

Short communication

Occurrence of the sexual morph of *Erysiphe macleayae* on *Chelidonium majus* in Romania

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Abstract – The sexual morph (chasmothecium) of *Erysiphe macleayae* on *Chelidonium majus* has been found in Romania for the first time. Although the asexual morph of this powdery mildew on *C. majus* is known from many countries, the occurrence of chasmothecia on this host is rather rare. A description of morphological characters and an analysis of the phylogenetic relationship of ITS sequences of the Romanian specimens are presented.

Key words: greater celandine, powdery mildew, chasmothecia, ITS

Introduction

Erysiphe macleayae was originally described from China as a parasite of *Macleaya cordata* (Willd.) R. Br. and *Papaver nudicaule* L. (Zheng and Chen 1981). Besides these hosts, it was also reported as causal agent of powdery mildew on *Macleaya microcarpa* (Maxim.) Fedde (Park et al. 2012), *Meconopsis cambrica* (L.) Vig. (Schmidt and Scholler 2011, Braun and Cook 2012), and *Chelidonium majus* L. (Jiang et al. 2015), all of them plant species of the family Papaveraceae. Outside this family, it was recently found on *Torenia fournieri* Linden ex E. Fourn. (Men et al. 2014), a species of Linderniaceae.

In the last decade, the powdery mildew of *Chelidonium majus* (greater celandine) has been a disputed topic in the context of the extension of its range of distribution and the absence, until recently, of the sexual morph. The first report of this disease based on DNA analysis of the anamorph was published by Jankovics (2007) from Hungary, who identified the powdery mildew on *C. majus* as *Oidium neolycopersici*. In a study of the powdery mildews of Papaveraceae species in Germany, Schmidt and Scholler (2011) mentioned that the powdery mildew of *C. majus* may be related to or even conspecific with *E. macleayae*. Later, Pastirčáková and Pastirčák (2013) confirmed on the basis of morphological characteristics that the anamorph of the *C. majus* powdery mildew in the Czech Republic and Slovakia is caused by a *Pseudoidium* species. Finally, Jiang et al. (2015) published the first collection of the chasmothecia on *C. majus* in China and identified the causal agent as *Erysiphe macleayae*. Fruiting bodies (chasmo-

thecia) were also found in Germany in 2014 (Braun 2014) and Slovakia in 2014 and 2015 (Pastirčáková et al. 2016). Recently, the anamorph of *E. macleayae* on *C. majus* was also reported from Ukraine (Heluta and Kravchuk 2015).

Based on a collection from Moldavia (Romania), the anamorph of this powdery mildew on *C. majus* was described by Iacob and Drobotă (2008) as a new species, *Oidium chelidonii* Iacob. Because of the absence of both a Latin diagnosis and a holotype, this name is invalid (Pastirčáková et al. 2016).

The aim of this paper is to report the new occurrence of the teleomorph of *E. macleayae* on *C. majus*, which is new for Romania. Furthermore, a description of the Romanian specimens and a phylogeny inferred including the newly obtained ITS sequences are provided.

Materials and methods

Leaves of *Chelidonium majus* infected with powdery mildew were collected from the urban area of Iași, Romania. For light microscopic examination, fresh samples were mounted in 2% KOH. The sexual and asexual features (chasmothecia, asci, ascospores, conidia, and conidiophores) were described, measured, and photographed. The given size ranges of all structures are based on 30 measurements. Statistical data (length and width) are given as arithmetic mean \pm standard deviation (SD). For the examination of the conidial germination, the moist chamber method with saturated aqueous solution of potassium sulphate as described in Braun and

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Cook (2012) was used. The analyzed specimens are deposited in the Herbarium of Alexandru Ioan Cuza University of Iasi, Romania (Index Herbariorum abbreviation: I).

For the molecular characterization, DNA was extracted from the chasmothecia (voucher specimen I 183221) and the anamorph (voucher specimen I 183222). The nuclear ribosomal internal transcribed spacer (ITS) region was amplified using primers ITS1F 5'-CTTGGTCATTTAGAGGAAG-TAA-3' (Gardes and Bruns 1993) and ITS4, 5'-TCCTCC-GCTTATTGATATGC-3' (White et al. 1990) and sequenced at Alvalab (Santander, Spain). The newly obtained sequences were deposited in GenBank (accession numbers KU756266 and KU756267). For the phylogenetic analysis, sequences of the powdery mildew specimens isolated from *Chelidonium majus* (Jankovics 2007, Jiang et al. 2015, Kovács et al. 2011, Pastirčáková et al. 2016, Takamatsu et al. 2015), *Macleaya cordata* (Jiang et al. 2015, Pastirčáková et al. 2016, Takamatsu et al. 2015), *Macleaya microcarpa* (Park et al. 2012), and *Torenia fournieri* (Men et al. 2014) were downloaded from GenBank and aligned with the ClusatlW algorithm (Larkin et al. 2007). Sequences of *Erysiphe aquilegiae* DC., *E. magnoliae* (Sawada) U. Braun & S. Takam. from Takamatsu et al. (2015), and *E. deutziae* (Bunkina) U. Braun & S. Takam. from Denton and Henricot (2007) were used as outgroup taxa as these species were recovered as basal to the *E. aquilegiae* clade in the comprehensive phylogenetic analysis of Takamatsu et al. (2015). A list of all the 35 sequences used in the analysis is included in the On-line Suppl. Tab. 1. Maximum likelihood (ML) analysis was used as a tree building method. The K2 (Kimura 2-parameter) model of nucleotide substitution was selected using BIC (Bayesian Information Criterion). The reliability of phylogenetic relationships was evaluated using a non-parametric bootstrap analysis with 1000 replicates and ML bootstrap values (BP) are presented at branch nodes. The bootstrap values exceeding 75 were considered well supported. All computations were performed using MEGA v6.0.5 (Tamura et al. 2013).

Results and discussion

In the autumn of 2014, several specimens of *C. majus* with powdery mildew were collected in Iasi, Romania, including a single leaf with a few young chasmothecia. A year later, alongside a building wall near the headquarters of the Alexandru Ioan Cuza University of Iași, a powdery mildew specimen on *C. majus* with mature chasmothecia on basal leaves was found. Of the 12 leaves of the plant, nine showed disease symptoms. Besides this, although other specimens of greater celandine with powdery mildew were investigated, only a single additional leaf with chasmothecia on another specimen was found. The collected specimens belong to *E. macleayae*. The occurrence of the teleomorph of this powdery mildew is new for Romania.

Erysiphe macleayae R.Y. Zheng & G.Q. Chen, Sydowia 34: 290 (1981)

Mycelium white, amphigenous, but mostly epiphyllous, covering the entire surface of the older basal leaves, or form-

ing patches mainly on the upper lobes of the leaf (Fig. 1A). Hyphae 4–6 μm wide, with lobed or nipple-shaped appressoria. Conidiophores straight or slightly curved, hyaline, 70–135 (mean 99.8 ± 14.7) \times 8–12 μm (mean 10.0 ± 0.9), foot-cells cylindrical, followed by 1–2 shorter cells. Conidia formed singly, ellipsoid, doliiform-cylindrical, hyaline, 25–50 (mean 38.7 ± 5.4) \times 10–20 μm (mean 14.6 ± 2.7). Germ tubes formed subapically (*Pseudoidium* type) (Fig. 1D). Chasmothecia scattered to gregarious, subglobose, 75–116 μm (mean 92.3 ± 10.2) in diameter, initially light brown, then dark brown. Appendages 15–25 per chasmothecium, simple, often tortuous-sinuous, up to 700 μm long, light brown (Fig. 1B). Asci 3–6 per chasmothecium, 45–70 (mean 57.1 ± 7.6) \times 30–55 μm (mean 37.4 ± 7.9), obovoid-saccate, short-stalked, 3–6-spored. Ascospores 16–26 (mean 21.6 ± 2.7) \times 10–13 μm (mean 10.7 ± 0.8), ellipsoid-ovoid (Fig. 1C).

Specimens examined: on living leaves of *Chelidonium majus*, ROMANIA, Iasi, Anastasie Fătu Botanic Garden, Dumbrava Roșie Street, 7 Oct. 2014, leg. et det. V. Chinan, voucher specimen I 183224 (anamorph and immature chasmothecia); Iasi, Pinului Street, near the Alexandru Ioan Cuza University, 10 Oct. 2015, leg. et det. V. Chinan, voucher specimens: I 183221 (teleomorph, GenBank accession number KU756266), I 183222 (anamorph, GenBank accession number KU756267); 20 Oct. 2015, leg. et det. V. Chinan, voucher specimen I 183223 (teleomorph).

Phylogenetic analysis: the sequences obtained from the chasmothecia (KU756266) and the anamorph (KU756267) of the Romanian specimens are identical, and positioned well with the other sequences of *Erysiphe macleayae* parasitizing *C. majus*, *M. cordata*, *M. microcarpa* and *T. fournieri*

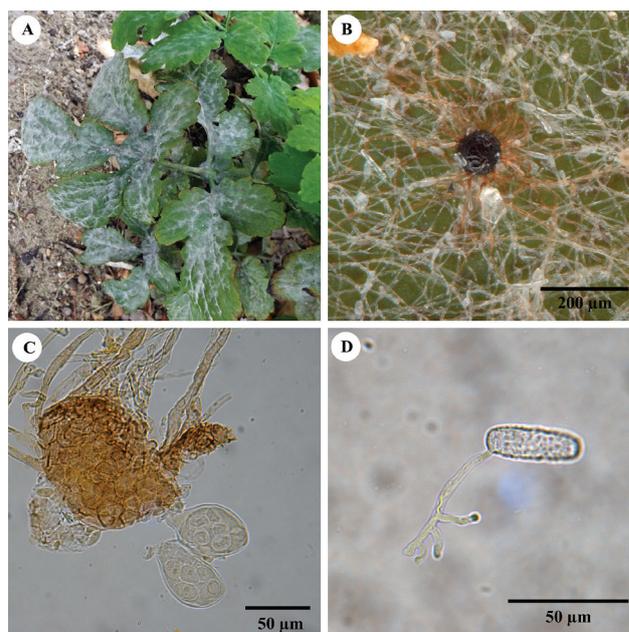


Fig. 1. *Erysiphe macleayae*: A – symptoms on *Chelidonium majus*, B – chasmothecium, conidia and mycelium on the leaf surface, C – crushed mature chasmothecium, asci with ascospores, D – germinated conidium.

(BP = 96) (Fig. 2). These results confirm the species identity and the conspecificity of the asexual and sexual morphs found on *C. majus* in Romania. The intraspecific molecular variability of *E. macleayae* is very low: specimens of diverse geographical origins differ at most in two substitutions (0–0.4% uncorrected pairwise distance).

Both the sexual and the asexual morph of the Romanian collections agree well morphologically with the descriptions of *E. macleayae* from *M. cordata* (Zheng and Chen 1981) and *C. majus* (Jiang et al. 2015, Pastirčáková et al. 2016).

It is noteworthy that in Europe chasmothecia of *E. macleayae* on *C. majus* were found in autumn 2014, simultaneously in Germany (Braun 2014), Slovakia (Pastirčáková et al. 2016) and Romania (in this study). In these countries, the anamorph on this host was first found in 2003 (Jage et al. 2010), 2006 (Pastirčáková and Pastirčák 2013), and 2007 (Iacob and Drobotá 2008) respectively. This confirms that ma-

ny powdery mildew fungi do not produce chasmothecia for several years after extending their geographical areas or host ranges (Kiss 2002, Kiss et al. 2002). The annual monitoring of this powdery mildew made in Slovakia by Pastirčáková et al. (2016), in the period 2006–2015 fully supports this observation. Furthermore, homo- or heterothallism of the species concerned may play an important role. Heterothallic species often need more time to find compatible thalli to be able to reproduce sexually. The individual life cycle of *E. macleayae* is still unknown, but heterothallism appears to prevail among powdery mildews (Wolfenbarger et al. 2015).

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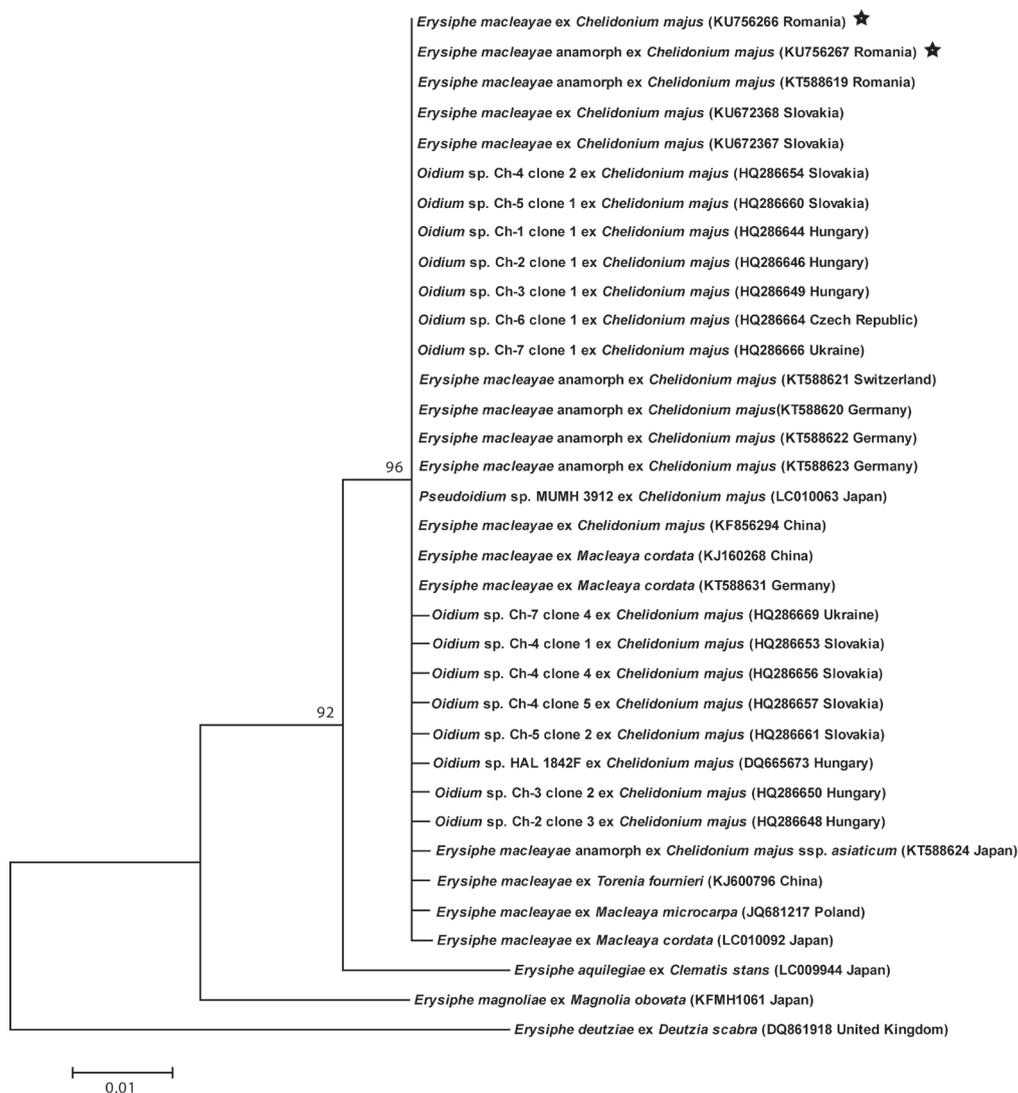


Fig. 2. Rooted maximum likelihood tree based on ITS sequences. The sequences of *Erysiphe macleayae* from Romania obtained in this work are indicated by a star. Bootstrap support values from 1000 pseudoreplicates are reported above nodes.

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