenclature for algae, fungi, and plants (Melbourne Code)* (McNeill *et al.*, 2012), the correct name for all known strains isolated from *Potentilla* spp. is *Taphrina tormentillae*, while strains parasitizing *G. montanum* are assigned to a novel species, *Taphrina gei-montani*.

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