




Powdery mildew of ash trees caused by the non-native *Erysiphe salmonii* in Hungary

Alexandra Pintye¹  · Orsolya Molnár¹ · Anita Z. Soós¹ · Diána Seress¹ · János Ágoston² · Márk Z. Németh¹

Received: 7 November 2023 / Accepted: 9 December 2023 / Published online: 5 January 2024
© The Author(s) 2024

Abstract

Fraxinus ornus and *F. excelsior* are naturally occurring woodland trees and widely cultivated ornamental plants in Hungary. Leaves with powdery mildew symptoms observed unusually on adaxial side of leaves of *F. ornus* and *F. excelsior* were collected from two locations in Hungary. We identified the causing fungi based on their morphological characteristics and molecular phylogenetic analysis. Numerous chasmothecia were found on a part of the samples, which were spherical, 83–120 µm in diameter, and the apices of the appendages uncinated or spirally curved. Anamorphs were characterized by conidiophores developing conidia singly, and by hyphae with lobed appressoria, characteristic of *Erysiphe* spp. The internal transcribed spacer region of the nrDNA was amplified, and the BLAST searches showed 100% similarity with *Erysiphe salmonii* sequences in GenBank. In the phylogenetic analysis the sequences of the Hungarian samples grouped in one clade with the sequences of other *E. salmonii* specimens collected in Central and Eastern Europe and Asia. This is the first report of the non-native *E. salmonii* causing powdery mildew on *Fraxinus* sp. in Hungary.

Keywords Ash · Powdery mildew · *Phyllactinia* · Infection · Plant pathogen

Introduction

Ashes are woodland tree species in Hungary and widely cultivated as ornamental plants. These species are often infected with powdery mildew (PM), and until recently (Heluta et al. 2017), only *Phyllactinia* PM species were described from ash trees, such as *P. fraxini*, *P. fraxinicola*, *P. japonica*, and *P. fraxini-longicuspis* (Maeda et al. 2021). However, a new causal agent, *Erysiphe salmonii*, has been discovered in several European countries since 2015 (Heluta et al. 2017). This species was originally described from Japan, and was known also from China, infecting different *Fraxinus* and *Syringa* species (Braun and Cook 2012). In Europe, *E. salmonii* was first found on *F. excelsior* and *F. pennsylvanica* in Ukraine by Heluta et al. (2017). Later on, it was also described in Switzerland (Beenken and Brodtbeck 2020) infecting *F. ornus*; in Austria on *F. excelsior* and *F. ornus* (Voglmayr

et al. 2021); in Romania (Chinan and Dascălu 2022); in Italy (Hofbauer and Braun 2023), and in Slovenia (Brglez et al. 2023).

In 2022 and 2023, powdery mildew symptoms were observed on the adaxial side of leaves of *F. ornus* and *F. excelsior* plants in several locations in Hungary. The present study aimed to identify and characterize the causal agent of these symptoms on *Fraxinus* plants in Hungary.

Materials and methods

The first infected *F. ornus* leaves were collected in Budapest in October 2022, and the others in the vicinity of Budapest in September 2023 from forested areas. The infected *F. excelsior* leaves were sampled in Budapest in September 2023 from urban areas (Table 1). All but one of the infected plants were young, no more than two years old, and heavily infected. The fifth sample, PM339 was collected from a mature tree, which was moderately infected. Samples were placed in plastic bags and transferred to the laboratory for further investigation. Chasmothecia were examined covered in lactic acid; while the anamorphs were studied after boiling in lactic acid (Shin and La 1993). The morphological

✉ Alexandra Pintye
pintye.alexandra@atk.hun-ren.hu

¹ Plant Protection Institute, Centre for Agricultural Research, HUN-REN, Budapest, Hungary

² PhatoPlant-Lab, Széchenyi István University, HUN-REN-SZE, Mosonmagyaróvár, Hungary

Table 1 Data of the samples collected in the present study

Designation	Host plant	Date of collection	GPS coordinates	Herbarium inventory no	Herbarium barcode	GenBank ITS accession no
PM265	<i>Fraxinus ornus</i>	Oct 2022	47.51759, 18.95899	BP112648	HNHM-MYC 032041	OR610788
PM333	<i>F. ornus</i>	Sept 2023	47.53584, 18.87133	BP112652	HNHM-MYC 032045	OR610789
PM335	<i>F. ornus</i>	Sept 2023	47.47719, 18.93126	BP112653	HNHM-MYC 032046	OR643689
PM338	<i>F. excelsior</i>	Sept 2023	47.52309, 18.96326	BP112655	HNHM-MYC 032048	OR858889
PM339	<i>F. excelsior</i>	Sept 2023	47.52168, 18.96818	BP112656	HNHM-MYC 032049	OR643690

characteristics of the fungal structures were examined with phase contrast microscopy using a ZEISS AxioScope2 microscope (Germany) equipped with an AxioCam ICc5 camera (Zeiss). At least, 20 measurements were made for each fungal structure. The pathogen was identified based on morphology (Braun and Cook 2012) and based on sequences of the nrDNA internal transcribed spacer (ITS) (see below). The PM infected leaves were deposited at the Herbarium of the Hungarian Natural History Museum, Budapest, Hungary (Table 1).

In those cases when chasmothecia were found on the leaves the genomic DNA was extracted from a single chasmothecium following a described protocol (Pintye et al. 2020). In the case of the other samples, a piece of cellotape was used for the collection of the mycelium, afterwards the cellotape was incubated in 200 µl TE buffer (Lonza) at 97 °C for 10 min (Pintye et al. 2023).

The ITS region was amplified using general (ITS4 and ITS5; White et al. 1990) and powdery mildew specific primers (PM5 and PM6; Kiss et al. 2001; Takamatsu and Kano 2001). All PCR amplifications were performed in a final volume of 20 µL. Reaction components included 1 µL of 10 µM forward and reverse primers (Thermo Fisher Scientific Inc), 1 µL DNA template and 10 µL Phusion Green Hot Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific). The cycling times and temperatures for both primer pairs (PM5-ITS4 and ITS5-PM6) were as follows: 98 °C for 2 min, followed by 35 cycles of 10 s at 98 °C, 20 s at 58 °C and 21 s at 72 °C, and a final extension step at 72 °C for 5 min. The obtained sequences were compared with the accessions in the National Center for Biotechnology Information database (NCBI, <http://www.ncbi.nlm.nih.gov/Blast.cgi>) using the BLAST search (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) (Altschul et al. 1990). The resulting sequences were deposited in GenBank (Table 1).

Two of the newly obtained sequences were aligned with 30 other sequences (Table 2) retrieved from GenBank using the online version of MAFFT 7 (Kato and Standley 2013) with the E-INS-i method. The alignments were examined and edited using MEGA 7 (Kumar et al. 2016). The dataset

consisted of 32 sequences and 462 characters; *Erysiphe aphananthes* was used as outgroup. Maximum likelihood (ML) phylogenetic analysis was carried out with the raxmlGUI 1.5 implementation (Silvestro and Michalak 2012; Stamatakis 2014). A GTR + G nucleotide substitution model was used with ML estimation of base frequencies. Maximum likelihood bootstrap (BS) analysis with 1000 replicates was used to test the support of the branches. Phylogenetic tree was visualized and edited in TreeGraph (Stöver and Müller 2010).

Results

Numerous chasmothecia and only a few conidiophores were found on samples PM265 from *F. ornus* and PM339 from *F. excelsior*, while only mycelium and a few conidia were observed on other samples. The morphological features of the specimens were identical to those of *Erysiphe salmonii* described by Braun and Cook (2012). Chasmothecia measured 83–120 µm in diameter, had 10–30 appendages, 103–147 µm long, straight or curved. The apices of the appendages were uncinated, spirally curved (Fig. 1a). Chasmothecia did not contain asci. Mycelium was epiphytic with moderately lobed hyphal appressoria (Fig. 1b, c). The conidiophores were straight and produced single conidia, hyaline, ellipsoid-ovoid, measuring 25–33 × 9–13 µm, without fibrosin bodies. Germ tubes were subterminal and the conidial appressoria were lobate and multilobate (Fig. 1d).

The sequences of the Hungarian specimens were identical, and showed 100% identity to sequences of *E. salmonii*. The phylogenetic analysis revealed (Fig. 2) that the Hungarian samples grouped with the epitype of *E. salmonii* (MUMH4167; accession no. LC577619), other specimens from Japan (LC028981), Ukraine (LC259501), Romania (MW633028), Austria (OK383397), and from Switzerland (MW265935) infecting *F. mandshurica*, *F. rhynchophylla*, *F. excelsior*, *F. pennsylvanica*, *F. sieboldiana* and *F. ornus*. Grouping was supported by high bootstrap value (96). Thus, both morphological examination and phylogenetic analysis

Table 2 List of powdery mildew sequences obtained from GenBank and used for phylogenetic analysis

Fungal species	GenBank ITS accession number	Sample designation	Year of collection	Place of collection	Host species	References
<i>Erysiphe salmonii</i>	LC259500	MUMH6789	2015	Ukraine	<i>Fraxinus excelsior</i>	Heluta et al. (2017)
<i>E. salmonii</i>	LC259501	MUMH6790	2015	Ukraine	<i>F. rhynchophylla</i>	Heluta et al. (2017)
<i>E. salmonii</i>	LC259502	MUMH6792	2015	Ukraine	<i>F. pennsylvanica</i>	Heluta et al. (2017)
<i>E. salmonii</i>	MH880101	CNUFC-PWF1	2018	Korea	<i>F. chinensis</i> subsp. <i>rhynchophylla</i>	Lee and Nguyen (2019)
<i>E. salmonii</i>	MW265935	ZTMyc 64441	2020	Switzerland	<i>F. ornus</i>	Beenken and Brodtbeck (2020)
<i>E. salmonii</i>	MW265934	ZTMyc 64438	2020	Switzerland	<i>F. ornus</i>	Beenken and Brodtbeck (2020)
<i>E. salmonii</i>	LC577616	MUMH3923	2005	Japan	<i>Fraxinus</i> sp.	Yamaguchi et al. (2021)
<i>E. salmonii</i>	LC577619	MUMH4167	2005	Japan	<i>F. sieboldiana</i>	Yamaguchi et al. (2021)
<i>E. salmonii</i>	LC577624	MUMH5355	2011	Japan	<i>F. sieboldiana</i>	Yamaguchi et al. (2021)
<i>E. salmonii</i>	LC028981	MUMHs96	1995	Japan	<i>F. mandshurica</i>	Yamaguchi et al. (2021)
<i>E. salmonii</i>	OK383397	WU:44783	2021	Austria	<i>F. excelsior</i>	Voglmayr et al. (2021)
<i>E. salmonii</i>	OK324154	WU:44779	2021	Austria	<i>F. ornus</i>	Voglmayr et al. (2021)
<i>E. salmonii</i>	OK324155	WU:44780	2021	Austria	<i>F. ornus</i>	Voglmayr et al. (2021)
<i>E. salmonii</i>	MW633027	I 186268	2020	Romania	<i>F. excelsior</i>	Chinan and Dascălu (2022)
<i>E. salmonii</i>	MW633028	I 186269	2020	Romania	<i>F. excelsior</i>	Chinan and Dascălu (2022)
<i>E. fraxinea</i>	LC577606	MUMH487	1998	Japan	<i>F. lanuginosa</i>	Yamaguchi et al. (2021)
<i>E. fraxinea</i>	LC577608	MUMH4020	2005	Japan	<i>F. lanuginosa</i>	Yamaguchi et al. (2021)
<i>E. fraxinea</i>	LC577610	MUMH7087	2017	Japan	<i>F. lanuginosa</i>	Yamaguchi et al. (2021)
<i>E. fraxinea</i>	LC577609	MUMH4418	2006	Japan	<i>F. lanuginosa</i>	Yamaguchi et al. (2021)
<i>E. fraxinea</i>	LC028977	MUMH173	1996	Japan	<i>F. lanuginosa</i>	Yamaguchi et al. (2021)
<i>E. fraxinea</i>	LC028982	MUMH488	1998	Japan	<i>F. apertisquamifera</i>	Yamaguchi et al. (2021)
<i>E. fraxinea</i>	LC577605	MUMH480	1998	Japan	<i>F. apertisquamifera</i>	Yamaguchi et al. (2021)
<i>E. fraxinea</i>	LC577611	MUMH7089	2017	Japan	<i>F. lanuginosa</i>	Yamaguchi et al. (2021)
<i>E. fraxinea</i>	LC577607	MUMH3552	2004	Japan	<i>F. lanuginosa</i>	Yamaguchi et al. (2021)
<i>E. fraxinicola</i>	LC577627	MUMH5282	2011	Japan	<i>F. longicuspis</i>	Yamaguchi et al. (2021)
<i>E. fraxinicola</i>	LC577629	MUMH5650	2010	Japan	<i>F. longicuspis</i>	Yamaguchi et al. (2021)
<i>E. fraxinicola</i>	LC577628	MUMH5608	2010	Japan	<i>F. longicuspis</i>	Yamaguchi et al. (2021)
<i>E. fraxinicola</i>	LC577630	MUMH7092	2017	Japan	<i>F. longicuspis</i>	Yamaguchi et al. (2021)
<i>E. fraxinicola</i>	LC028979	MUMH211	1996	Japan	<i>F. longicuspis</i>	Yamaguchi et al. (2021)
<i>E. aphananthes</i>	LC028971	MUMH4648	2007	Japan	<i>Aphananthe aspera</i>	Takamatsu et al. (2015)

have clearly shown that the newly occurring species of powdery mildew that infects ash trees is *E. salmonii*.

Discussion

Only *Phyllactinia* species were reported to infect ash trees (Heluta et al. 2017; Yamaguchi et al. 2021) in Europe until 2015 (Heluta et al. 2017). After this first report (Heluta et al. 2017), a rapid spread of *E. salmonii* through the Eastern and Central regions of Europe was described (e.g. Beenken and Brodtbeck 2020; Chinan and Dascălu 2022). A similar pattern of spread of a PM fungus originating also from Asia was observed in the case of hazel powdery mildew in

Europe. An epiphytic species, *E. corylacearum* appeared alongside a widespread hemiendophytic species (*P. guttata*) commonly visible on the lower side of leaves on the same host plant (Heluta et al. 2019; Sezer et al. 2017). This species, *E. corylacearum* was also recently introduced to Hungary (Kalmár et al. 2023). *E. salmonii* is presumably an invasive species, which has recently migrated to Eastern Europe (Heluta et al. 2017), and the source of inoculum could be the chasmothecia accumulated on goods, people's clothes or imported plants as it was assumed for *E. kenjiana* by Heluta et al. (2009).

The present study represents the first report of *E. salmonii* infecting ash trees in Hungary, and provides another example for the geographical expansion potential of PM fungi.

Fig. 1 *Erysiphe salmonii* on *Fraxinus ornus*. **a** Chasmothecium with uncinated, spirally curved appendages. **b, c** Moderately lobed, hyphal appressoria. **d** Germinating conidium with multilobed appressorium. Bars: a 100 μ m; b–d 12 μ m

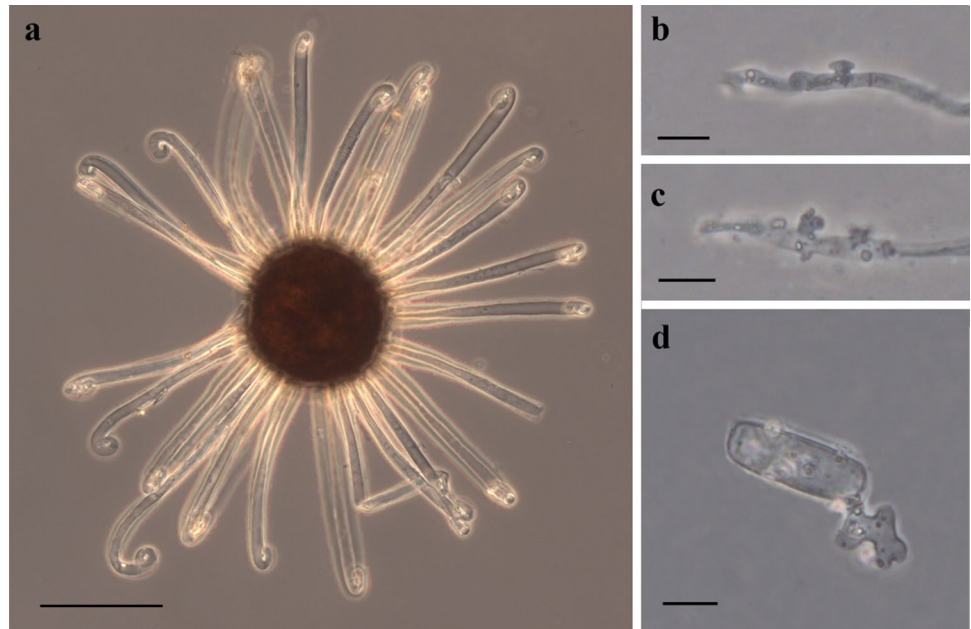


Fig. 2 Maximum likelihood tree based on nrDNA internal transcribed spacer (ITS) sequences of *Erysiphe* species infecting ash trees. The ITS sequence of *E. aphananthes* served as outgroup. The bootstrap values presented as percentages, below 70% are not shown. The data set comprised 462 characters. Samples collected in this work are shown in boldface. Bar indicates 0.01 expected change per site per branch. The country of origin is provided with two-letter code. (HT: ex holotype; ET: ex epitype)



Acknowledgements This research was supported by the Hungarian Scientific Research Fund (NKFIH OTKA FK142735 and FK142971), by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (awarded to Márk Z. Németh; BO/00221/21/4) and by the

ÚNKP-23-5 New National Excellence Program of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund (ÚNKP-23-5-ELTE-497). The research of the HUN-REN-SZE PhatoPlant-Laboratory was supported by the

Hungarian Research Network TKI (HUN-REN TKI) (Project Number: 3200107).

Funding Open access funding provided by HUN-REN Centre for Agricultural Research.

Declarations

Conflict of interest All authors declare that no competing interests exist.

Human and animal rights and informed consent This article does not contain any studies with human participants or animals performed by any of the authors. It is original and has not been published elsewhere.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215:403–410
- Beenken L, Brodtbeck T (2020) First record of *Erysiphe salmonii* causing powdery mildew on *Fraxinus ornus* in Switzerland. *New Dis Rep* 42:22–22
- Braun U, Cook R (2012) Taxonomic manual of the Erysiphales (powdery mildews). CBS-KNAW Fungal Biodiversity Centre, Utrecht
- Brglez A, Piškur B, Ogris N (2023) First report of *Erysiphe salmonii* on *Fraxinus ornus* and *F. excelsior* in Slovenia. *New Dis Rep* 47:e12159
- Chinan V-C, Dascălu M-M (2022) First report of *Erysiphe salmonii* causing powdery mildew on *Fraxinus excelsior* in Romania. *J Plant Dis Prot* 129:193–196
- Heluta V, Takamatsu S, Voytyuk S, Shiroya Y (2009) *Erysiphe kenjiana* (Erysiphales), a new invasive fungus in Europe. *Mycol Prog* 8:367–375
- Heluta VP, Takamatsu S, Siahaan SaS (2017) *Erysiphe salmonii* (Erysiphales, Ascomycota), another East Asian powdery mildew fungus introduced into Ukraine. *Український ботанічний журнал* 212–219
- Heluta VP, Makarenko N, Al-Maali GA (2019) First records of *Erysiphe corylacearum* (Erysiphales, Ascomycota) on *Corylus avellana* in Ukraine. *Український ботанічний журнал* 76:252–259
- Hofbauer WK, Braun U (2023) New discoveries of powdery mildew species from Austria and Italy. *Schlechtendalia* 40:272–277
- Kalmár K, Desiderio F, Varjas V (2023) First report of *Erysiphe corylacearum* causing powdery mildew on hazelnut in Hungary. *Plant Dis* 107:579
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 30:772–780
- Lee HB, Nguyen TTT (2019) First report of powdery mildew caused by *Erysiphe salmonii* on *Fraxinus rhynchophylla* in Korea. *Plant Dis* 103:769. <https://doi.org/10.1094/PDIS-09-18-1572-PDN>
- Levente KI, Roger TA, Saenz GS, Cunnington JH, Takamatsu S, Pascoe I, Bardin M, Nicot PC, Sato Y, Rossmann AY (2001) Identification of two powdery mildew fungi, *Oidium neolycopersici* sp. Nov. and *O. lycopersici*, infecting tomato in different parts of the world. *Mycol Res* 105:684–697
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874
- Maeda M, Meeboon J, Heluta VP, Liu S-Y, Tang S-R, Takamatsu S (2021) Phylogeny and taxonomy of *Phyllactinia* species (powdery mildew: Erysiphaceae) occurring on the ash trees (*Fraxinus* spp.). *Mycoscience* 62:268–280
- Pintye A, Németh MZ, Molnár O, Horváth ÁN, Spitzmüller Z, Szalóki N, Pál K, Váczy KZ, Kovács GM (2020) Improved DNA extraction and quantitative real-time PCR for genotyping *Erysiphe necator* and detecting the DMI fungicide resistance marker A495T, using single ascocarps. *Phytopathol Mediterr* 59:97–106
- Pintye A, Németh MZ, Molnár O, Horváth ÁN, Matolcsi F, Bókony V, Spitzmüller Z, Pálfi X, Váczy KZ, Kovács GM (2023) Comprehensive analyses of the occurrence of a fungicide resistance marker and the genetic structure in *Erysiphe necator* populations. *Sci Rep* 13:15172
- Sezer A, Dolar FS, Lucas SJ, Köse Ç, Gümüş E (2017) First report of the recently introduced, destructive powdery mildew *Erysiphe corylacearum* on hazelnut in Turkey. *Phytoparasitica* 45:577–581
- Shin HD, La YJ (1993) Morphology of edge lines of chained immature conidia on conidiophores in powdery mildew fungi and their taxonomic significance. *Mycotaxon* 46:445–451
- Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. *Org Divers Evol* 12:335–337
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313
- Stöver BC, Müller KF (2010) TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinform* 11:1–9
- Takamatsu S, Kano Y (2001) PCR primers useful for nucleotide sequencing of rDNA of the powdery mildew fungi. *Mycoscience* 42:135–139
- Takamatsu S, Ito H, Shiroya Y, Kiss L, Heluta V (2015) First comprehensive phylogenetic analysis of the genus *Erysiphe* (Erysiphales, Erysiphaceae) II: the *Uncinula* lineage. *Mycologia* 107:903–914
- Voglmayr H, Jaklitsch W, Kirisits T (2021) First report of powdery mildew caused by *Erysiphe salmonii* on *Fraxinus excelsior* and *F. ornus* in Austria. *New Dis Rep* 44:12049
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: Guide Methods Appl* 18:315–322
- Yamaguchi Y, Meeboon J, Heluta VP, Liu S-Y, Feng J, Takamatsu S (2021) Phylogeny and taxonomy of *Erysiphe* species (powdery mildew: Erysiphaceae) occurring on the ash trees (*Fraxinus* spp.). *Mycoscience* 62:115–123

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.