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Variability of *Puccinia komarovii* var. *komarovii* isolates from *Impatiens parviflora* DC. and its occurrence in the Drawa and Wigry National Parks in Poland

Anna Baturo-Cieśniewska [1*], Katarzyna Patejuk [2],
Agata Kaczmarek-Pieńczewska [2], Marta Jurga-Zotow [2]

[1]Department of Biology and Plant Protection, Laboratory of Molecular Mycology, Phytopathology and Entomology, Bydgoszcz University of Science and Technology, Al. Prof. S. Kaliskiego 7, 85-796 Bydgoszcz, Poland

[2]Department of Plant Protection, Division of Plant Pathology and Mycology, Wrocław University of Environmental and Life Sciences, Pl. Grunwaldzki 24a, 50-363 Wrocław, Poland

[*] To whom correspondence should be addressed. Email: baturo-a@pbs.edu.pl

Abstract

Small balsam (*Impatiens parviflora* DC.), an invasive plant native to central Asia, is currently one of the most extensive invasive plants in Central Europe, including Poland. Its expansion may affect populations of native species, such as touch-me-not balsam (*Impatiens noli-tangere*). It is particularly dangerous in legally protected areas, where the possibilities of eradicating it are very limited. There is some hope for stopping its expansion by using pathogens as a biological weapon. In this case, useful may be host-specific rust fungus *Puccinia komarovii* var. *komarovii*.

Our three-year observations of *I. parviflora* infection severity by *P. komarovii* var. *komarovii* carried out in Poland in Drawa National Park (DNP) and Wigry National Park (WNP) showed the higher incidence of rust on leaves in DNP, where the median blade infestation for two years was 51% of leaf coverage. In contrast, for three years in WNP, the median blade infestation amounted to only 25%. The most severe symptoms occurred during the summer months. However the percentage of leaf coverage by pathogen exhibited fluctuations depending on the year, implying an influence of environmental conditions that stabilized the infection each season.

Molecular analyses, in addition to confirming the infection of the tested plants by *P. komarovii* var. *komarovii*, enabled the characterization of its population based on comparative analysis of ITS and LSU sequences. Clear differences were found between the studied isolates and *P. komarovii* from *I. glanduliferae* and *I. brachycentra*.

There was no diversity among our isolates, but they differed from the sequences of Polish isolates originating from *I. parviflora* available in GenBank, which indicates some diversity of rust in a relatively small area. It probably results from sexual reproduction and may enable rapid adaptation of the pathogen to the region or host plant, or it may indicate certain "entry points" to Poland, where the isolates arrived from their native place of occurrence.

Keywords

Puccinia komarovii; *Impatiens parviflora*; invasive plant; internal transcribed spacer; large subunit

1. Introduction

Impatiens parviflora DC., commonly known as small balsam, is an invasive plant native to the mountains of central Asia. Intentionally introduced for botanical reasons, this species turned out to be one of Central Europe's most widespread invasive plants (Langmaier & Lapin, 2020; Tanner et al., 2015a). Despite the fact that the factors affecting its spread are still debatable and unclear, it was found that it is able to

penetrate into species-rich habitats, which makes it a potential threat to biodiversity (Adamowski & Bomanowska, 2016; Florianova & Münzbergova, 2018).

Most scientific reports concern the invasive species *Impatiens glandulifera* Royle, known as Himalayan balsam, which is invasive throughout Europe and can also seriously reduce native plant diversity (Ab Razak et al., 2023; Coakley & Petti, 2021; Tanner et al., 2015a; Tanner & Gange, 2020). It negatively affects whole ecosystems where the native biodiversity of invertebrates and microbial communities is disturbed (Coakley & Petti, 2021).

Much less is said about *Impatiens parviflora*, although it has been noted in 34 European countries, as reported already in 2008 by Lambdon et al. (2008). It has been observed at many sites in European temperate broadleaf forests (Langmaier & Lapin, 2020), such as the Czech Republic (Florianová & Münzbergová, 2017; Hejda, 2012) and Slovakia (Jarčuška et al., 2016) or Lithuania (Krokaitė et al., 2022).

The first localities of *I. parviflora* in Poland were recorded in 1850 in northern regions near Gdańsk (Krawiecowa, 1951), and since then, this species has spread very expansively (Piskorz & Klimko, 2006). It occurs, among others, in Polish legally protected areas such as Wigry National Park (Pusz et al., 2020), Kampinos National Park (Adamowski & Bomanowska, 2014), Wielkopolski National Park (Piskorz & Klimko, 2006, 2007), or Białowieża National Park with Białowieża Primeval Forest (Adamowski & Keczyński, 1998; Chmura & Sierka, 2007). However, it should be expected in the future that the rate of invasion of *I. parviflora* may decrease in our country as a result of occupation of almost the entire ecological niche.

The ability of small balsam to tolerate less favorable conditions under a dense tree canopy gives it an advantage over other species (Langmaier & Lapin, 2020; Lanta et al., 2022), although, as noted by Bobulská et al. (2016) *I. parviflora* can also develop in diverse conditions because sunlight and soil moisture do not have a significant impact on its population. It may, therefore, be a threat to the *Impatiens noli-tangere*, which is more sensitive to conditions and native to Europe, which was noted in Belgium (Godefroid & Koedam, 2010; Vervoort & Jacquemart, 2012). Glushakova et al. (2015) found that *I. parviflora* leads to a serious decrease in biodiversity of the plant species. It also influences the structure of soil microbiocenoses (Chmura & Gucwa-Przepióra, 2012).

Mechanical control of small balsam is tedious (Adamowski & Bomanowska, 2016). There is some hope for stopping the expansion of invasive *Impatiens* by using pathogens or pests that limit their populations. A possible biological weapon against small balsam may be rust fungus *Puccinia komarovii* Tranzschel ex P. Syd. & Syd. (Piskorz & Klimko, 2006; Tanner et al. 2015b; Rolnikuj.pl, 2017) which has been a parasite of *I. parviflora* and *Impatiens amphorata* in Central Asia and the northern Himalayas. It probably came to Europe with *I. parviflora* seeds in the early 20th century. In Poland, where it is considered a common species, this rust was reported for the first time in the area of Silesia and Beskidy in 1934, while in other European countries is treated as invasive (Majewski, 1979; Piskorz & Klimko, 2006). According to the observations of Tanner et al. (2015b), in Europe, it infects *I. parviflora*

mainly in wooded habitats, and the percentage of infected plants may reach even 90% (Piskorz & Klimko, 2006).

Puccinia komarovii is an autoecious rust and had been known to be host-specific rust, infecting only *Impatiens parviflora* in both its native and European ranges, and was described originally from this *Impatiens* species (Piskorz & Klimko, 2006; Tanner et al., 2015a). It turned out that *I. glandulifera* can also be its host (Tanner et al., 2015b). However, there is host specificity. Hence, isolates infecting *I. parviflora* are referred to as *P. komarovii* var. *komarovii*, unlike *P. komarovii* var. *glanduliferae* inhabiting *I. glandulifera* (Tanner et al., 2015a).

Due to the above facts, observations of infection severity of *I. parviflora* by *P. komarovii* var. *komarovii* in two Polish national parks - Drawa National Park (DNP) and Wigry National Park (WNP) were carried out. Also, molecular analyses of pathogen isolates were performed to confirm the species identification of the rust fungus, diagnosed on the basis of morphology and symptoms on small balsam as *P. komarovii* var. *komarovii* and preliminary characterization of the population of this fungus based on comparative analysis of rDNA region sequences.

2. Material and methods

2.1. Field research

The subjects of this investigation comprised *I. parviflora* specimens grown within Drawa National Park (DNP), situated in northwestern Poland, at the confluence of Greater Poland, Lubusz, and West Pomeranian Voivodeships and specimens from Wigry National Park (WNP), which is situated in the northeastern region of Poland within Podlaskie Voivodeship (Figure 1). At both study locations, 20 plots measuring 10×10 m were selected within mixed forests (Table 1) characterized by dense *I. parviflora* coverage. From each of these locations, 10 plants were chosen for observation, and the dynamics of rust occurrence were monitored at 4-week intervals, commencing from May to June, depending on the growth rate of *I. parviflora*, and continuing until September. These observations spanned three plant vegetation seasons (2020–2022) in WPN and two seasons (2022–2023) in DNP. Monthly assessments of the percentage of leaf coverage affected by etiological symptoms of *Puccinia komarovii* var. *komarovii* were conducted based on an average of 10 plants per plot. Descriptive statistics and graphical representations depicting the dynamics of rust occurrence were generated using Tableau (2020.2.4 Professional Edition).

2.2. Molecular analyses

The research material for molecular analyses were leaves of *I. parviflora* with clear disease symptoms indicating infection with the fungus *P. komarovii*. Material was taken in August because, during this period, the most pronounced symptoms caused by uredinia and telia were observed. Leaves were collected from 14 small balsam sites in 2022, from 11 sites in 2023 in DNP, and from six sites in 2022 in WNP. In this way, 31 samples were obtained, from which 31 DNA samples were obtained for analyses of rDNA regions of *P. komarovii* var. *komarovii* (Table 2).
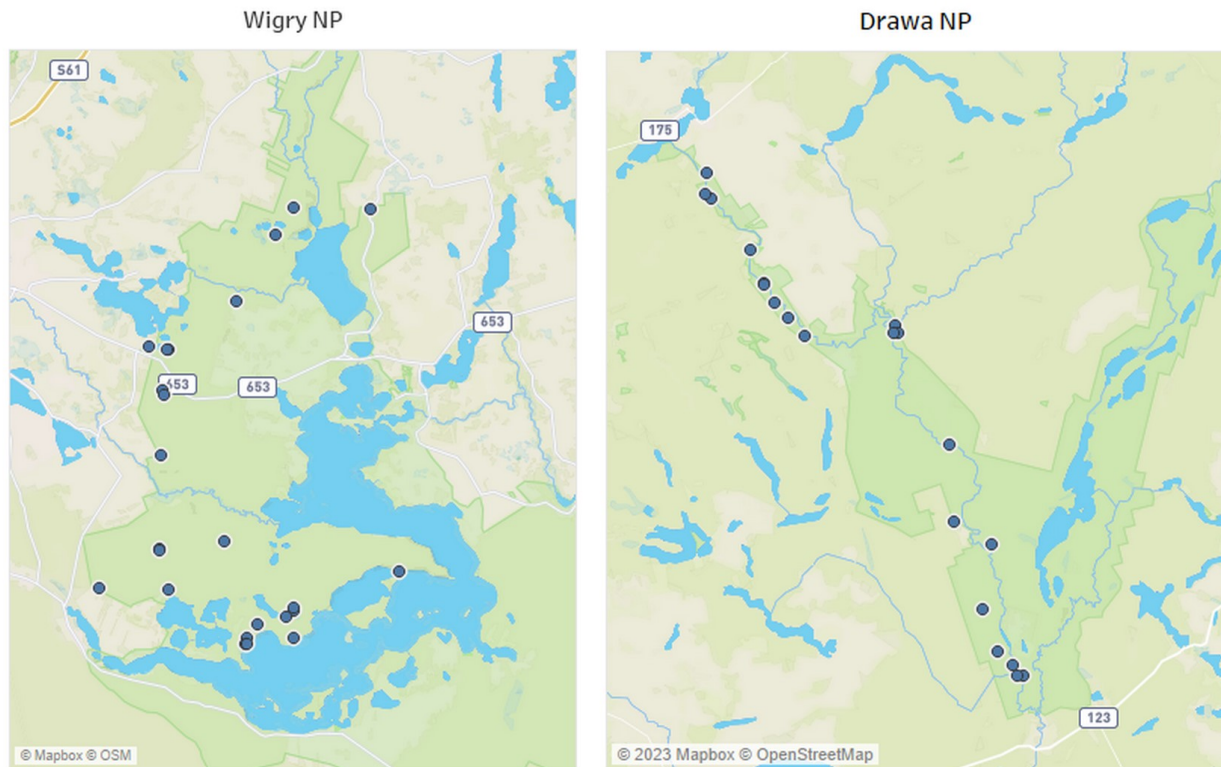
Figure 1 Locations of sample collection in Wigry and Drawa National Parks.

2.3. DNA extraction and PCR assays

Leaf fragments with disease symptoms were freeze-dried in a CoolSafe device (ScanVac, Denmark). From symptomatic leaves collected at 31 sites, small fragments measuring 25–49 mm^2 with numerous clusters of spores were cut out and homogenized to fine powder with quartz beads in a MagnaLyser homogenizer (Roche, Switzerland). DNA extracted from forty-gram samples using the Bead-Beat Micro AX Gravity kit (A&A Biotechnology) was measured fluorometrically on a Quantus device (Promega, USA) and diluted to 10 ng·μL^{-1} in ddH$_2$O for further analyses.

Amplifications of the ITS (*internal transcribed spacer*) and 28S (LSU - *large subunit*) of rDNA were done in 37.5 μL w an Eppendorf EP Mastercycler (Eppendorf, Germany). The final concentration of reagents was as follows: 1× PCR Mix Plus (A&A Biotechnology, Poland), DNA of individual samples at a concentration of 4 ng, and rust-specific primers, respectively ITS5-u/ITS4-u (Pfunder & Schürch, 2001) and LRust1R/LR6 (Beenken et al., 2012; White et al., 1990), each at a concentration of 0.8 pM.

The PCR began with an initial denaturation step of 5 min at 95 °C, followed by 35 cycles of 95 °C for 1 min, an annealing temperature of 53 °C for 50 s and 72 °C for 50 s (ITS) or 90 s (LSU), followed by 10 min at 72 °C for a final extension.

The presence of amplicons was verified by electrophoretic separation of 2 μL of the postreaction mixture in tris-borate-EDTA (TBE) buffer on a 1.2% agarose gel (Pronadisa, Spain) stained with SimplySafe (EUR$_X$, Poland) and visualized using the INTAS set (Germany) consisting of a transilluminator, camera and computer with software.

2.4. Sequencing of PCR products and data analysis

Amplification products were purified and sequenced by Genomed (Poland). Both strands were sequenced. FinchTV 1.4 (Geospiza) was used to analyze the obtained sequences.

Alignments were carried out using ClustalW through the Molecular Evolutionary Genetics Analysis Version 11 (MEGA11) Toolbar (Tamura et al., 2021). For species identification based on ITS and LSU sequences, the *Basic Local Alignment Search Tool* (BLAST) in the NCBI (The National Center for Biotechnology Information) database was used (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome).

The sequences of the analyzed isolates were compared with the *P. komarovii* sequences available in GenBank NCBI. In the case of the ITS regions, there were 16 sequences of isolates from *I. parviflora* (seven Polish and nine Chinese), 91 Indian sequences from *I. brachycentra* and *I. glandulifera* (respectively seven and 84) and eight Pakistani ones from *Impatiens* sp.

For comparative analyses of the LSU region, 20 sequences of isolates from *I. parviflora* (one from the UK, 12 - from Hungary, seven- from Poland), seven from *I. brachycentra* (all from India), seven from *I. glandulifera* (all from India) and seven from *Impatiens* sp. (Pakistan) were taken.

Dendrograms were constructed to visualize the genetic diversity between the 31 *P. komarovii* var. *komarovii* isolates tested and the sequences of 44 or 41 *P. komarovii* isolates for ITS and LSU, respectively, from GenBank NCBI. For technical reasons, the number of Indian sequences of the ITS region

Table 1 Characteristics of sample locations.

Park	Spot	Latitude	Longitude	Altitude
DPN	imp1	53.030808	15.943017	44
DPN	imp2	53.030569	15.946634	55
DPN	imp3	53.034328	15.940096	78
DPN	imp4	53.038949	15.931640	127
DPN	imp5	53.052938	15.923821	89
DPN	imp6	53.082094	15.907726	62
DPN	imp7	53.074817	15.929012	55
DPN	imp8	53.145335	15.874101	81
DPN	imp9	53.145382	15.876771	92
DPN	imp10	53.147957	15.875093	68
DPN	imp11	53.198473	15.770029	98
DPN	imp12	53.191505	15.768883	84
DPN	imp13	53.190218	15.772351	95
DPN	imp14	53.172944	15.794380	106
DPN	imp15	53.161211	15.801845	102
DPN	imp16	53.161561	15.802129	66
DPN	imp17	53.155033	15.808092	114
DPN	imp18	53.150353	15.815414	116
DPN	imp19	53.144262	15.824962	92
DPN	imp20	53.107942	15.905081	73
WPN	Imp1	54.085482	23.011387	118
WPN	Imp2	54.074747	23.017392	154
WPN	Imp3	54.075605	23.016813	118
WPN	Imp4	54.031918	22.992823	99
WPN	Imp5	54.061473	23.016067	151
WPN	Imp6	54.040413	23.015180	115
WPN	Imp7	54.040600	23.015155	161
WPN	Imp8	54.042395	23.039790	117
WPN	Imp9	54.031488	23.018655	98
WPN	Imp10	54.023907	23.052587	112
WPN	Imp11	54.025770	23.062945	102
WPN	Imp12	54.027507	23.065835	107
WPN	Imp13	54.026848	23.065927	113
WPN	Imp14	54.020893	23.065773	124
WPN	Imp15	54.019693	23.048608	102
WPN	Imp16	54.019600	23.048107	98
WPN	Imp17	54.020872	23.048547	98
WPN	Imp18	54.119767	23.07865	145
WPN	Imp19	54.115747	23.095185	106
WPN	Imp20	54.084697	23.017995	133

DPN - Drawa National Park; WNP - Wigry National Park.

obtained from *I. glandulifera* was limited to 13 differentiated representatives.

The dendrograms were constructed in MEGA11 using the Maximum Likelihood algorithm. The Tamura 3-parameter model was applied (Tamura, 1992).

3. Results

3.1. Field research

During field studies, the highest incidence of rust infection on *I. parviflora* leaves was recorded in DNP, where the

median blade infestation for 2022–2023 was 51% of leaf coverage. In contrast, after three years of observation in WNP, the median blade infestation amounted to only 25%. These findings are supported by the yearly average infection rate of *I. parviflora* in DNP, where the median yearly infection for 2022 was 21%, increasing to 68% in 2023. Notably, in 2022, spot imp3 in DNP showed the highest median rust infection (50%), while most other locations fell within the interquartile range of 13–40%. In 2023, DNP experienced the highest leaf blade coverage of rust in any observation year, with the interquartile range doubling from the previous year, ranging from 55% to 87%. Spot imp6, in particular, consistently exhibited a median 100% coverage of rust throughout the entire year.

In WNP, the median yearly infection never exceeded 50% and, for the years 2020–2023, constituted 14%, 34%, and 16%, respectively. The highest yearly median rust infection in WNP was observed in 2022 at spots imp2 (68%) and imp17 (66%). Infection ranges in 2020 and 2022 in WNP were similar, with interquartile ranges falling between 8–36% in 2020 and 8–43% in 2022. The peak infection in WNP was observed in 2021, with Q1 starting at 22% and Q3 finishing at 44%. This is shown in graphs (Figure 2).

Field studies indicated that the most severe symptoms caused by *P. komarovii* var. *komarovii* on *I. parviflora* leaves occurred during the summer months. However, the peak of rust presence varied between years. In WNP, the median peak of rust presence was observed in September 2020 at 50%, July 2021 at 30%, and September 2022 at 25%. In DNP, the maximum rust presence was observed in July 2022 at 20% and in August 2023 at 100%. In some growing seasons, premature death of *I. parviflora* was observed, with entire plant populations in certain locations perishing by September. Such instances were documented in both WNP in 2021 and DNP in 2023.

3.2. Molecular analyses

Comparative analysis of the ITS and LSU sequences of the tested samples clearly showed the presence of the rust fungus *P. komarovii* var. *komarovii* on the leaves of *I. parviflora* collected in 31 locations. The sequences of the fungal isolates found in individual samples were deposited in GenBank NCBI. Their accession numbers are listed in Table 2. Access to the sequences in the GenBank database is available at https://www.ncbi.nlm.nih.gov/nucleotide/.

The ITS sequences of the 31 *P. komarovii* var. *komarovii* isolates obtained over two years from the DPN and WPN did not differ from each other. Comparative analysis with sequences from *I. parviflora* available in GenBank NCBI showed that all our sequences showed 100% identity with the sequences of seven isolates - three from central Poland with accession numbers KC430771 KC430772 and KC430775 and four sequences KC430842, KC430839 KC430838 and KC4 30837 from China. One nucleotide distinguished them from the Polish sequences KC430769, KC430773 and KC430774 and the Chinese KC430840, which meant an identity level of 99.84%. They differed by two nucleotides from KC430841, KC430843, and KC430844 from China, and the Polish KC430770 (identity 99.69%), and by four from the Chinese KC430845 (identity 99.37%).

Comparing our sequences with seven sequences obtained from *Impatiens brachycentra* (spurless balsam), it was found that they differed from single isolates by one (KC430763), three (KC430759) or four nucleotides (KC430758), which corresponded to an identity level of 99.84, 99.53 and 99.37%, respectively. Our isolates differed much more from the remaining four isolates obtained from this species of *Impatiens*, i.e., by 8–10 nucleotides, with an identity level of 98.76–98.29%.

The similarity of *P. komarovii* sequences obtained from *I. glandulifera* to the ITS sequences of our isolates was even lower. The sequences differed by at least six nucleotides and by a maximum of 14 nucleotides, which resulted in an identity of 99.07–97.83%.

However, the greatest differences were found in Pakistani *P. komarovii* isolates from the unknown *Impatiens* species. Our isolates were similar to them only by approximately 88%.

Dendrograms based on ITS region sequences show relationships between our *P. komarovii* isolates and GenBank NCBI isolates from *I. parviflora* (Figure 3) and between our isolates and *P. komarovii* isolates from *Impatiens* species other than *I. parviflora* (Figure 4).

Also, the sequences of our 31 isolates encoding LSU did not differ from each other. However, they did not show 100% identity with any Polish isolate of *I. parviflora* from Greater Poland Voivodeships available in GenBank NCBI. They were identical to the sequences of the KC460259 isolate from Hungary and LC494104 from the United Kingdom.

The tested isolates differed from the sequences of the Polish isolate KC460243 and the Hungarian KC460260 by one nucleotide, which was equivalent to a degree of identity of 99.91%, or by two nucleotides in KC460254 and KC460256 from Hungary and KC460239 from Poland, with a degree of identity of 99.81%. In the case of the remaining Polish sequences in GenBank, the diversity was greater and concerned three (KC460240) to seven (KC460244) nucleotides, and in the case of the remaining Hungarian sequences ranged from three (KC466553 and KC460255) to ten (KC460263) nucleotides. Therefore, for the LSU region, greater diversity between Polish isolates was found in the ITS regions.

Comparisons of our LSU sequences with *P. komarovii* isolates derived from other species *Impatiens* than *I. parviflora* showed a large range of diversity. Our isolates differed by two nucleotides from only one sequence of *P. komarovii* (KC460250) originating from the Indian *I. glandulifera*. From the remaining six sequences derived from this Impatiens species, available in GenBank - by four or even seven nucleotides. Comparing our isolates with sequences of Indian isolates derived from *I. brachycentra*, the differences were even greater and ranged from four to 12 nucleotides. Pakistani isolates originating from *Impatiens* sp. differed from our isolates by three nucleotides and even 14 ones (identity 98.71%).

The relationships between the LSU sequences described above are graphically presented in dendrograms, which, similarly to the ITS sequences, show relationships between our *P. komarovii* isolates and *I. parviflora* isolates from GenBank NCBI (Figure 5) and between our isolates and *P. komarovii* isolate derived from *Impatiens* species other than *I. parviflora* (Figure 6).
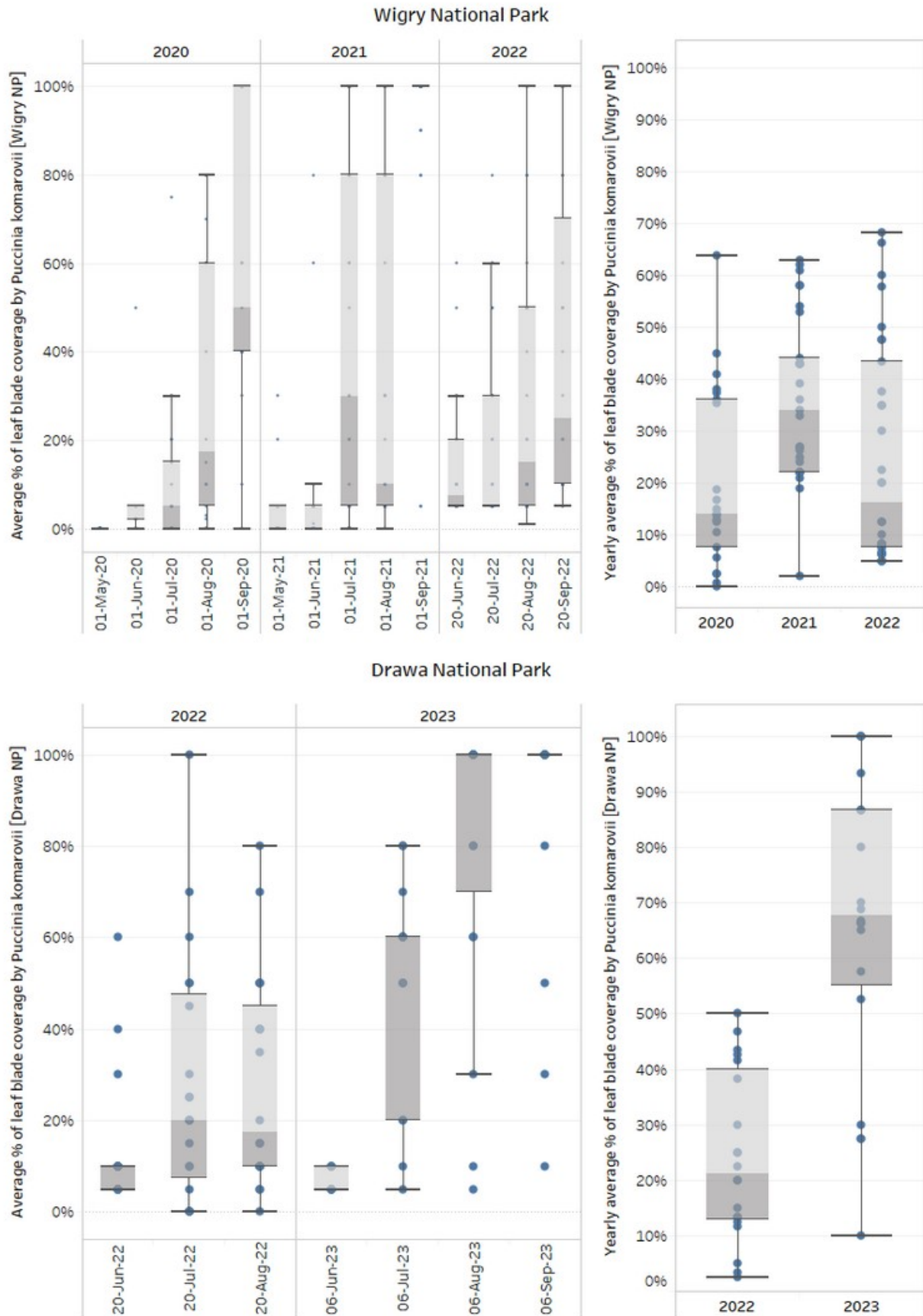
Figure 2 Monthly and yearly average of *I. parviflora* leaf blades' coverage by *Puccinia komarovii* var. *komarovii* in Wigry and Drawa National Parks in 2020–2023.

Table 2 A list of 31 *P. komarovii* var. *komarovii* isolates originated from *Impatiens parviflora* in Wigry National Park (WNP) in 2022 and in Drawa National Park (DNP) in 2022 and 2023 with accession numbers of their ITS and LSU sequences deposited in GenBank NCBI.

Code of *P. komarovii* isolate	ITS GenBank accession No	LSU GenBank accession No
Pk22DPN1	OP682901	OP683207
Pk22DPN8	OP683196	OP683206
Pk22DPN10	OP683195	OP683209
Pk22DPN11	OP682899	OP687971
Pk22DPN16	OP682929	OP683224
Pk22DPN19	OP683198	OP687973
Pk22DPN20	OP683201	OP687974
Pk22DPNx1	OP682904	OP683204
Pk22DPNx2	OP682923	OP683223
Pk22DPNx3	OP683193	OP683235
Pk22DPNx4	OP683202	OP683216
Pk22DPNx5	OP682900	OP683203
Pk22DPNx6	OP683197	OP683236
Pk22WPN2	OP683194	OP687975
Pk22WPN3	OP682902	OP683234
Pk22WPN4	OP682928	OP683213
Pk22WPN5	OP682927	OP687958
Pk22WPN11	OP687976	OP683222
Pk22WPN14	OP687970	OP683208
Pk22WPN22	OP687972	OP683205
Pk23DPN1	OR475551	OR473652
Pk23DPN2	OR475552	OR473633
Pk23DPN3	OR475553	OR473641
Pk23DPN4	OR475554	OR473642
Pk23DPN5	OR475555	OR473649
Pk23DPN10	OR475556	OR473650
Pk23DPN11	OR475557	OR475550
Pk23DPN12	OR475558	OR473651
Pk23DPN15	OR475559	OR475549
Pk23DPN18	OR475560	OR473654
Pk23DPN20	OR475561	OR473655

4. Discussion

Most scientific reports concern the invasive species *Impatiens glandulifera* (Ab Razak et al., 2023; Coakley & Petti, 2021; Tanner et al., 2015a), which is infected by *P. komarovii* var. *glanduliferae*. This rust fungus causes significant damage of infected plants at the seedling stage (that usually leads to plant death) and leaves of maturing plants (Varia et al., 2016).

Evans and Ellison (2005) emphasize that understanding the biology of a pathogen that may be a potential biological control agent before its large-scale introduction into an ecosystem is an essential element of any weed biocontrol program. It is equally important to prove its specificity for the unwanted, problematic plant species that is our target (Barton, 2012). According to Tanner et al. (2015a), in the *P. komarovii* population, there are several pathotypes specific to a particular plant species. *I. glandulifera* shows immunity to inoculations with *P. komarovii* obtained from *I. parviflora* and vice versa. Therefore, host specificity predisposes *P. komarovii* var. *glanduliferae* to the role of fungal agent for the biological control of Himalayan balsam (Tanner et al., 2015a). For this reason, this rust fungus has been introduced into the UK for biological control of this weed, where was the subject of an intensive biological control research program (Currie et al., 2020; Varia et al., 2016). Ellison et al. (2020) revealed the first field results that clearly showed the potential for successful biological control of *Impatiens glandulifera*.

Similar hopes are associated with *P. komarovii* var. *komarovii* in relation to *I. parviflora* (Pusz et al., 2020). Already 17 years ago, Piskorz and Klimko (2006) reported on the rapid reproduction of *Impatiens* in Poland and other Central European countries. This is facilitated by its high adaptabil-
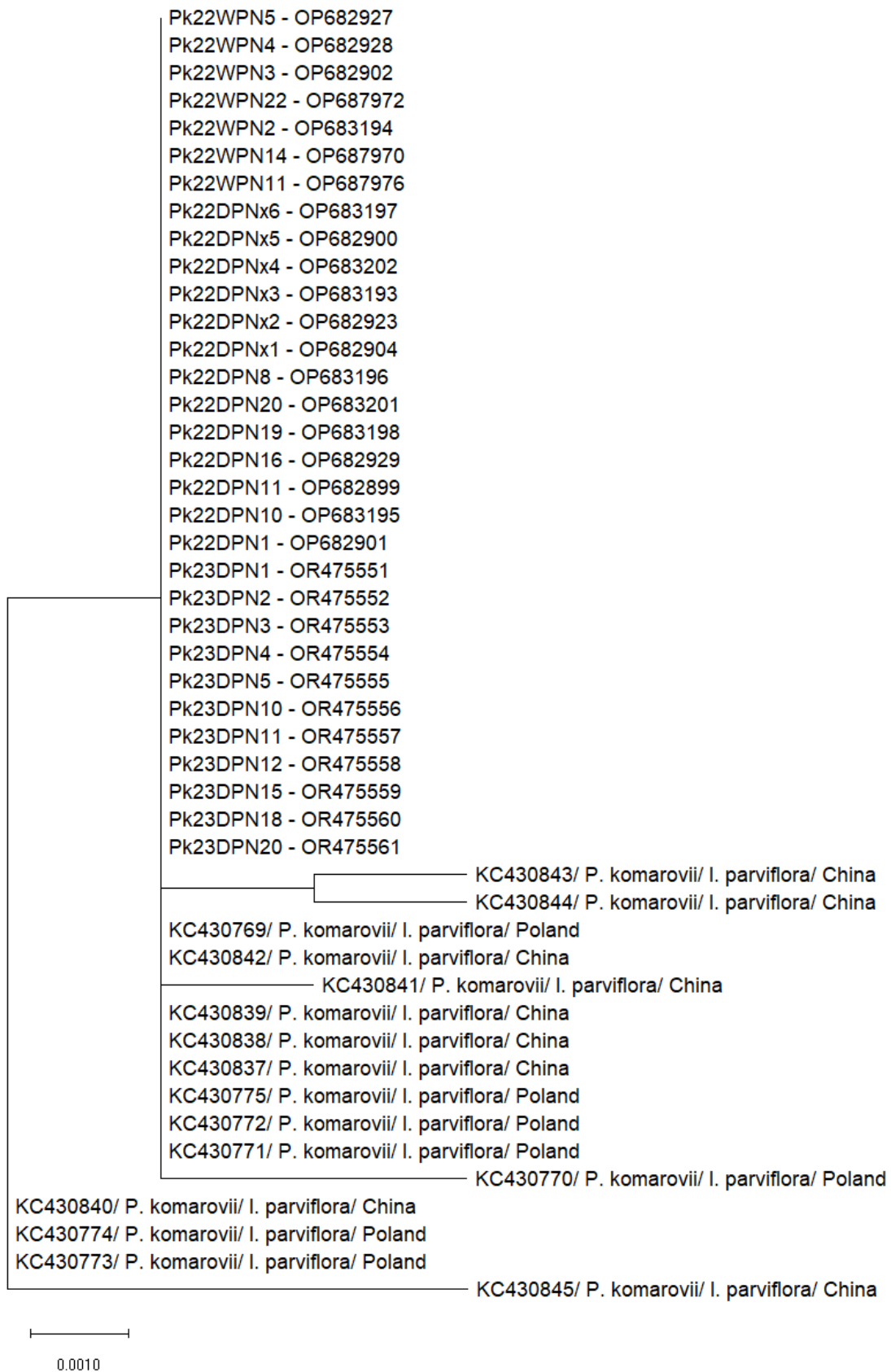
Figure 3 Dendrogram based on ITS sequences showing the relationships between the studied 31 *Puccinia komarovii* var. *komarovii* isolates and 16 *P. komarovii* var. *komarovii* isolates from GenBank NCBI derived from *I. parviflora*.

Pk22WPN5 - OP682927
Pk22WPN4 - OP682928
Pk22WPN3 - OP682902
Pk22WPN22 - OP687972
Pk22WPN2 - OP683194
Pk22WPN14 - OP687970
Pk22WPN11 - OP687976
Pk22DPNx6 - OP683197
Pk22DPNx5 - OP682900
Pk22DPNx4 - OP683202
Pk22DPNx3 - OP683193
Pk22DPNx2 - OP682923
Pk22DPNx1 - OP682904
Pk22DPN8 - OP683196
Pk22DPN20 - OP683201
Pk22DPN19 - OP683198
Pk22DPN16 - OP682929
Pk22DPN11 - OP682899
Pk22DPN10 - OP683195
Pk22DPN1 - OP682901
Pk23DPN1 - OR475551
Pk23DPN2 - OR475552
Pk23DPN3 - OR475553
Pk23DPN4 - OR475554
Pk23DPN5 - OR475555
Pk23DPN10 - OR475556
Pk23DPN11 - OR475557
Pk23DPN12 - OR475558
Pk23DPN15 - OR475559
Pk23DPN18 - OR475560
Pk23DPN20 - OR475561
KC430763/ P. komarovii/ I. brachycentra/ India
KC430759/ P. komarovii/ I. brachycentra/ India
KC430758/ P. komarovii/ I. brachycentra/ India
KC430805/ P. komarovii/ I. glandulifera/ India
KC430826/ P. komarovii/ I. glandulifera/ India
KC430785/ P. komarovii/ I. glandulifera/ India
KC430751/ P. komarovii/ I. glandulifera/ India
KC430765/ P. komarovii/ I. brachycentra/ India
KC430761/ P. komarovii/ I. brachycentra/ India
KC430810/ P. komarovii/ I. glandulifera/ India
KC430779/ P. komarovii/ I. glandulifera/ India
KC430768/ P. komarovii/ I. brachycentra/ India
KC430834/ P. komarovii/ I. glandulifera/ India
KC430827/ P. komarovii/ I. glandulifera/ India
KC430819/ P. komarovii/ I. glandulifera/ India
KC430807/ P. komarovii/ I. glandulifera/ India
KC430767/ P. komarovii/ I. brachycentra/ India
KC430832/ P. komarovii/ I. glandulifera/ India
KC430817/ P. komarovii/ I. glandulifera/ India
KC430824/ P. komarovii/ I. glandulifera/ India
KC430744/ P. komarovii/ Impatiens sp./ Pakistan
KC430742/ P. komarovii/ Impatiens sp./ Pakistan
KC430748/ P. komarovii/ Impatiens sp./ Pakistan
KC430746/ P. komarovii/ Impatiens sp./ Pakistan
KC430747/ P. komarovii/ Impatiens sp./ Pakistan
KC430745/ P. komarovii/ Impatiens sp./ Pakistan
KC430743/ P. komarovii/ Impatiens sp./ Pakistan
KC430741/ P. komarovii/ Impatiens sp./ Pakistan

0.01

Figure 4 Dendrogram based on ITS sequences showing the relationships between the studied 31 *Puccinia komarovii* var. *komarovii* isolates and 28 *P. komarovii* isolates from *Impatiens* species other than *I. parviflora*.
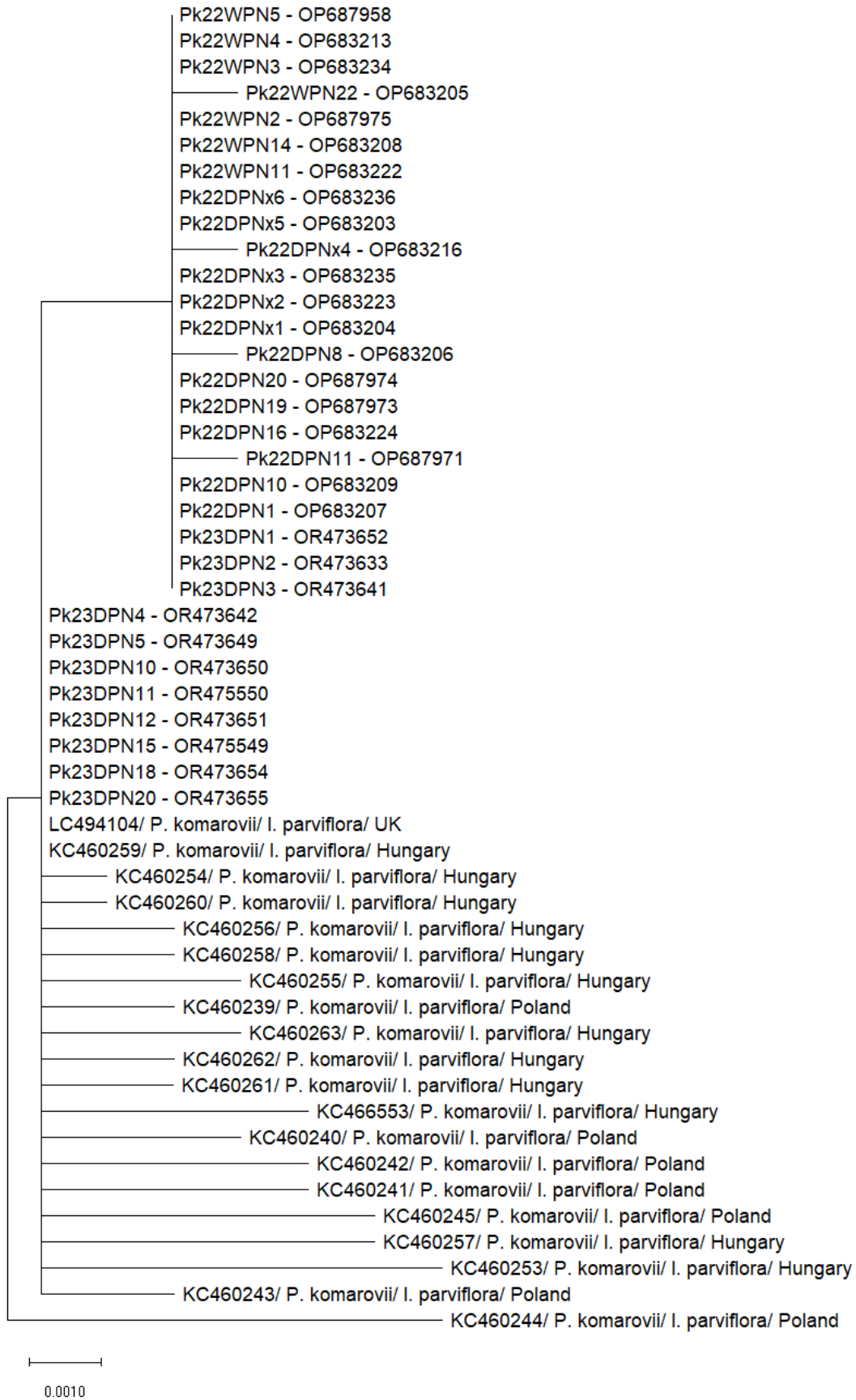
Pk22WPN5 - OP687958
Pk22WPN4 - OP683213
Pk22WPN3 - OP683234
Pk22WPN22 - OP683205
Pk22WPN2 - OP687975
Pk22WPN14 - OP683208
Pk22WPN11 - OP683222
Pk22DPNx6 - OP683236
Pk22DPNx5 - OP683203
Pk22DPNx4 - OP683216
Pk22DPNx3 - OP683235
Pk22DPNx2 - OP683223
Pk22DPNx1 - OP683204
Pk22DPN8 - OP683206
Pk22DPN20 - OP687974
Pk22DPN19 - OP687973
Pk22DPN16 - OP683224
Pk22DPN11 - OP687971
Pk22DPN10 - OP683209
Pk22DPN1 - OP683207
Pk23DPN1 - OR473652
Pk23DPN2 - OR473633
Pk23DPN3 - OR473641
Pk23DPN4 - OR473642
Pk23DPN5 - OR473649
Pk23DPN10 - OR473650
Pk23DPN11 - OR475550
Pk23DPN12 - OR473651
Pk23DPN15 - OR475549
Pk23DPN18 - OR473654
Pk23DPN20 - OR473655
LC494104/ P. komarovii/ I. parviflora/ UK
KC460259/ P. komarovii/ I. parviflora/ Hungary
KC460254/ P. komarovii/ I. parviflora/ Hungary
KC460260/ P. komarovii/ I. parviflora/ Hungary
KC460256/ P. komarovii/ I. parviflora/ Hungary
KC460258/ P. komarovii/ I. parviflora/ Hungary
KC460255/ P. komarovii/ I. parviflora/ Hungary
KC460239/ P. komarovii/ I. parviflora/ Poland
KC460263/ P. komarovii/ I. parviflora/ Hungary
KC460262/ P. komarovii/ I. parviflora/ Hungary
KC460261/ P. komarovii/ I. parviflora/ Hungary
KC466553/ P. komarovii/ I. parviflora/ Hungary
KC460240/ P. komarovii/ I. parviflora/ Poland
KC460242/ P. komarovii/ I. parviflora/ Poland
KC460241/ P. komarovii/ I. parviflora/ Poland
KC460245/ P. komarovii/ I. parviflora/ Poland
KC460257/ P. komarovii/ I. parviflora/ Hungary
KC460253/ P. komarovii/ I. parviflora/ Hungary
KC460243/ P. komarovii/ I. parviflora/ Poland
KC460244/ P. komarovii/ I. parviflora/ Poland

0.0010

Figure 5 Dendrogram based on LSU sequences showing the relationships between the studied 31 *Puccinia komarovii* var. *komarovii* isolates and 20 *P. komarovii* var. *komarovii* isolates from GenBank NCBI derived from *I. parviflora*.
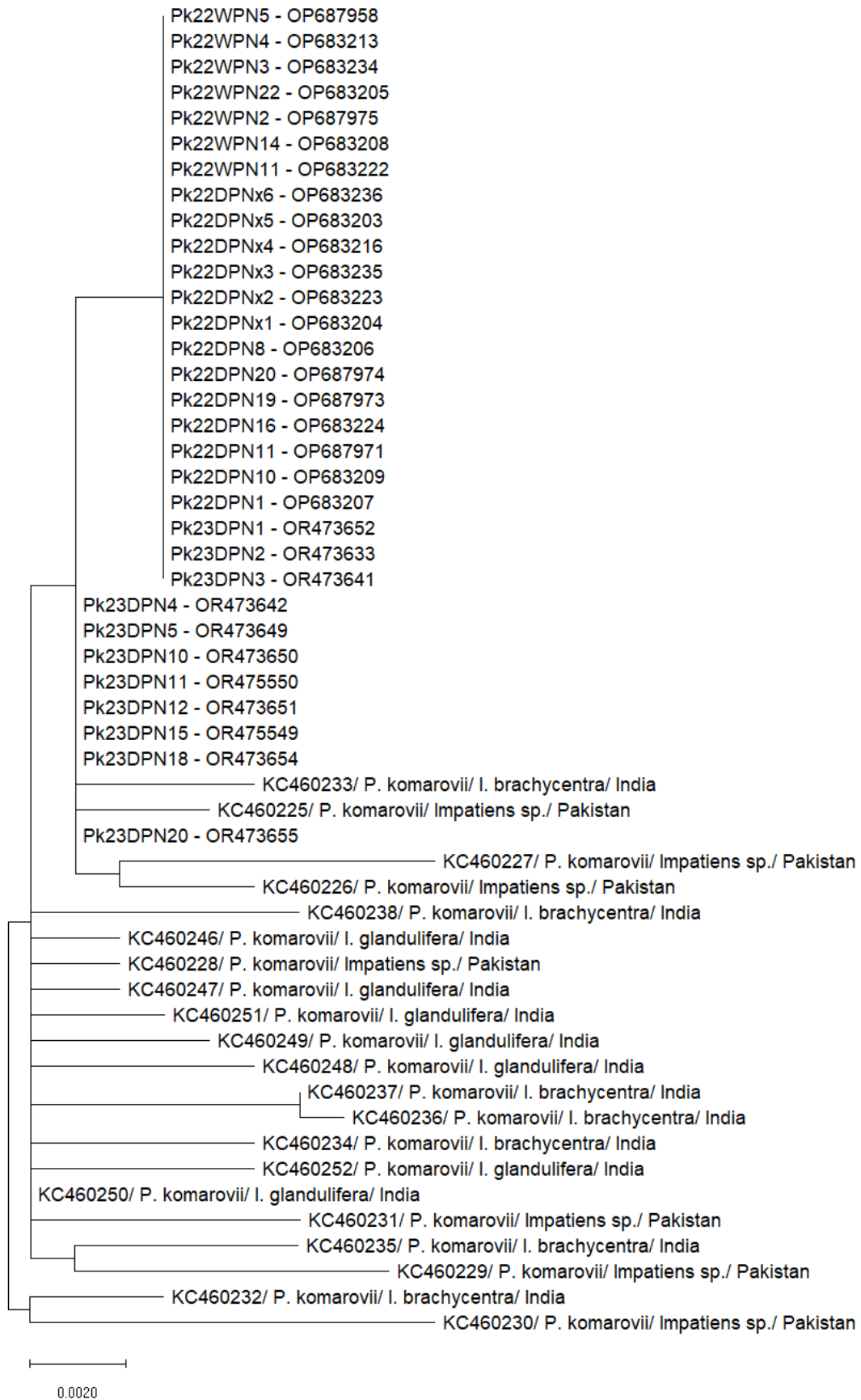
Figure 6 Dendrogram based on LSU sequences showing the relationships between the studied 31 *Puccinia komarovii* isolates and 21 *P. komarovii* isolates from *Impatiens* species other than *I. parviflora*.

ity to temperature, humidity, soil reaction, and nutrients (Reczyńska et al., 2015). Observations carried out in the Czech Republic by Florianová and Münzbergová (2018) showed an almost twofold increase in the intensity of the occurrence of *I. parviflora* on the observed plots in the period 2011–2016. These studies confirmed that *I. parviflora* tolerates acidic soil reactions and is tolerant (except for seedlings) to herb layer cover. This is consistent with the work of Lanta et al. (2022), and Langmaier and Lapin (2020), who summarized that the successful reproduction of *I. parviflora* is due to tolerating less favorable conditions under a dense tree canopy and thus avoids competition by this shade-tolerant plant species. However, seedlings are more sensitive, which also results in susceptibility to pathogens. Bacigálová et al. (1998) reported up to 100% mortality of this weed seedlings infected with aeciospores of *P. komarovii* var. *komarovii*. *P. komarovii* var. *komarovii* holds promise concerning its relationship with *I. parviflora*, as previously suggested by Pusz et al. (2020). Our observations have revealed a substantial infection of small balsam leaf blades by this rust, occasionally resulting in symptoms manifesting on 100% of the leaf surface, leading to premature *I. parviflora* mortality and population decline by September. Unfortunately, we did not observe an annual escalation in the occurrence of rust driven by inoculum growth in the environment. The percentage of leaf coverage by *P. komarovii* var. *komarovii* exhibited fluctuations, depending on the year, implying an influence of environmental conditions that stabilized the infection each year. As a result, a consistent increase in its occurrence or a fixed peak of infection was not observed; both parameters varied annually and varied on every spot. Therefore there appears the need to understand the genet diversity of *P. komarovii* var. *komarovii* population occurring in Poland, which might predispose its usage as a biological agent against small balsam.

As noted by Pollard et al. (2021) on the occasion of research on the biological control of Himalayan balm, for successful control, it is essential that plant biotypes are matched to the most virulent rust strains. Strains from the native range may be useful, among which it is reasonable to distinguish those to which local biotypes will not be resistant. Although in the case of *I. glandulifera* control in the UK, the most effective strain was *Puccinia komarovii* var. *glanduliferae* from India (Pollard et al., 2019), local strains can also potentially naturally limit weeds, whose expansiveness can be particularly dangerous in legally protected areas, where the possibilities of human intervention aimed at controlling the spread of plants are very limited. For this reason, it is advisable to monitor the dynamics of the development of *P. komarovii* var. *komarovii* in Poland, observing its genetic variability and impact on the *I. parviflora* population.

Due to the host-specificity described by Tanner et al. (2015a), there is no danger that this rust will pose a threat to touch-me-not balsam (*Impatiens noli-tangere*) native in Poland. No symptoms of *P. komarovii* have been observed on *I. noli-tangere* during three years of research conducted by us in DNP and WNP (data not published). Also, there is no information about such infections in the available literature.

The *P. komarovii* var. *komarovii* isolates analyzed in this study did not show any genetic differentiation within the analyzed rDNA regions, but they differed from the sequences of isolates from the Great Poland Voivodeship available in GenBank

NCBI. In the case of ITS regions, this difference was a maximum of two nucleotides, while in the case of LSU - even seven ones. Greater differences were found between our isolates and isolates derived from *I. glanduliferae* and *I. brachycentra*, an Asian species which, unlike *I. parviflora* and *I. glandulifera*, do not occur in Poland (POWO, 2023). This is consistent with the observations of Tanner et al. (2015a), who observed variation in both ITS and LSU sequences of the rust-infecting *I. glandulifera* compared to accessions on other hosts.

Our research indicates some differentiation of *P. komarovii* var. *komarovii* in a relatively small area, i.e., in Poland. This genetic diversity probably results from sexual reproduction and may enable rapid adaptation of the pathogen to the region or host plant. Adaptation of pathogens to plant species is often due to the generation of novel allele combinations (Covo, 2020). Or it may indicate certain "entry points" to Poland where the isolates arrived from their native place of occurrence. While in the case of crop plant pathogens, genetic diversity means a threat because it may be related to the mentioned adaptive abilities, in the case of invasive plant pathogens, the development of which we would like to limit, genome instability seems to be desirable, because the pathogen has a greater chance of overcoming the possible resistance of the host plant and adaptation to the environment.

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