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NEW DISEASE REPORT

Rust disease on *Nymphoides peltata* caused by *Puccinia* sp. in Korea

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Nymphoides peltata (Asteraceae), commonly known as 'yellow floating heart', is an aquatic plant with buoyant leaves. It is native to East Asia and commonly cultivated in garden ponds and lakes for ornamental purposes. In other regions, for example in North America, *N. peltata* is an unwanted species, posing a high ecological risk due to its ability to densely cover water surface, shade submersed plants and reduce gas exchange (Plant Epidemiology and Risk Analysis Laboratory, 2012).

In June 2023, during surveys for candidate biological control agents for *Nymphoides*, typical aecial symptoms of rust disease were found on *N. peltata* leaves at the Kumho River in Daegu (35°53'10"N, 128°30'01"E) and Mankyong river in Wanju (35°54'21.4"N, 127°07'32.6"E) in Korea. Disease incidence was more than 90% (Figure 1). Symptoms included round, yellowish, discoloured spots on the upper surface of the leaves. Subsequently, these spots developed into yellow to orange aecia. A representative sample was deposited at the Kunsan National University Herbarium (acc. no. KSNUH2120), Gunsan, Korea.

The rust-infected leaves and the causal agent were examined under a dissecting microscope (M205C; Leica, Germany), a differential interference contrast microscope (Axio Imager 2; Carl Zeiss, Germany) and a scanning electron microscope (S-4800+EDS; Hitachi, Japan). Spermogonia were epiphyllous, subepidermal, centrally surrounded by



FIGURE 1 Rust symptoms (yellow chlorotic spots and aecia) caused by *Puccinia* sp. on the upper surface of *Nymphoides peltata* leaves.

aecia, bright yellow to brown, round, and 100–150 μm in diameter (Figure 2). Aecia were epiphyllous, densely grouped, yellow to orange, cup-shaped with peridia, and 150–300 μm in diameter (Figure 3).

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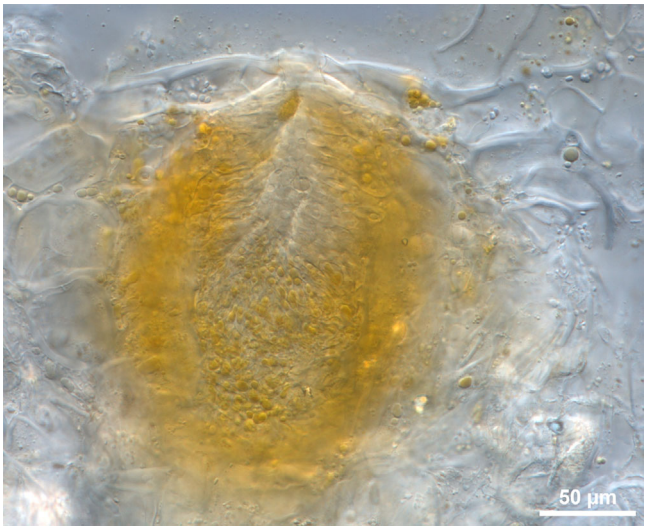


FIGURE 2 Cross-section of a spermogonium of *Puccinia* sp. on *Nymphoides peltata* observed under a differential interference contrast microscope.

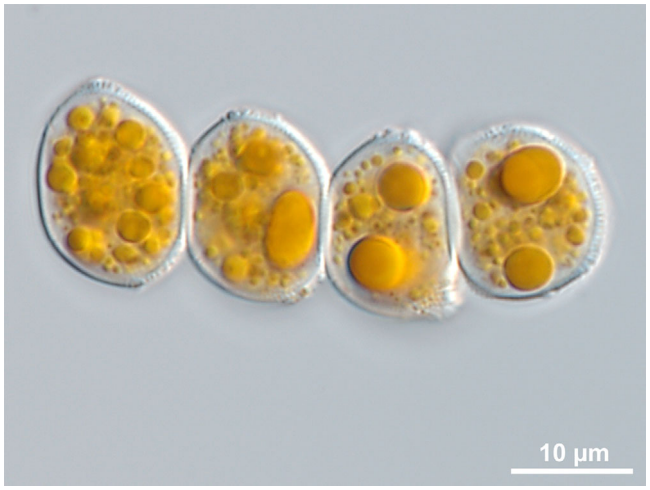


FIGURE 4 Aeciospores of *Puccinia* sp. on *Nymphoides peltata* observed under a differential interference contrast microscope.

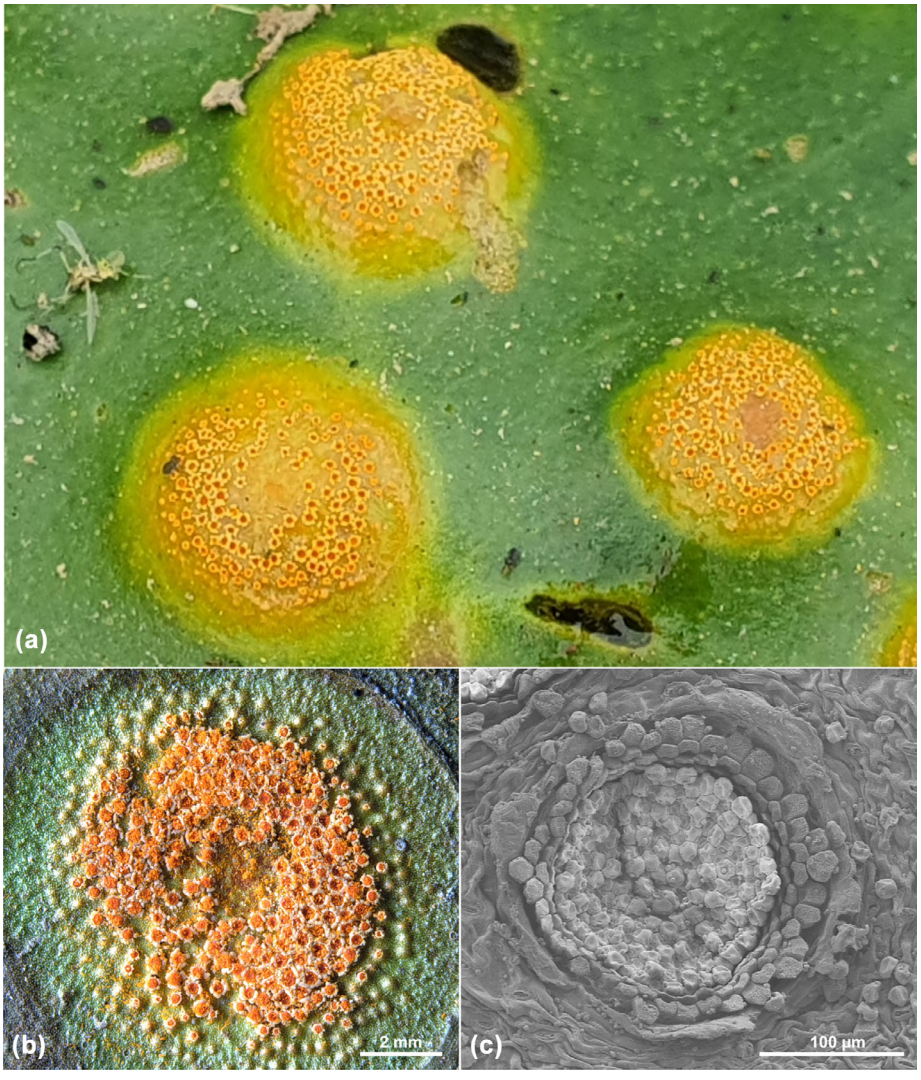


FIGURE 3 Aecia of *Puccinia* sp. on *Nymphoides peltata* observed in the field (a), under a dissecting microscope (b), and under a scanning electron microscope (c).

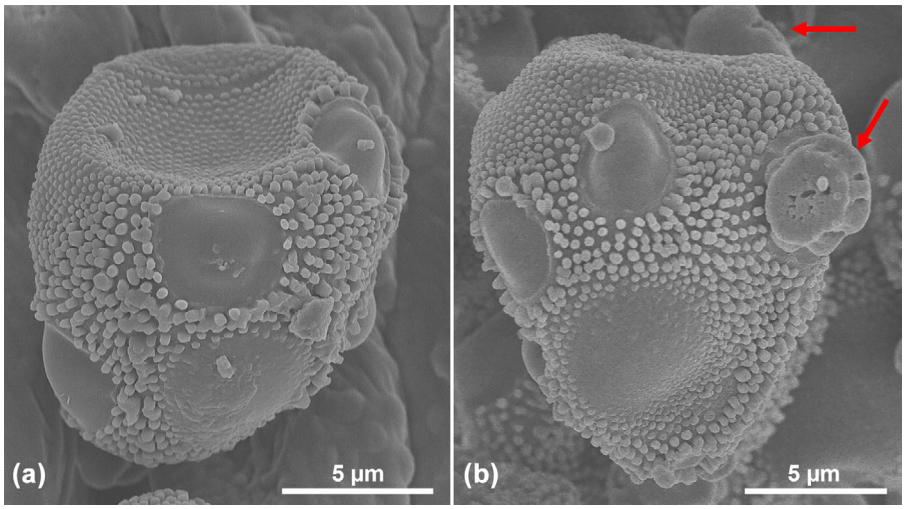


FIGURE 5 Aeciospore of *Puccinia* sp. on *Nymphoides peltata* observed under a scanning electron microscope, with verrucose wall ornamentation (a) and large granules (b; red arrows).

TABLE 1 The morphological characteristics of rust species previously reported on *Nymphoides* species. Some authors treat these as synonyms of *Puccinia scirpi*, however, morphological differences have been reported.

		Aeciospores		
Rust fungus	**Host plant**	**Colour**	**Size (μm)**	**Reference**
Puccinia sp.	*Nymphoides peltata*	yellow	15.0-21.3 × 13.0-17.6	This study
Dicaeoma scirpi (= *P. scirpi*)	*N. grayanum*	pale yellow or colourless	16-23	(Arthur & Kern, 1920)
P. scirpi	*N. indica*	yellow or hyaline	15.0-23.0 × 20.0-26.0	(Soares et al., 2006)
Aecidium limnanthemi (= *P. scirpi*)	*Limnanthemi nymphoidis* (= *N. peltata*)	hyaline	20-28	(Saccardo, 1888)
A. nymphoides (= *P. scirpi*)	*Limnanthemi nymphoidis* (= *N. peltata*)	yellow	12-20	(Saccardo, 1888)

The peridial cells were hyaline, rhomboidal with a verrucose, thick-walled and 18.8–34.5 × 15.1–21.6 μm. Aeciospores were yellowish, subglobose or suboblate and (15.0–)16.9–19.4(–21.3) × (13.0–)14.0–16.4(–17.6) μm (av. 18.2 × 15.2 μm) (Figure 4). The Aeciospore wall was verrucose with large granules and 0.5–1.2 μm thick (Figure 5). These morphological characteristics are similar to those of *Puccinia scirpi* found on *Nymphoides indica* in Brazil (Soares et al., 2006; Table 1).

To confirm the morphological identification, genomic DNA was extracted from one specimen (KSNUH2120) using a MagListo 5M plant Genomic DNA Extraction Kit (Bioneer; Daejeon, Korea). The internal transcribed spacer (ITS) and large subunit (LSU) regions of the rDNA, and the cytochrome oxidase 3 (*cox*3) mtDNA region were amplified and sequenced with primers ITS5u/ ITS4rust, LRust1R/LRust3, and CO3-F1/CO3-R1, respectively (Vialle et al., 2009). The resulting sequences were deposited in GenBank (Accession. Nos. OR821997, OR821998 and OR830028) and compared with other reference sequences using BLASTn analysis. The ITS and LSU sequences of the Korean specimen had ten and eight nucleotide differences, respectively, with those of *P. scirpi* on *N. indica* (KX999892). The *cox*3 sequence differed at two nucleotides with *P. scirpi* on *N. indica* (KX999929). In maximum-likelihood phylogenetic trees inferred from a concatenated alignment of ITS, LSU, and *cox*3 sequences (Figure 6), the Korean specimen formed a clade with the reference sequence of *P. scirpi*. However, due to poor support and multiple sequence differences, we report this rust fungus here as *Puccinia* sp. It is possible that it represents a new species or one of the *Puccinia* species that have no publicly available sequence data.

To our knowledge, this is the first report of *Puccinia* sp. on *Nymphoides peltata* in Korea. Given its high disease incidence, this pathogen could be a potential concern for *N. peltata* in Korea and further research is required to determine its identity.

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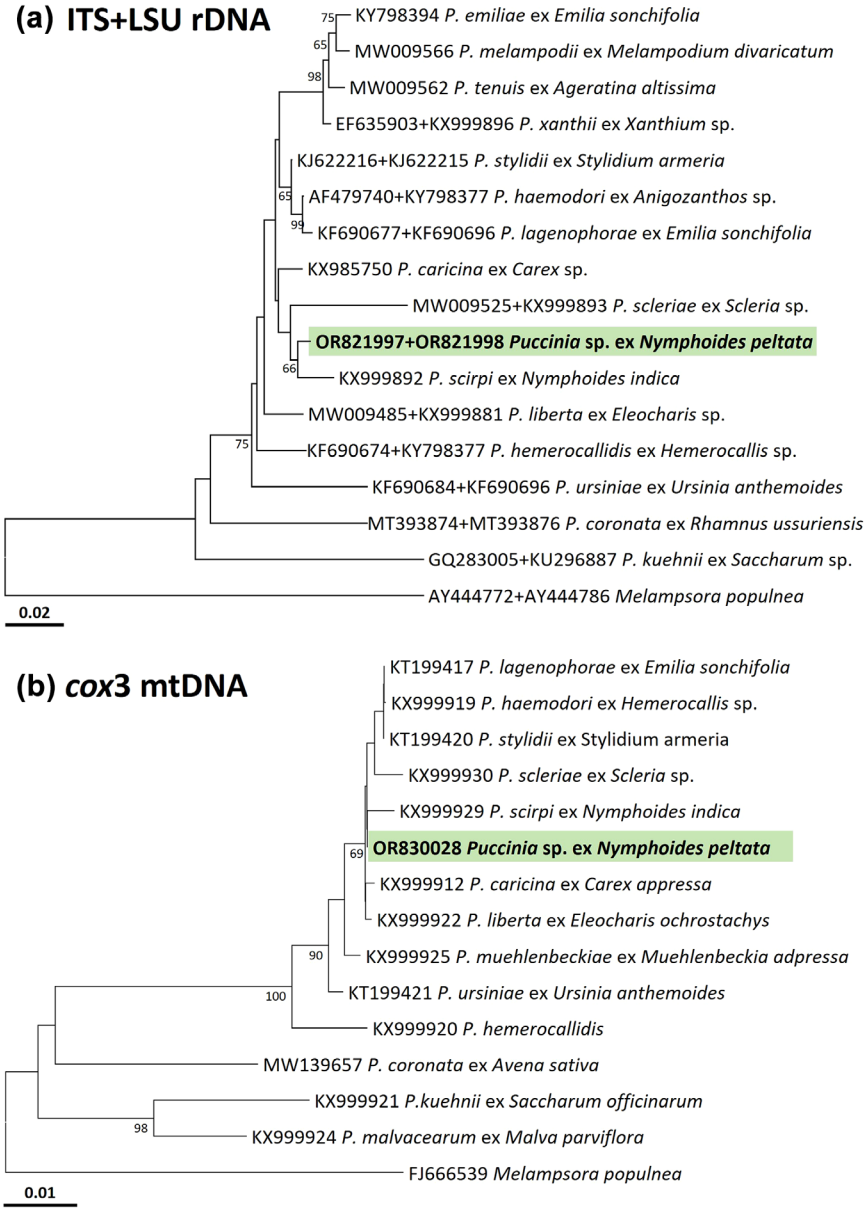


FIGURE 6 Minimum evolution phylogenetic trees between *Puccinia* sp. on *Nymphoides peltata* and related *Puccinia* species based on a concatenated alignment of ITS and LSU rDNA (a), and an alignment of *cox*3 mtDNA (b) sequences. Bootstrap values based on 1,000 replications are indicated at each branch. A Korean specimen is shown in a green box.

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