Eur J Plant Pathol
https://doi.org/10.1007/s10658-026-03250-1

Pustula echinaceae sp. nov. (Albuginales, Oomycota), a new species causing white blister rust disease on the ornamental plant *Echinacea purpurea* (L.) Moench

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Received: 22 January 2026 / Accepted: 21 May 2026
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Abstract The order *Albuginales* includes the causal agents of white blister rust disease, which affect a wide range of weedy plants, both cultivated and wild. The genus *Pustula* is one of the five major lineages of white blister rusts identified to date. It causes disease on asterids, the largest group of flowering plants. *Pustula* is represented by several phylogenetically distinct, host-specific lineages, many of which remain formally undescribed. In this study, samples of white blister rust collected from the economically valuable ornamental plant *Echinacea purpurea* were investigated. Phylogenetic analysis based on *COX2* mtDNA, supported by ITS and LSU rDNA sequence comparisons, combined with detailed morphological investigations, revealed a distinct and independent lineage, separate from previously known species of *Pustula*. As a result, a new species, *Pustula echinaceae* sp. nov., infecting *Echinacea purpurea*, is described and illustrated.

Keywords *Albuginaceae* · *Asteraceae* · *COX2* · Phylogeny · Purple coneflower

Introduction

White blister rusts (WBR) are a cosmopolitan group of obligate biotrophic oomycetes that infect a wide range of weedy flowering plants (Thines & Spring, 2005; Thines, 2010). While principally parasitic on eudicots, exceptions have been documented on *Piperaceae* and *Orchidaceae* (Walker and Priest, 2007; Thines & Voglmayr, 2009). White blister pathogens can infect various cultivated and wild plants, causing

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Published online: 06 June 2026

significant reductions in crop yield and substantial economic losses in some cases (Ploch et al., 2011; Xu et al., 2016, 2018).

Historically, WBR species identification primarily relied on morphological features, particularly sporangium size and oospore wall ornamentation (Choi et al., 2007; Thines, 2014). Furthermore, before these criteria were widely adopted, species were often assigned based on the assumption of host family specificity (Săvulescu, 1946; Biga, 1955; Choi & Priest, 1995). Previously, all WBR were classified within the single genus *Albugo* sensu lato, readily distinguishable from other oomycetes based on their unique morphological characteristics (Thines, 2010; Xu et al., 2018).

The advent of molecular phylogeny based on DNA nucleotide sequences demonstrated the distant phylogenetic relationship between *Albugo* and other oomycetes (Riethmüller et al., 2002; Hudspeth et al., 2003). Consequently, a new order, *Albuginales*, was established to accommodate all white blister rusts (Thines & Spring, 2005). Further phylogenetic analyses, combined with morphological examinations, revealed a high level of diversity within *Albugo* sensu lato and identified several distinct lineages within the *Albuginales* (Thines & Spring, 2005; Voglmayr & Riethmüller, 2006; Thines & Voglmayr, 2009; Mirzaee et al., 2021c). As a result, three independent genera, *Albugo* sensu stricto, *Pustula*, and *Wilsoniana*, were proposed based on molecular phylogeny, cytological differences, and variations in sporangial and oospore morphology (Thines & Spring, 2005).

The three identified lineages within *Albuginales* are distinguishable not only by molecular data and morphology but also by their general host range. *Albugo* sensu stricto is primarily parasitic on *Brassicales*, with only one confirmed exception on *Fabaceae* (Choi et al., 2011; Ploch et al., 2018; Mirzaee et al., 2013, 2021a). *Wilsoniana* parasitizes *Caryophyllales* (Thines & Spring, 2005; Thines & Voglmayr, 2009), while *Pustula* causes disease on various *Asterales* and some related orders (Thines & Spring, 2005; Ploch et al., 2011; Choi et al., 2012; Mirzaee et al., 2021a, b). A fourth distinct phylogenetic lineage is currently assigned to the genus *Albugo*, which contains the species *A. occidentalis*, which infects spinach (*Spinacia oleracea*) of the order *Caryophyllales* (Thines & Spring, 2005; Thines, 2014). In addition to these four lineages, another lineage infecting *Convolvulaceae* remains assigned to the genus *Albugo*, even though it is morphologically quite divergent, and characterized by thick sporangium walls and densely ornamented oogonia. Further morphological, cytological, or ultrastructural studies are needed to determine if these latter two lineages also warrant elevation to the genus level (Thines, 2014).

Recent molecular phylogenetic studies of the genus *Pustula*, focusing on plants within the *Asteranae* superorder (*Araliaceae, Asteraceae, Goodeniaceae*, and *Gentianaceae*) based on *COX*2 sequences, clearly demonstrate that the genus comprises several distinct lineages, often exhibiting host specificity at the genus level (Rost & Thines, 2012; Xu et al., 2016, 2018; Mirzaee, 2021a, 2021b, 2023a). These findings were first revealed and highlighted by Ploch et al., (2011), who examined 46 specimens from 21 plant genera and identified 18 distinct lineages within *Pustula*, suggesting its division into more than a dozen distinct species.

Subsequently, *P. obtusata* and *P. centaurii* from *Tragopogon orientalis*, and *Centaurium* sp., respectively, were accommodated in *Pustula* (Choi et al., 2012). Thereafter, several new species within *Pustula* have been described based on *COX2* phylogeny combined with morphological observations, including *P. helianthicola* from *Helianthus annuus* (Rost & Thines, 2012); *P. junggarensis* from *Takhtajaniantha pusilla* (Xu et al., 2016); *P. cancriniae* and *P. xinyuanensis* from *Cancrinia discoidea* and *Convolvulus gortschakovii*, respectively (Xu et al., 2018); *P. lactucae* and *P. persica* from *Lactuca undulata* and *Gymnarrhena micrantha*, respectively (Mirzaee et al., 2021a; Mirzaee et al., 2021b); *Pustula senecionis* from *Senecio* spp. and *Pustula thinesii* from *Xeranthemum inapertum* (Mirzaee et al., 2023a).

As asterids comprise the largest group of flowering plants, encompassing over 80,000 species, representing approximately one-third of all flowering plant species (Bremer et al., 2004; Angiosperm Phylogeny Group et al., 2016; Mandel et al., 2019), it is most likely that a considerably larger number of *Pustula* species than currently documented exist. White blister rust has been reported on only a small number of plants within the *Asteraceae* family worldwide (Farr & Rossman, 2026), but this limited reporting may be due to underreporting of the disease in non-crop plants. This suggests an underestimation of *Pustula* biodiversity within

Asterids, implying the existence of numerous yet-to-be-described species within this genus.

The genus *Echinacea* (Asteraceae) consists of a small number of herbaceous perennial plants native to parts of North America (Wichtl, 2004; Barnes et al., 2005) and is restricted to areas east of the Rocky Mountains and within the Atlantic drainage area, predominantly the Great Plains, the central United States, and adjacent areas of Canada (Binns et al., 2002). It includes nine species, three of which are particularly notable for their economic importance and diverse applications (Parsons et al., 2018): *Echinacea purpurea* (L.) Moench, *E. pallida* (Nutt.) Nutt., and *E. angustifolia* DC. These species are highly valued medicinal plants, owing to the presence of several bioactive compounds with pharmacological activities (Manayi et al., 2015). Among them, *E. purpurea* commonly known as the (eastern) purple coneflower, hedgehog coneflower, or American coneflower (McKeown, 1999), has gained considerable attention for its multifaceted applications. This species is cultivated globally and is widely used in pharmaceuticals and dietary supplements (Barrett et al., 1999, 2010). Additionally, *E. purpurea* is a popular ornamental plant in temperate regions, ideal for curbs, walkways, and garden beds (Letchamo et al., 2002; Ault, 2007; Koenig & Roehr, 2006).

To date, several reports document white blister rust disease on *Echinacea* species, all originating from the United States (Rogerson, 1958; du Toit & Derie, 2014; Spies et al., 2016), yet none of these studies identified the causal agent to species level. Recent taxonomic revisions within white blister rusts, coupled with accumulating evidence of strict host specificity among *Pustula* lineages on *Asteraceae*, combined with the economic importance of *Echinacea* spp. highlight this genus might harbor a hitherto undescribed species of *Pustula*. For investigating this possibility, the present study aimed at providing a detailed morphological analysis, which, together with an evaluation of the phylogenetic relationships to known *Pustula* species, would enable its formal description.

Materials and methods

Specimen sampling and morphological examination

A sample exhibiting typical symptoms of white blister rusts, was received from the Landwirtschaftliches Technologiezentrum Augustenberg (Agricultural Technology Center Augustenberg) located in Karlsruhe, Baden-Württemberg, Germany on August 07, 2024 (Table 1). Pathogen structures were mounted onto glass slides in drops of 70 % aqueous lactic acid solution, gently heated to the boiling point and covered with a coverslip. Morphological observations of sporangiophores, sporangia, and oospores were made using a compound microscope equipped with Differential Interference Contrast (DIC) (Imager M2, Carl Zeiss Göttingen, Germany). Measurements were obtained using AxioVision v7 (Zeiss, Oberkochen, Germany) with calibrated photographs captured by an AxioCam (Zeiss, Oberkochen, Germany). Measurements were performed at ×1000 and ×400 magnification. Data are presented as (minimum–)mean minus standard deviation–mean–mean plus standard deviation(–maximum) and shown in Table 2. In the taxonomic descriptions, the range of the standard deviation, rounded to the nearest 0.5 µm is given.

DNA extraction, PCR and sequencing

Genomic DNA was extracted from sori formed on infected leaf tissue of dried specimens using the innuPREP Plant DNA kit (IST innuscreen GmbH, Berlin, Germany following the manufacturer's protocol. The internal transcribed spacer (ITS) region, encompassing partial ITS1, ITS2 and the 5.8S rDNA, the large subunit (LSU) of the ribosomal RNA gene, and the cytochrome c oxidase subunit II (*COX2*) gene were amplified using the following primer pairs: ITS1-O (Bachofer, 2004) and LRO (reverse complement to LROR; Moncalvo et al., 1995), LR0R-O and LR6-O (Riethmüller et al., 2002), and *Cox2*-F and *Cox2*-R (Hudspeth et al., 2000), respectively. PCR amplifications for ITS, LSU and *COX*2 followed the protocol described in Ploch et al., (2018). PCR products were sequenced at the Senckenberg Biodiversity and Climate Research Centre (SBiK-F) laboratory centre in Frankfurt, Germany, using primers identical to those used for amplification.

Sequence analyses and phylogeny

Raw sequences were edited and assembled using Geneious (v. 5.6, Biomatters, Auckland, New Zealand). The edited sequences of each genomic region were compared with previously deposited DNA sequences

Table 1 The *Albuginaceae* species/specimens included in the phylogenetic analyses.

Species[a]	Host	Origin	Herbarium number	GenBank			Reference
				COX2	ITS	LSU	
Pustula cancriniae	*Cancrinia discoidea*	China	HMUT 4240	KY412452	KY421457	–	Xu et al., (2018)
	Cancrinia discoidea	China	HMUT 4241	KY412453	–	–	Ploch et al., (2011)
Pustula centaurii	*Eustoma grandiflorum*	Australia	DAR 51281	GU292173	–	–	Ploch et al., (2011)
	Centaurium erythraea	Australia	DAR 74543	GU292174	–	–	Ploch et al., (2011)
	Eustoma grandiflorum	Australia	VPRI 13352	GU292175	–	–	Ploch et al., (2011)
	Eustoma grandiflorum	Tasmania	DAR 72811	GU292176	–	–	Ploch et al., (2011)
***Pustula echinaceae* sp. nov.**	***Echinacea purpurea***	**Germany**	**FR-0046198**	**PX833722**	**PX831737**	**PX831738**	**This study**
	Echinacea angustifolia	USA	DAOM242817	KT257466	–	–	Spies et al., (2016)
	Echinacea angustifolia	USA	E2	KF981439	–	–	du Toit & Derie, (2014)
Pustula helianthicola	*Helianthus annuus*	Australia	DAR 45536	GU292165	–	–	Ploch et al., (2011)
	Helianthus annuus	Australia	HOH-HUH 679	GU292166	–	–	Ploch et al., (2011)
Pustula junggarensis	*Takhtajaniantha pusilla*	China	HMUT T-1601	KX589298	KX589300	–	Xu et al., (2016)
	Takhtajaniantha pusilla	China	HMUT T-16022	KX589299	–	–	Xu et al., (2016)
	Takhtajaniantha pusilla	China	HMUT T-16023	KX589297	–	–	Xu et al., (2016)
	Takhtajaniantha pusilla	Iran	IRAN17969F	MZ491097	MZ671828	–	Mirzaee et al., (2023b)
Pustula lactucae	*Lactuca undulata*	Iran	RAN17919FT	MZ182085	MZ494190	MZ494190	Mirzaee et al., (2021a)
	Lactuca undulata	Iran	RAN17944F	MZ182086	MZ494191	MZ494191	Mirzaee et al., (2021a)
Pustula obtusata s.lat.	*Tragopogon graminifolius*	Iran	FR0046065	MW450686	–	–	Mirzaee et al., (2021a)
	Tragopogon graminifolius	Iran	FR0046066	MW450687	–	–	Mirzaee et al., (2021a)
Pustula persica	*Gymnarrhena micrantha*	Iran	FR0046081	MW450684	–	–	Mirzaee et al., (2021b)
	Gymnarrhena micrantha	Iran	FR0046083	MW450685	–	–	Mirzaee et al., (2021b)
Pustula thinesii	*Xeranthemum inapertum*	Iran	IRAN17982F	OK205226	OR286578	–	Mirzaee et al., (2023a)
Pustula thinesii	*Xeranthemum inapertum*	Iran	IRAN17981F	OK205227	–	–	Mirzaee et al., (2023a)
Pustula senecionis	*Senecio hybridus*	Australia	VPRI 18267	GU292197	–	–	Ploch et al., (2011)
	Senecio vulgaris	Australia	VPRI 32491	GU292198	–	–	Ploch et al., (2011)

Table 1 (continued)

Species[a]	Host	Origin	Herbarium number	GenBank			Reference
				COX2	ITS	LSU	
	Senecio vulgaris	Australia	VPRI 24987	GU292196	–	–	Ploch et al., (2011)
	Senecio lautus	Australia	DAR 69348	GU292195			Ploch et al., (2011)
	Senecio vulgaris	Australia	VPRI 30458	GU292194			Ploch et al., (2011)
	Senecio vulgaris	Australia	VPRI 30457	GU292193	–	–	Ploch et al., (2011)
	Senecio vulgaris	Australia	DAR 16766	GU292192	–	–	Ploch et al., (2011)
	Senecio cineraria	USA	CUP 064965	GU292188	–	–	Ploch et al., (2011)
	Senecio inaequidens	Germany	HOH-HUH 936	GU292190	–	–	Ploch et al., (2011)
	Senecio vulgaris	Germany	HOH-HUH 880b	GU292191	–	–	Ploch et al., (2011)
	Senecio vernalis	Iran	IRAN17929F	MZ491098	–	–	Mirzaee et al., (2023a)
	Senecio vernalis	Iran	IRAN18311F	MZ491100	–	–	Mirzaee et al., (2023a)
	Senecio vernalis	Iran	IRAN17993F	MZ491099	–	–	Mirzaee et al., (2023a)
Pustula spinulosa	*Cirsium segetum*	China	SAU 1391	GU292178	–	–	Ploch et al., (2011)
	Cirsium arvense	Tasmania	DAR 26334	GU292179	–	–	Ploch et al., (2011)
	Cirsium oleraceum	Austria	GZU 112000	GU292180	–	–	Ploch et al., (2011)
Pustula sp.	*Trachymene* sp.	Australia	DAR 10111	GU292187	–	–	Ploch et al., (2011)
Pustula sp.	*Ozothamnus diosmifolius*	Australia	VPRI 20887a	GU292185	–	–	Ploch et al., (2011)
	Ozothamnus diosmifolius	Australia	DAR 75447	GU292186	–	–	Ploch et al., (2011)
	Ozothamnus diosmifolius	Australia	VPRI 20887b	GU292184	–	–	Ploch et al., (2011)
Pustula sp.	*Podolepis jaceoides*	Australia	DAR n.l.	GU292205	–	–	Ploch et al., (2011)
Pustula sp.	*Helichrysum bracteatum*	Australia	VPRI 15895	GU292199	–	–	Ploch et al., (2011)
	Helichrysum sp.	Australia	DAR 71914	GU292200	–	–	Ploch et al., (2011)
	Helichrysum bracteatum	Australia	DAR 16980a	GU292201	–	–	Ploch et al., (2011)
	Helichrysum sp.	Australia	DAR 48862	GU292202	–	–	Ploch et al., (2011)
	Helichrysum sp.	Australia	VPRI 18446	GU292203	–	–	Ploch et al., (2011)
	Helichrysum sp.	Australia	DAR 69962	GU292204	–	–	Ploch et al., (2011)
Pustula sp.	*Gnaphalium affine*	Korea	KUS-F 20200	GU292183	–	–	Ploch et al., (2011)
Pustula sp.	*Cotula australis*	Australia	DAR 16763	GU292182	–	–	Ploch et al., (2011)
	Callistephus chinensis	Australia	DAR 61418	GU292181	–	–	Ploch et al., (2011)
Pustula sp.	*Ambrosia artemisiifolia*	Unknown	VPRI 5652	GU292170	–	–	Ploch et al., (2011)
	Solenogyne bellioides	Australia	VPRI 92	GU292162	–	–	Ploch et al., (2011)
Pustula sp.	*Scorzonera hispanica*	Bulgaria	SOMF 16582	GU292171	–	–	Ploch et al., (2011)

Table 1 (continued)

Species[a]	Host	Origin	Herbarium number	GenBank			Reference
				COX2	ITS	LSU	
	Goodenia lunata	Australia	DAR 33573	GU292172	–	–	Ploch et al., (2011)
Pustula sp.	*Gazania* sp.	Australia	VPRI 10667	GU292177	–	–	Ploch et al., (2011)
Pustula sp.	*Ageratum conyzoides*	Venezuela	CUP 000006	GU292163	–	–	Ploch et al., (2011)
	Ageratum conyzoides	Venezuela	CUPvz 001006	GU292164	–	–	Ploch et al., (2011)
Pustula tragopogonis	*Tragopogon pratensis*	Germany	HOH-HUH 725	EU826101	–	–	Ploch et al., (2011)
	Tragopogon porrifolius	Australia	DAR 51374	GU292167	–	–	Ploch et al., (2011)
	Tragopogon porrifolius	Tasmania	DAR 30294	GU292168	–	–	Ploch et al., (2011)
	Tragopogon porrifolius	Australia	VPRI 16030	GU292169	–	–	Ploch et al., (2011)
Pustula xinyuanensis	*Convolvulus gortschakovii*	China	HMUT 3475	KY412454	KY412459	–	Xu et al., (2018)
Pustula xinyuanensis	*Convolvulus gortschakovii*	China	HMUT 3473	KY412455	–	–	Xu et al., (2018)
Pustula xinyuanensis	*Convolvulus gortschakovii*	China	HMUT 3476	KY412456	–	–	Xu et al., (2018)
Wilsoniana bliti	*Amaranthus viridis*	Australia	DAR 76991	GU292161	–	–	Ploch et al., (2011)

BPI: National Fungus Collections, Beltsville, Maryland, USA; CUP: Plant Pathology Herbarium, Cornell University; DAR: Herbarium of the Orange Agricultural Institute; FR: Senckenberg Forschungsinstitut und Naturmuseum; GZU: Herbarium, Institut für Pflanzenwissenschaften, Karl-Franzens-Universität Graz; HMUT: Mycological Herbarium of Tarim University; HOH: The Herbarium of the University of Hohenheim; KUS-F: The Korea University Herbarium; IRAN: Iranian Fungal Culture Collection at the Iranian Research Institute of Plant Protection, Tehran, Iran; SOMF: Bulgarian Academy of Sciences Mycological Collection; STE: Department of Plant Pathology, University of Stellenbosch; VPRI: Victorian Department of Environment and Primary Industries.

The specimen sequenced in this study is indicated in bold.

using the BLAST tools implemented in the NCBI GenBank database (www.ncbi.nlm.nih.gov/genbank/). Sequence data from type and reference strains of known *Pustula* species were retrieved from GenBank (Table 1). The retrieved sequences were added to the edited sequences in Geneious (version 5.6) and aligned using the latest version of MAFFT with the Q-INS-I algorithm (MAFFT Development Team, 2025). Subsequently, after removing leading and trailing gaps, phylogenetic analyses were performed using the TrEase webserver (Mishra et al., 2023) for the *COX*2 dataset. Analyses included RAxML v8 (Stamatakis, 2014) for Maximum Likelihood (ML), and MrBayes 3.2 (Ronquist et al., 2012) for Bayesian inference (BI). For the Bayesian analysis, the GTR model was selected and run for 1,000,000 generations. The first 30% of trees were discarded, and posterior probabilities were calculated from the remaining trees sampled during the stationary phase. In RAxML the GTRGAMMA algorithm was used for phylogenetic inference. Minimum Evolution (ME) analysis was performed separately using MEGA7 (Kumar et al., 2016) using the Tamura-Nei nucleotide substitution model, with pairwise deletion of gaps. The robustness of internal branches was assessed through 1,000 bootstrap replicates (Felsenstein, 1985), providing statistical support for the inferred phylogeny. The remaining parameters were kept at their default settings in the program. The sequences obtained in this study were deposited in GenBank, with accession numbers provided in Table 1. The full alignment of dataset has been submitted to TreeBASE (http://purl.org/phylo/treebase/phylo

Table 2 Morphological characterisation of *Pustula echinaceae*

	Length			Width		
	min-max	mean	(minimum–) mean minus standard deviation–mean–mean plus standard deviation (–maximum)	min-max	mean	(minimum–) mean minus standard deviation–mean–mean plus standard deviation (–maximum)
Primary Sporangia	13.99–29.19	20.16	(13.99–)17.41–20.16–22.91(–29.19)	11.07–24.51	17.81	(11.07–)15.39–17.8–20.22(–24.51)
	Length			**Width**		
	min-max	**mean**	**(minimum–) mean minus standard deviation–mean–mean plus standard deviation (–maximum)**	**min-max**	**mean**	**(minimum–) mean minus standard deviation–mean–mean plus standard deviation (–maximum)**
Secondary Sporangia	14.38–24.68	20.18	(14.38–)18.23–20.18–22.13(–24.68)	15.45–24.38	19.76	(15.45–)17.98–19.76–21.54(–24.38)
	Length			**Width**		
	min-max	**mean**	**(minimum–) mean minus standard deviation–mean–mean plus standard deviation (–maximum)**	**min-max**	**mean**	**(minimum–) mean minus standard deviation–mean–mean plus standard deviation (–maximum)**
Oospores	45.1–62.2	53.04	(45.1–)49.36–53–56.7(–62.2)	41.3–60.6	50.73	(41.3–)46.7–50.7–54.7(–60.6)
	Length			**Width**		
	min-max	**mean**	**(minimum–) mean minus standard deviation–mean–mean plus standard deviation (–maximum)**	**min-max**	**mean**	**(minimum–) mean minus standard deviation–mean–mean plus standard deviation (–maximum)**
Sporogenous hyphae	26.69–54.22	39.1	(26.69–)33.63–39.1–44.57(–54.22)	10.83–20.10	15.64	(10.83–)13.99–15.6–17.21(–20.10)
	min-max	**mean**	**(minimum–) mean minus standard deviation–mean–mean plus standard deviation (–maximum)**			
Wall thickness in Primary Sporangia	2.76–6.46	4.39	(2.76–)3.71–4.39–5.07(–6.46)			
Equatorial wall thickening (annulus)	1.51–4.4	2.48	(1.51–)2.42–2.48–3.01(–4.4)			

All measurements are given in micrometers (μm).

ws/study/TB2:S32476), and the new species described here has been registered in MycoBank (MB 861979).

Results

Phylogeny

ML and BI analyses based on *COX*2 mitochondrial DNA sequences from 66 specimens belonging to the genus *Pustula* exhibited congruent topologies with the ME analysis of the same dataset. Therefore, the ME tree was selected to represent the phylogeny with bootstrap support values from ML and posterior probabilities from BI added for comparison (Fig. 1). The *COX*2 dataset comprised 486 characters (including alignment gaps), with 130 variable and 356 constant characters.

In all phylogenetic trees based on *COX2* sequences, the *Pustula* specimen investigated in this study (PX833722) formed a distinct, robust monophyletic clade together with two previously reported specimens, E2 (KF981439; du Toit & Derie, 2014) and DAOM242817 (KT257466; Spies et al., 2016), both from *Echinacea angustifolia* (previously identified as *Pustula* sp. and *P. obtusata*, respectively). BLASTn searches confirmed 100% identity among these three sequences**,** while the next closest match was the ex-type of *P. helianthicola* (JF793557), showing 97.5% identity with 12 nucleotide differences. This clade, supported by maximal ME/ML bootstrap values (100/98) and Bayesian posterior probability (1), is sister to *P. helianthicola* from *Helianthus annuus*, strongly suggesting a distinct species on *Echinacea* spp. While the *COX2* sequences of this lineage revealed no sequence variation, about 2.5% sequence divergence was observed relative to *P. helianthicola*.

Due to the limited availability of ITS and LSU sequences of *Pustula* species in GenBank, phylogenetic analysis using these loci was not performed in this study. However, ITS and LSU sequences of the specimen collected in this study were generated and compared to previously deposited sequences in GenBank via BLAST. The ITS sequence (PX831737) exhibited 99.8% identity with no nucleotide differences and only one gap, when compared to the ITS sequence (KT257377) from *E. angustifolia*, previously identified as *P. obtusata*. The next closest match was *P. junggarensis* (KX589300), which showed 95.61% identity with 32 nucleotide differences. Similarly, LSU sequence (PX831738) analysis revealed 100% identity to KT257377 from *E. angustifolia*, with no nucleotide differences, while *P. tragopogonis* strain (EU826115) from *Cirsium oleraceum*, showed five nucleotide differences (96.3% identity).

Morphology

Measurements of morphological characteristics of *Pustula echinaceae* on *Echinacea purpurea* are summarized in Table 2. The main morphological characters that differentiate *Pustula* species are the size and shape of primary and secondary sporangia, wall thickness of the organs, and the size, shape, and surface ornamentation of oogonia and oospores. Sori of *Pustula* sp. on *Echinacea* are cream to yellowish-colored, and composed of densely packed sporogenous hyphae. They vary in size, with diameters ranging from less than 1 mm to 1–5 mm, sometimes merging to form patches exceeding 1 cm (Fig. 1a–c). The sori are roundish or irregular, distinct or confluent, often coalescing with satellite pustules covering large areas. Sporogenous hyphae are hyaline, clavate to cylindrical, straight to slightly curved or curved. Their length ranges from 26.5 to 54 µm and their width from 11 to 20 µm (mean ± SD = 39 ± 5.4 × 15.6 ± 1.6 µm, n = 85) (Fig. 1d). Sporangia are produced in basipetal chains and are hyaline, of two types. Primary sporangia are globose to subglobose with thickened walls, often polyangular or depressed due to pressure from neighboring sporangia and are attached to the plant epidermis. Their length ranges from 14 to 29 µm and their width from 11 to 24.5 µm (mean ± SD = 20.2 ± 2.8 × 17.8 ± 2.4 µm, n = 100) (Fig. 1e, f). The wall thickness of the primary sporangia ranges from 2.7 to 6.5 µm (mean ± SD = 4.4 ± 0.7 µm, n = 112), occasionally reaching up to 8 µm. Secondary sporangia are mainly globose to subglobose, with lengths from 14.5 to 24.5 µm and widths from 15.5 to 24.5 µm (mean ± SD = 20.2 ± 2.0 × 19.8 ± 1.8 µm, n = 122), with a length/width ratio of about 1.02. Secondary sporangia have an equatorial wall thickening as an annulus measuring 1.5 to 4.5 µm (mean ± SD = 2.5 ± 0.5 µm, n = 128) (Fig. 1g–i). Oogonia are globose to subglobose or irregular, hyaline, smooth-walled, and can reach up to 84 µm in diameter. Oospores are light ochre to dark brown, abundant within leaves, and predominantly globose. Their larger diameter

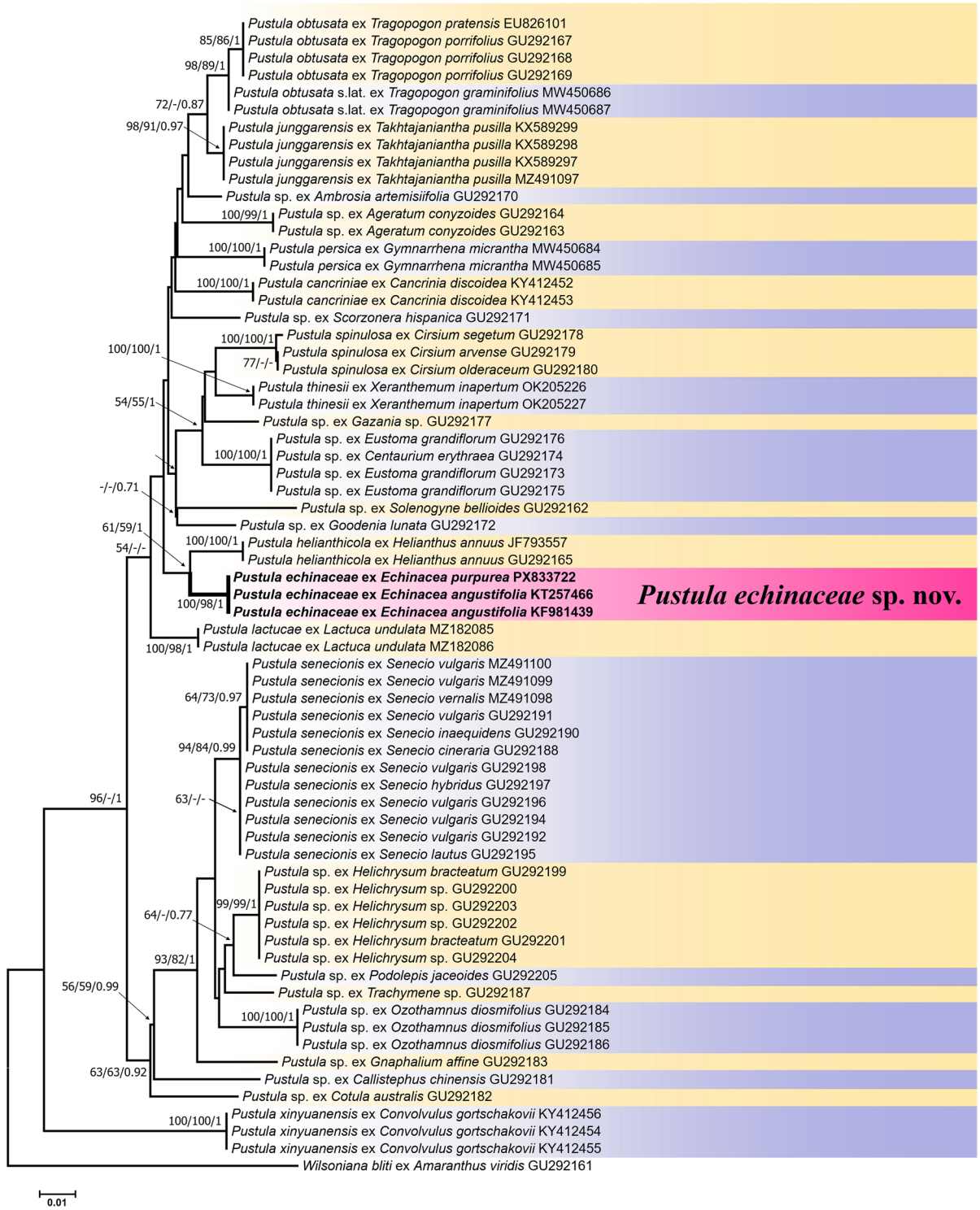


Fig. 1 Minimum Evolution tree of *Pustula* based on *COX*2 mtDNA sequences. Support values (Minimum Evolution bootstrap values >50%/Maximum Likelihood bootstrap values >50%/Bayesian posterior probabilities >0.70) are indicated along the branches. A dash indicates lack of support for the topology or for conflicting topologies. *Wilsoniana bliti* (GU29216) was used as outgroup. Sequence obtained in this study is highlighted in blue.


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◂**Fig. 2** *Pustula echinaceae*. (**a**, **b**) Disease symptoms on *Echinacea purpurea*; (**c**, **d**) cream to yellowish-colored sori on the undersides of leaves; (**e**) sporogenous hyphae; (**f**, **g**) primary sporangia; (**h**-**j**) secondary sporangia; (**k**–**m**) oospores; (**n**, **o**) prominent projections along the periphery of oospores; (**p**–**s**) ornamentations on the oospores surface. Scale bars: (**e**–**m**) = 20 µm, (**n**–**s**) =10 µm

ranges from 45.5 to 62 µm and their smaller diameter from 41.5 to 60 µm (mean ± SD = 53 ± 3.7 × 50.5 ± 4.0 µm, *n* = 60) (Fig. 1j–l, o–r). The ornamentation of oospores is predominantly reticulate, with frequent discontinuities, occasionally forming a broad net-like pattern. Sometimes oospore ornamentation appears as an irregularly bulging pattern. The outer margin of the oospore wall is irregularly undulate, with prominent denticulate (tooth-like) projections along the periphery, ranging from 1 to 2.5 µm in height, and variable in size and spacing (Fig. 1m, n).

Further details for the morphological characterization are provided in the taxonomy section and in Table 2.

Taxonomy

Pustula echinaceae Alizadeh & Thines, **sp. nov.,** MycoBank MB 861979 (Fig. 2.)

Etymology: The name "echinaceae" refers to the host genus, *Echinacea*, from which the species was described.

Diagnose:*P. echinaceae* can be discriminated from *P. helianthicola* by its slightly larger primary sporangia with thicker walls, a thicker annulus of secondary sporangia, larger oospores, and greater variability in oospore surface ornamentation.

Typification: Germany, Baden-Württemberg, Mannheim, from cultivated *Echinacea purpurea* received from a nursery, July 2024, Jan Hinrichs-Berger (FR-0046198, holotype), GenBank: *COX2* = PX833722; ITS = PX831737; LSU = PX831738.

Description: Mycelium intercellular. Sori on undersides of leaves, cream to yellowish-colored, composed of densely packed sporogenous hyphae. Size range of sori less than 1 mm to 1–5 mm diameter (Fig. 1a–c). Shape variable, roundish to irregular, distinct or confluent, often coalescing with satellite pustules forming large areas >1 cm diameter. Sporogenous hyphae hyaline, clavate to cylindrical, straight, slightly curved to curved, 26.5–54 × 11–20 µm (av. 39 × 15.6 µm) (Fig. 1d). Sporangia produced in basipetal chains, hyaline, two types. Primary sporangia globose to subglobose with thickened wall, often polyangular or depressed due to mutual pressure from neighboring sporangia against epidermis inner surface; size 14–29 × 11–24.5 µm (av. 20.2 × 17.8 µm) (Fig. 1e, f). Wall thickness 2.7–6.5 µm (av. 4.4 µm), rarely up to 8 µm. Secondary sporangia mainly globose to subglobose, 14.5–24.5 × 15.5–24.5 µm (av. 20.2 × 19.8 µm), length/width ratio 1.02; equatorial wall thickening and annulus 1.5–4.5 µm (av. 2.5 µm) (Fig. 1g–i). Oogonia globose to subglobose or irregular, hyaline, smooth-walled, up to 84 µm diameter. Oospores yellowish to brown to dark brown, abundant within leaves, predominantly globose; size 45.5–62 × 41.5–60 µm (av. 53 × 50.5 µm) (Fig. 1j–l, o–r). Primary ornamentation predominantly reticulate, with frequent discontinuities in reticulum, occasionally forming broad net-like pattern. Secondary ornamentation irregularly bulging. Outer margin of oospore walls irregularly undulate, with prominent denticulate (tooth-like) projections along periphery, 1–2.5 µm in height, variable in size and spacing (Fig. 1m, n). Undulations generally non-uniform, some segments relatively uniform, contributing to distinct overall ornamentation of oospore.

Notes: The phylogenetic analysis revealed a close phylogenetic relationship between *Pustula* sp. infecting *Echinacea purpurea* and *P. helianthicola*, positioning them as closely related species within a sister group. However, distinct morphological features differentiate *P. echinaceae* from its closely related species. Primary sporangia in *P. echinaceae* tend to be slightly larger (average 20.1 × 17.8 µm) compared to *P. helianthicola* (average 17.8 × 14.9 µm). Furthermore, *P. echinaceae* exhibits thicker primary sporangial walls (average 4.39 µm) compared to *P. helianthicola* (average 3.0 µm). Secondary sporangia in both species exhibit comparable average sizes. However, the annulus of *P. echinaceae* secondary sporangia is generally thicker (1.5–4.5 µm, average 2.48 µm) than that of *P. helianthicola* (1.5–2.0 µm). Oospores in *P. echinaceae* are also typically larger (average 53 × 50.5 µm) than those of *P. helianthicola* (average 48.4 µm in diameter). *Pustula echinaceae* exhibits considerable variability in oospore surface ornamentation, ranging from a reticulate (net-like) pattern to irregular surfaces with uneven ridges and discontinuities within the reticulum. In contrast, oospores of *P. helianthicola* appear to show a more uniform surface

texture, with densely reticulate oospores (Rost & Thines 2012).

Morphological comparisons between *Pustula echinaceae* (from *Echinacea purpurea* in the present study) and *Pustula* sp. (from *Echinacea angustifolia*, reported by du Toit and Derie, 2014) revealed some differences. The primary sporangia of *P. echinaceae* averaged 20 × 18 µm, ranging from 14 to 29 µm in length and 11 to 24.5 µm in width, with a wall thickness averaging 4.4 µm. Secondary sporangia measured on average 20 × 20 µm, ranging from 14.5 to 24.5 µm in length and 15.5 to 24.5 µm in width. In contrast, the sporangia of *Pustula* sp. from *E. angustifolia* were smaller, with an average size of 21 × 20 µm, length ranging from 16 to 25 µm and width from 16 to 24 µm, and a wall thickness averaging 2.6 µm. Oospores also differed in size, with *Pustula* sp. from *E. angustifolia* producing larger oospores (av. 75 × 63 µm) compared to *P. echinaceae* (av. 53 × 50.5 µm). If the observed differences are in the natural range of the *P. echinaceae* or are indicative of some genetic differentiation as a result of specialization to another host species will need to be clarified by future population genetic studies.

Discussion

Studies of the past 15 years have started to shed light on the diversity of *Pustula* across the asterid host clade—the largest group of flowering plants (Ploch et al., 2011; Rost & Thines, 2012; Xu et al., 2016, 2018; Mirzaee, 2021a, 2021b, 2023a). The presence of multiple distinct and host-specific lineages within the genus *Pustula*, at least host-specific at the genus level (Ploch et al., 2011; Mirzaee, 2021a, 2021b, 2023a), revealed that a substantial number of *Pustula* species have not been described so far. This pattern is also consistent with the *Echinacea*-associated lineage being clearly distinct from its sister lineage, the sunflower pathogen *P. helianthicola* (Rost & Thines, 2012).

The mitochondrial *COX2* locus has been widely used as a reliable marker for species identification and phylogenetic inference in oomycetes (Thines et al., 2008; Langer et al., 2021; Mu et al., 2024a, b; Tsai & Thines, 2025). Comparative studies have demonstrated that *COX*2 exhibits higher interspecific divergence compared to alternative loci such as *COX*1, making it particularly suitable for species delimitation (Choi et al., 2015). Furthermore, *COX*2 shows high amplification success across diverse oomycete lineages and has been consistently applied in taxonomic studies of *Albuginales*, including *Pustula* (Ploch et al., 2011; Xu et al., 2016, 2018; Mirzaee et al., 2021a, 2023a). Previous studies on *Pustula* and related *Albuginales* have shown that *COX*2 provides sufficient resolution for species delimitation, even when additional loci are unavailable. Although a multilocus framework would be desirable, the available sequence data for *Pustula* remains too limited to support such analyses. Thus, *COX*2 represents the most informative and widely comparable marker currently available for species-level delimitation within the genus.

Hence, following the approach of recent studies on *Pustula* (Xu et al., 2016, 2018; Mirzaee, 2021a, 2021b, 2023a), the phylogenetic tree was inferred based on *COX2* sequences, while ITS and LSU data were included for comparison due to the limited availability of comparable sequences in public databases. The ITS and LSU sequences obtained in this study were congruent with the *COX2*-based phylogenetic placement, further supporting the reliability of *COX2* for species-level resolution.

Based on morphology and nucleotide sequences of *COX*2 and ITS, the two previously reported occurrences of *Pustula* on *E. angustifolia* (du Toit & Derie, 2014; Spies et al., 2016) and *E. purpurea* (Spies et al., 2016), as well as a newly collected specimen from Germany belong to the same species. *COX2* data revealed complete sequence identity within this lineage, while showing a clear genetic separation from *P. helianthicola*. The observed ~2.5% *COX2* sequence divergence from *P. helianthicola* falls within the range of interspecific divergence reported for *Pustula* and related *Albuginales* taxa, whereas intraspecific variation in this locus is typically very low or absent (Ploch et al., 2011; Mirzaee, 2021a, 2021b, 2023a). The LSU sequence showed complete identity with previously reported *Echinacea*-associated specimens, while the ITS sequence exhibited near-complete identity, differing only by a single gap. This contrast between interspecific divergence and the absence of intraspecific variability is consistent with the delimitation of this lineage as a separate species in *Pustula*, despite only subtle morphological differences. Key morphological differences include slightly larger primary sporangia, thicker sporangial walls, and greater

variability in oospore ornamentation (see Results, Table 2). Taken together, the combined molecular, morphological, and host-associated evidence supports the recognition of this lineage as a distinct species.

Of the nine species of *Echinacea*, three are of economic importance, because of diverse applications (Parsons et al., 2018): *Echinacea purpurea* (L.) Moench, *E. pallida* (Nutt.) Nutt., and *E. angustifolia* DC. All three species are medicinal plants, with several bioactive compounds (Manayi et al., 2015). Among the three species, *E. purpurea* commonly has gained considerable attention for its multifaceted applications (Barrett et al., 1999, 2010), including its use as an ornamental plant in temperate regions (Letchamo et al., 2002; Ault 2007; Koenig & Roehr, 2006). To date, 44 reports of fungi and oomycetes affecting *Echinacea* species have been documented (USDA Fungal Databases, accessed January, 2026). Among these, a single historical record reported *Albugo tragopogonis* on *E. angustifolia* in the United States as early as 1883 (Rogerson, 1958). Additionally, there are three reports involving two *Pustula* sp. on *E. angustifolia* (du Toit & Derie 2014; Spies et al. 2016) and one on *E. purpurea* (du Toit & Derie 2014), all from the United States. However, the pathogen was also found in Germany in this study, indicating that the *Echinacea*-associated *Pustula* lineage is not restricted to North America. The occurrence of this pathogen in Germany may reflect a trans-Atlantic introduction, potentially associated with the trade of asymptomatic plants (Ploch & Thines, 2011) or infested seeds, similar to the situation observed for other biotrophic oomycete pathogens that have emerged over the past decades (Görg et al., 2017; Thines et al., 2009a, 2009b, 2019). Nevertheless, the origin of the German occurrence and the possible introduction pathways remain unclear and require further epidemiological and population-level studies.

For an early intervention in such emerging threats, the identification of the causative pathogens is considered important for developing effective control strategies for plant diseases (Alizadeh et al., 2022). Failure to accurately identify pathogens at the species level can lead to significant consequences, as demonstrated by *Peronospora belbahrii* (Thines et al., 2009b). Initially misclassified as *Peronospora lamii* due to its association with the same plant family, its spread was underestimated. Consequently, no quarantine measures were implemented, allowing the pathogen to spread rapidly through the distribution of infected seeds and natural migration, and also to colonise new hosts (Thines et al., 2018). Today, *Peronospora belbahrii* is a globally established pathogen (Ronco et al., 2009). Likewise, the new occurrence of *Pustula* on *Echinacea* spp. deserves attention. However, the present study primarily represents a taxonomic contribution, and broader plant pathological or biosecurity implications should be supported by targeted epidemiological and population-level investigations.

As mentioned in the Taxonomy section, a morphological comparison between *P. echinaceae* collected from *E. purpurea* in this study reported from *E. angustifolia* by du Toit and Derie (2014) revealed several notable differences. As Toit and Derie (2014) did not specify whether their sporangial measurements referred to primary or secondary sporangia, and how oospore sizes were measured, direct comparisons should be made with caution. Nevertheless, the observed morphological differences may reflect variation related to the distinct host species, similar to the situation in downy mildews (Runge & Thines 2012) or already reflect some genetic differentiation according to the host species in a radiation process after a host jump (Thines 2019). In addition, Constantinescu and Thines (2006) observed that primary and secondary sporangia can vary in size within a sorus, and some sori may contain sporangia larger than average. This variability, along with differences in the number of structures measured in previous studies (Constantinescu & Thines, 2006), may also explain the morphological differences reported, and is one of the reasons why sequence-based identification is the method of choice in white blister pathogens.

It is well established that plant pathogens are often introduced to new continents and regions along with their hosts (Thines et al., 2023; Schertler et al., 2024). Thus, it is important to better understand the diversity of plant pathogens, including the genus *Pustula*, as a basis for future assessments of pathogen spread and associated risks.

Acknowledgements The authors gratefully acknowledge funding by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project number 547786015.

Authors' contributions Conceptualization: Alireza Alizadeh, Marco Thines. Methodology: Alireza Alizadeh. Formal analysis and investigation: Alireza Alizadeh. Writing – original draft preparation: Alireza Alizadeh. Writing – review

and editing: All authors. Funding acquisition: Marco Thines, Alireza Alizadeh (DFG grant). Resources: Jan Hinrichs-Berger, Lindsey J. du Toit, Young-Joon Choi

Funding Open Access funding enabled and organized by Projekt DEAL. This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project number 547786015. Alireza Alizadeh and Marco Thines acted as Co-investigators.

Data availability The sequences obtained in this study were deposited in GenBank (accession numbers in Table 1), the alignment and phylogenetic trees in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S32476), and the new species *Pustula echinaceae* was registered in MycoBank (MB 861979).

Declarations

Competing interests The authors declare that they have no competing interests.

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