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First records of *Pyralis cardinalis* Kaila, Huemer, Mutanen, Tyllinen & Wikström, 2020 in the Romanian fauna (Lepidoptera: Pyralidae)

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Abstract

Pyralis cardinalis Kaila, Huemer, Mutanen, Tyllinen & Wikström, 2020, was recently described based on material originating from northern Europe to South Korea and Japan. Here this species is reported for the first time from various localities in north-eastern Romania, where it can occur in sympatry and synchrony with the morphologically similar *P. regalis* ([Denis & Schiffermüller], 1775). Adult external morphology, male genitalia and DNA barcodes of Romanian specimens are examined. To the best of our knowledge, the Romanian specimens represent the southernmost confirmed records of *P. cardinalis* in Europe and highlight the need to better document the distribution of this taxon.
Keywords: Lepidoptera, Pyralidae, distribution, DNA barcoding, haplotypes, scientific collections, sympatry, Romania.

Primeros registros de *Pyralis cardinalis* Kaila, Huemer, Mutanen, Tyllinen & Wikström, 2020 en la fauna rumana (Lepidoptera: Pyralidae)

Resumen

Pyralis cardinalis Kaila, Huemer, Mutanen, Tyllinen & Wikström, 2020, ha sido descrita recientemente basado en material procedente del norte de Europa hasta Corea del Sur y Japón. Aquí esta especie se reporta por primera vez en varias localidades del noreste de Rumanía, donde puede ocurrir en simpatría y sincronía con *P. regalis* ([Denis & Schiffermüller], 1775), una especie morfológicamente similar. Se examinan la morfología externa de adultos, los genitales masculinos y los códigos de barras de ADN de especímenes rumanos. Hasta donde sabemos, los especímenes rumanos representan los registros confirmados de *P. cardinalis* más al sur de Europa y resaltan la necesidad de documentar mejor la distribución de este taxón.
Palabras clave: Lepidoptera, Pyralidae, distribución, códigos de barras de ADN, haplotipos, colecciones científicas, simpatría, Rumanía.

Introduction

Until recently, the genus *Pyralis* Linnaeus, 1758 included seven recognized species in Europe: *P. farinalis* Linnaeus, 1758, *P. regalis* [Denis & Schiffermüller], 1775, *P. perversalis* (Herrich-Schäffer, 1849), *P. lienigialis* (Zeller, 1843), *P. kacheticalis* (Christoph, 1893), *P. manihotalis* (Guenée, 1854) and

P. pictalis (Curtis, 1834) (Slamka, 2006). However, a recent revision (Wikström et al. 2020) based on morphology and mitochondrial DNA (DNA barcodes) added two species to the European fauna, namely *Pyralis sagarrai* Leraut, 2005 (previously regarded as a subspecies of *P. regalis*) and *Pyralis cardinalis* Kaila, Huemer, Mutanen, Tyllinen & Wikström, 2020.

While *P. sagarrai* appears restricted to south-western Europe (Iberia and the French Pyrenees) (Slamka, 2006), *P. cardinalis* is transpalearctic, ranging from northern Europe to South Korea and Japan (Wikström et al. 2020). *Pyralis cardinalis* appears to be expanding at least in northern Europe (Wikström et al. 2020), but its recent discovery and similarity to the widespread *P. regalis* mean that its distribution is still poorly known.

Material and methods

Specimens: We examined 86 specimens from Romania with phenotypes corresponding to *P. regalis* or *P. cardinalis*. Identifications based on external morphology were done using the identification keys provided by Wikström et al. (2020) and Larysz (2020). Specimens were collected during the night using 160W mercury vapour lamps (230V) and/or 8W actinic or black light tubes (12V).

Genitalia examination: Male genitalia of three specimens (two *P. cardinalis*, one *P. regalis*) were prepared largely following the protocol outlined by Robinson (1976). The abdomen was detached from the thorax and was softened in 10% potassium hydroxide. The dissection was flushed with water, the genitalia structures were cleaned in Euparal essence and then embedded in Euparal resin. Male genitalia were mounted in a standard dorso-ventral position. The drawings were made from printed photographs. The genitalia were photographed with a BTC BIM 105B microscope coupled with a Canon EOS 1300D DSLR camera.

DNA barcoding: DNA barcodes (i.e. a 658 base-pair long fragment of the 5' end of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene) were obtained from six Romanian *Pyralis* specimens, out of 15 analysed. Genomic DNA was extracted from one or two legs using the DNeasy Blood and Tissue Kit (Qiagen). DNA barcodes were amplified by polymerase chain reaction using the primers HybLCO (5'-TAATACGACTCACTATAGGGGGTCAACAAATCATAAAGATATTGG-3') and HybHCO (5'-ATTAACCCTCACTAAAGTAAACTTCAGGGTGACCAAAAAATCA-3') (Wahlberg & Wheat, 2008). Double-stranded DNA was amplified in 10-L reactions containing 5.7 µL autoclaved Milli-Q water, 1 µL 10X buffer, 1 µL 25 mM MgCl2, 0.2 µL, 10 mM dNTPs, 0.5 µL of each primer (10 M), 0.1 µL Ampli Taq Gold DNA polymerase (Applied Biosystems), and 1 µL of extracted DNA. The typical thermal cycling profile was: first denaturation at 95 ºC for 300 s, followed by 39 cycles of 95 ºC for 30 s, 50 ºC for 30 s, and 72 ºC for 120 s, with a final extension at 72 ºC for 120 s.

Laboratory work and DNA sequencing were carried out at the University of Oulu, Finland. DNA barcodes and associated information are publicly available in DS-PYRECA (https://doi.org/10.5883/DS-PYRECA) in the Barcode of Life Data Systems (BOLD; Ratnasingham & Hebert, 2007).

The six Romanian sequences were aligned with *Pyralis* DNA barcodes published by Wikström et al. (2020), resulting in a dataset of 94 COI sequences. A neighbor-joining (NJ) tree was generated using MEGA version X (Kumar et al. 2018), using pairwise deletion and 1,000 bootstrap replicates.

Results

Romanian records: Among the 86 specimens of *Pyralis* from Romania examined we identified 27 individuals belonging to *P. cardinalis* and 59 belonging to *P. regalis* (Figure 1a-e).

P. cardinalis Kaila, Huemer, Mutanen, Tyllinen & Wikström, 2020

Botoşani county, Cătămăreşti (oak forest), 215 m, 47.748 N, 26.558 E, 24-VI-1994, 3 spec., C. Corduneanu leg. and coll.; Botoşani county, Ionăşeni (deciduous forest edge), 175 m, 47.696 N, 26.994

E, 04-VI-2016, 1 spec., C. Corduneanu leg. and coll., 22-VII-2017, 1 spec. (DNA barcode amplification failed), C. Corduneanu leg., V. Dincă coll.; Botoşani county, Leorda (oak forest), 245 m, 47.826 N, 26.405 E, 13-VII-2007, 1 spec., C. Corduneanu leg. and coll.; Botoşani county, Orăşeni Vale (riparian forest), 160 m, 47.645 N, 26.649 E, 26-VI-1998, 1 spec., C. Corduneanu leg. and coll., 01-VII-2005, 2 spec., C. Corduneanu leg. and coll., 15-VI-2007, 1 spec., C. Corduneanu leg. and coll., 21-VI-2007, 1 spec. (DNA barcode amplification failed), C. Corduneanu leg., V. Dincă coll., 02-VII-2008, 1 spec., C. Corduneanu leg., MINGA coll., 02-VII-2008, 1 spec., C. Corduneanu leg., L. Rákosy coll., 02-VII-2008, 1 spec. (DNA barcode amplification failed), C. Corduneanu leg., V. Dincă coll., 03-VII-2010, 1 spec. (DNA barcode amplification failed), C. Corduneanu leg., V. Dincă coll., 05-VII-2013, 1 spec., C. Corduneanu leg. and coll., 01-VII-2022, 1 spec. (BOLD process ID VEDS004-23), C. Corduneanu leg. and coll., 01-VII-2022, 1 spec. (BOLD process ID VEDS005-23), C. Corduneanu leg. and coll., 01-VII-2022, 1 spec. (BOLD process ID VEDS006-23), C. Corduneanu leg. and coll.; Botoşani county, Schit Orăşeni (garden), 185 m, 47.642 N, 26.685 E, 05-VII-1993, 1 spec. (genit. prep. 2879/Rákosy), C. Corduneanu leg., L. Rákosy coll., 15-VI-1995, 1 spec. (DNA barcode amplification failed), C. Corduneanu leg., V. Dincă coll., 12-VII-2023, 1 spec., C. Corduneanu leg. and coll.; Botoşani county, Schit Orăşeni (oak forest), 260 m, 47.632 N, 26.660 E, 17-VII-1995, 1 spec., C. Corduneanu leg. and coll., 12-VII-1999, 1 spec., C. Corduneanu leg. and coll.; Botoşani county, Şupitca (garden), 165 m, 47.606 N, 26.782 E, 20-VII-2015, 1 spec., I. Surugiu leg. and coll.; Botoşani county, Vorona (oak forest), 275 m, 47.607 E, 26.660 E, 28-VI-1997, 1 spec., C. Corduneanu leg. and coll.; Suceava county, Rădăuţi (wet meadow), 375 m, 47.854 N, 25.885 E, 30-VII-2005, 1 spec. (genit. prep. 2878/Rákosy), C. Corduneanu leg., L. Rákosy coll.

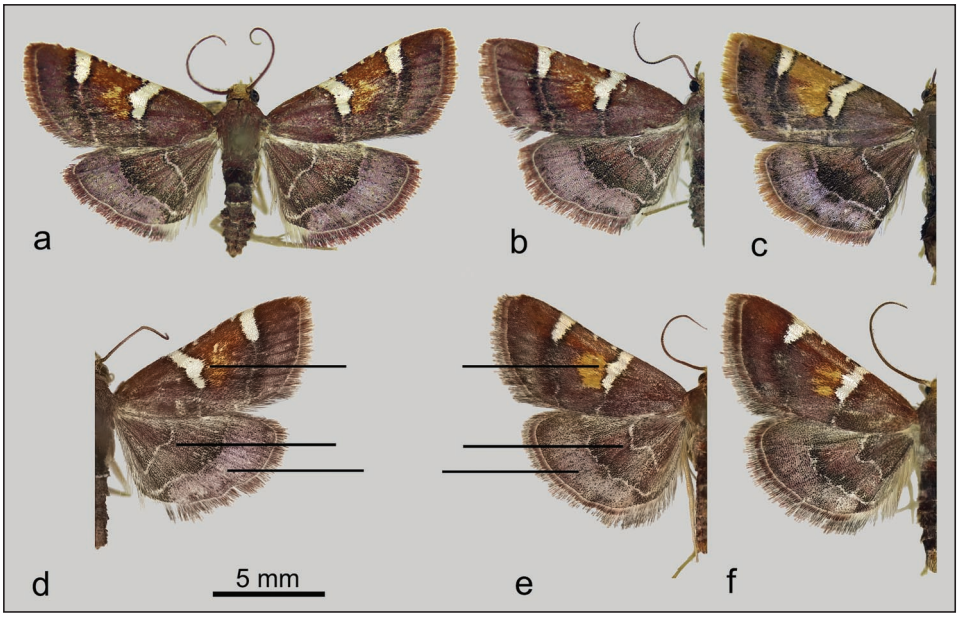

Figure 1. Specimens of *P. cardinalis* and *P. regalis* from north-eastern Romania (Botoşani county). Black lines in d and e indicate characters useful for the identification of the two species. **a.** *P. cardinalis*, Ionăşeni, 4-VI-2016. **b.** *P. cardinalis*, Orăşeni Vale, 26-VI-1998. **c.** *P. cardinalis*, Vorona, 28-VI-1997. **d.** *P. cardinalis*, Orăşeni Vale, 1-VII-2022, BOLD process ID VEDS005-23. **e.** *P. regalis*, Schit Orăşeni, 12-VII-2023. **f.** *P. regalis*, Schit Orăşeni, 24-VII-2023. Photos: C. Corduneanu.

P. regalis ([Denis & Schiffermüller], 1775)

Bihor county, Groşi (beech forest), 320 m, 47.068 N, 22.468 E, 07-VIII-2019, 1 spec., C. Corduneanu leg. and coll., 1 spec. (DNA barcode amplification failed), C. Corduneanu leg., V. Dincă coll.; Botoşani county, Ionăşeni (deciduous forest edge), 190 m, 47.701 N, 26.993 E, 31-VII-2014, 2 spec., C. Corduneanu leg. and coll., 22-VII-2015, 1 spec. (BOLD process ID VEDS001-23), C. Corduneanu leg., V. Dincă coll.; Botoşani county, Ionăşeni (deciduous forest edge), 175 m, 47.696 N, 26.994 E, 23-VIII-2015, 1 spec. (DNA barcode amplification failed), C. Corduneanu leg., V. Dincă coll., 04-VI-2016, 1 spec., C. Corduneanu leg. and coll., 30-VI-2016, 2 spec., I. Surugiu leg. and coll., 30-VI-2016, 1 spec. (DNA barcode amplification failed), C. Corduneanu leg., V. Dincă coll., 21-VIII-2016, 1 spec., C. Corduneanu leg. and coll., 21-VIII-2016, 1 spec. (BOLD process ID VEDS003-23), C. Corduneanu leg., V. Dincă coll., 06-IX-2016, 1 spec. (DNA barcode amplification failed), C. Corduneanu leg., V. Dincă coll.; Botoşani county, Orăşeni Vale (riparian forest), 160 m, 47.645 N, 26.649 E, 15-VI-2018, 1 spec. (BOLD process ID VEDS002-23), C. Corduneanu leg., V. Dincă coll.; Botoşani county, Schit Orăşeni (garden), 185 m, 47.642 N, 26.685 E, 12-VII-2023, 2 spec., C. Corduneanu leg. and coll., 24-VII-2023, 1 spec., C. Corduneanu leg. and coll.; Botoşani county, Şupitca (garden), 165 m, 47.606 N, 26.782 E, 08-IX-2014, 1 spec., I. Surugiu leg. and coll., 05-VII-2023, 1 spec., I. Surugiu leg. and coll.; Iaşi county, Breazu, comuna Rediu (meadow), 95 m, 47.238 N, 27.479 E, 26-VIII-2011, 1 spec., C. Balan leg., C. Corduneanu coll.; Botoşani county, Suliţa (deciduous forest edge), 130 m, 47.683 N, 26.992 E, 29-VIII-2011, 1 spec., C. Corduneanu leg. and coll.; Iaşi county, Stânca, comuna Comarna (garden), 50 m, 47.070 N, 27.804 E, 18-IX-2014, 1 spec., C.-O. Manci and C. Balan leg., C. Corduneanu coll.; Alba county, Cheile Runcului, 46.51 N, 23.44 E, 23-VII-1992, 1 spec., L. Rákosy leg. and coll.; Alba county, Ciuguzel, 46.28 N, 23.86 E, 06-VIII-2006, 1 spec., L. Rákosy leg. and coll.; Alba county, Rimetea, Piatra Secuiului, 950 m, 46.448 N, 23.586 E, 01-VII-1999, 1 spec., L. Rákosy leg. and coll.; Caraş-Severin county, Băile Herculane, 44.87 N, 22.41 E, 14-VII-1997, 1 spec., L. Rákosy leg. and coll., 15-VII-1997, 3 spec., L. Rákosy leg. and coll., 25-VII-1992, 1 spec., L. Rákosy leg. and coll.; Caraş-Severin county, Băile Herculane, Vârful Şuşcu, 44.89 N, 22.46 E, 15-VII-1997, 1 spec. (genit. prep. 2880/Rákosy), L. Rákosy leg. and coll.; Caraş-Severin county, Cheile Nerei, 44.86 N, 21.82 E, 30-VI-1994, 1 spec., L. Rákosy leg. and coll.; Caraş-Severin county, Cheile Nerei, Sasca Montană, 44.86 N, 21.82 E, 30-VI-1994, 1 spec., L. Rákosy leg. and coll.; Cluj county, Cheile Tureni, 46.61 N, 23.71 E, 05-VII-1980, 1 spec., L. Rákosy leg. and coll.; Cluj county, Cheile Turzii, 46.56 N, 23.68 E, 29-VII-1994, 1 spec., L. Rákosy leg. and coll., 02-VII-1990, 1 spec., L. Rákosy leg. and coll.; Cluj county, Mărişel-Colonie, 790 m, 46.69 N, 23.17 E, 28-VIII-1978, 1 spec., L. Rákosy leg. and coll.; Mehedinţi county, Dubova, 44.62 N, 22.25 E, 09-VI-1993, 4 spec., L. Rákosy leg. and coll.; Mehedinţi county, Dubova, Cazanele Mici, 44.62 N, 22.25 E, 16-VII-1997, 1 spec., L. Rákosy leg. and coll.; Mureş county, Criş, 46.14 N, 24.69 E, 17-VIII-2005, 1 spec., L. Rákosy leg. and coll.; Mureş county, Mihai Viteazul, 46.15 N, 25.02 E, 27-VII-2005, 1 spec., L. Rákosy leg. and coll.; Mureş county, Şaeş, 46.15 N, 24.77 E, 18-VIII-2007, 1 spec., L. Rákosy leg. and coll.; Mureş county, Sighişoara, 46.21 N, 24.79 E, 20-VIII-1977, 1 spec., L. Rákosy leg. and coll.; Tulcea county, Babadag, 44.85 N, 28.69 E, 07-VIII-1991, 2 spec., L. Rákosy leg. and coll.; Tulcea county, Greci, 45.20 N, 28.25 E, 15-VI-2006, 1 spec., L. Rákosy leg. and coll.; Tulcea county, Pădurea Horia, 230 m, 44.99 N, 28.45 E, 24-VII-1998, 1 spec., L. Rákosy leg. and coll.; Vâlcea county, Mt. Cozia, Mănăstirea Stânişoara, 680 m, 45.299 N, 24.338 E, 08-VI-2000, 1 spec., L. Rákosy leg. and coll.; Buzău county, Breaza, 335 m, 45.094 N, 26.537 E, 30-VIII-2022, 1 spec., V. Dincă leg. and coll., 20-IX-2023, 2 spec., V. Dincă leg. and coll.; Buzău county, Pietroasa Mică, 510 m, 45.116 N, 26.577 E, 27-VI-2023, 1 spec., V. Dincă leg. and coll.; Constanţa county, Negureni, 93 m, 44.088 N, 27.746 E, 29-IX-2023, 2 spec., V. Dincă leg. and coll.

The two species can usually be identified based on external morphology. In particular, the centrally widened outer edge of the white median fascia of the forewing, the distinct purple shade of the hindwing (Wikström et al. 2020) and the medially slightly bent whitish basal line of the hindwing (Larysz, 2020) distinguish *P. cardinalis* from other European congeneric taxa, including *P. regalis* (Figure 1a-e). However, due to intraspecific variability (e.g. fascia centrally slightly widened in certain specimens of *P. regalis*, worn specimens losing some of the purple shade etc.), it is advisable that the above mentioned characters are considered together (e.g. Figures 1d and 1e, where differences in fascia and purple shade are relatively subtle). Based on the specimens examined and in line with reports by Larysz (2020), we found that the shape of the whitish basal line of the hindwings is a useful diagnostic character, since in *P. regalis* this line is much more bent medially, having a more dentate appearance (Figure 1a-e).

Examination of male genitalia (Figure 2) confirmed identifications of *P. cardinalis* initially based on external morphology. In this species, some of the most obvious diagnostic characters are found in the phallus vesica, which has one large, spine-shaped and strongly sclerotized cornutus, as well as a smaller, slightly curved one, formed by a number of partially fused smaller spines. In *P. regalis*, the phallus vesica usually lacks clearly differentiated cornuti or spines (Wikström et al. 2020).

Additionally, the uncus in *P. cardinalis* is overall more or less triangular and sharper towards the apex, while in *P. regalis* it is that shaped and more rounded apically. The valvae in *P. cardinalis* are slightly longer and narrower than those of *P. regalis* (Figure 2) (Wikström et al. 2020).

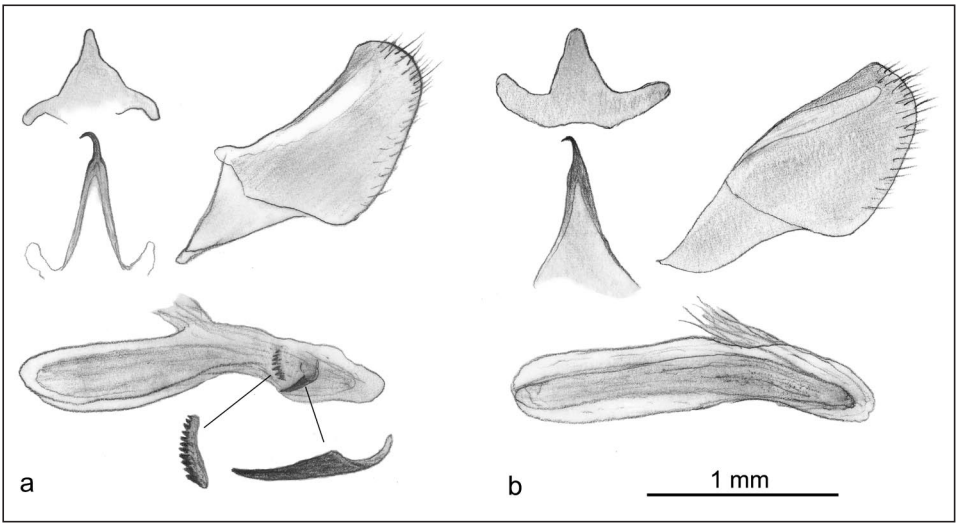

Figure 2. Elements of *Pyralis* male genitalia (uncus, gnathos, valva and phallus) from Romania. **a.** *P. cardinalis*, Schit Orăşeni (Botoşani county), 5-VII-1993, genit. prep. 2879/Rákosy. **b.** *P. regalis*, Băile Herculane, Vârful Şuşcu (Caraş-Severin county), 15-VII-1997, genit. prep. 2880/Rákosy.

All specimens of *P. cardinalis* were detected in north-eastern Romania, while *P. regalis* was much more widespread (Figure 3). We found *P. cardinalis* in various types of habitats, such as edges of deciduous forests, riparian forests or gardens. The maximum distance between sites is of approximately 85 km (Figure 3). The *P. cardinalis* specimens were collected between 1993 and 2023, with most records approximately between the last decade of June and mid-July, the earliest date being 4th of June and the latest 30th of July (Figure 4).

In four of the investigated sites, we identified both *P. cardinalis* and *P. regalis* (Figure 3), and synchrony was detected in two of these locations.

DNA barcoding

Of the six Romanian *Pyralis* specimens successfully barcoded, three belonged to *P. cardinalis* and three to *P. regalis* (Figure 5). Specimen assignment to species was unambiguous given the strong differentiation among taxa: minimum p-distance over 7% for *P. cardinalis* and almost 4% for *P. regalis* with respect to their nearest European neighbour (*P. sagarrai* in both cases) (Wikström et al. 2020). In contrast, intraspecific divergence in *P. cardinalis* was reported as low (maximum 0.7%) (Wikström et al. 2020) and the three Romanian specimens did not change this pattern. They represent two COI haplotypes that differ by a single mutation and are shared with other specimens of *P. cardinalis* (Figure 5).

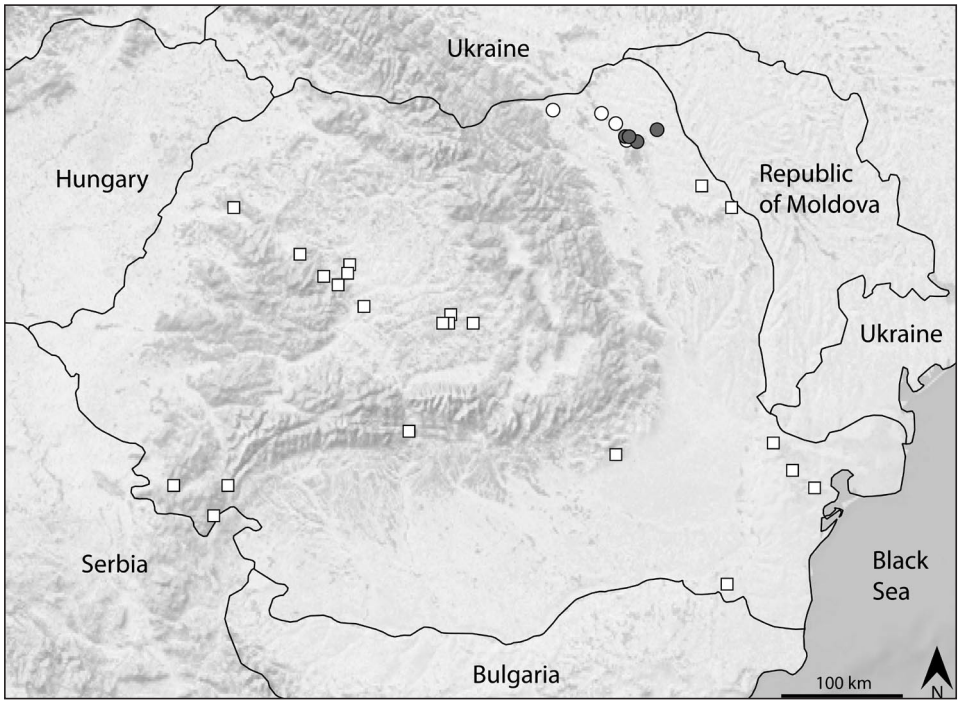

Figure 3. Distribution of *P. cardinalis* and *P. regalis* in Romania based on examined material. White dots, records of *P. cardinalis*; grey dots, detected sympatry between *P. cardinalis* and *P. regalis*; white squares, records of *P. regalis*.

Discussion

In Europe, the original description (Wikström et al. 2020) reported *P. cardinalis* from northern regions (Denmark, Sweden, Finland, Estonia, Latvia and Russia), but subsequent records from other countries have been relatively rapidly accumulating. This is the case of Poland (Larysz, 2020), Germany (Haslberger et al. 2021; Kormannshaus, 2022), Slovakia (Tokár et al. 2021) and Ukraine (Yepishin et al. 2021).

The examination of *Pyralis* specimens from Romania revealed the presence of *P. cardinalis* in the north-east, the findings representing the first records of this species in the country. The nearest reports

of *P. cardinalis* originate from Slovakia (Tokár et al. 2021) and Ukraine (Yepishin et al. 2021). To the best of our knowledge, the Romanian specimens represent the southernmost records of this species in Europe. Moreover, in north-eastern Romania the species was found in sympatry with *P. regalis*, phenomenon still rarely reported in Europe (Wikström et al. 2020). However, since *P. regalis* is widespread in southern regions, but also reaches at least some parts of central Europe, it is possible that re-evaluation of material present in collections combined with new field data will reveal more cases of sympatry. This likelihood probably increases also because *P. cardinalis* is apparently expanding at least in northern Europe (Wikström et al. 2020) and it is still unclear how far this species extends into southern Europe. The fact that *P. cardinalis* can, in addition to genitalia and DNA barcodes, also be identified based on external morphology should greatly facilitate the clarification of its distribution. In Romania for example, it is only similar to *P. regalis*, the other *Pyralis* species reported from the country (Rákosy & Goia, 2021) having clearly different morphology. Further investigations in this country may further extend the known distribution of this species.

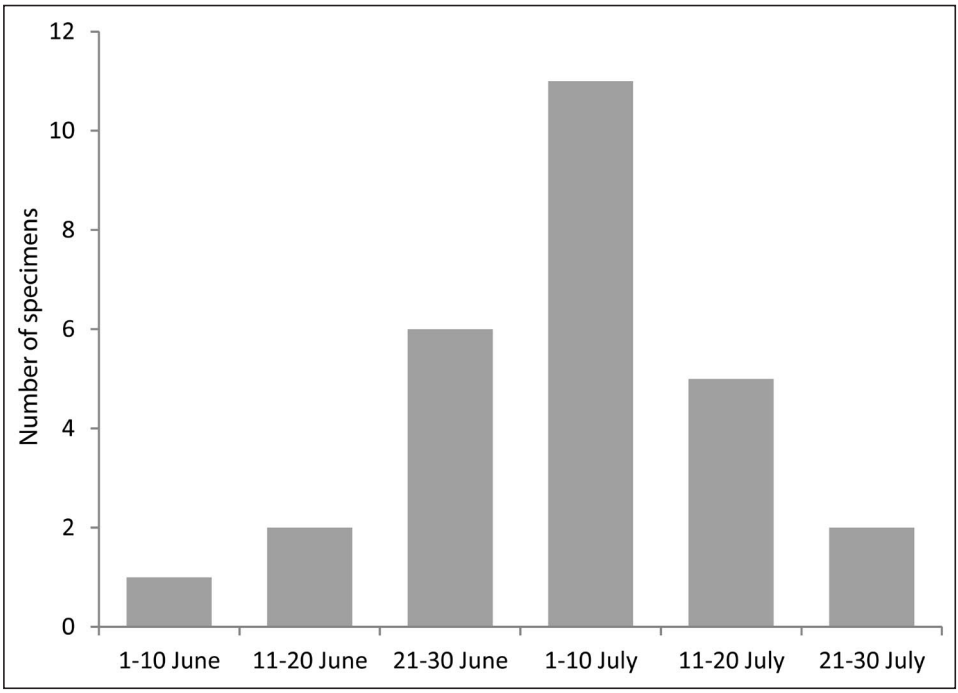

Figure 4. Phenology of *Pyralis cardinalis* adults inferred from 27 specimens originating from north-eastern Romania.

It is not clear what factors shape the range dynamics of *P. cardinalis*. The early stages and the biology of the species have been described recently (Buszko, 2022), the larvae feeding on dead leaves from the litter. Therefore, they do not appear to be strongly limited in terms of food availability. Using DNA to infer the origin of the contemporary distribution will likely require genomics, since DNA barcodes alone display very little variability in this species (Wikström et al. 2020).

The species is probably single brooded with a peak of the flight period during end June-July

(Wikström et al. 2020; Buszko, 2022), aspect supported by our preliminary data from Romania. It should be noted that, based on flight time data from Poland, Buszko (2022) hypothesized that a partial second brood may occur in August.

Conclusion

We documented the presence of *P. cardinalis* in north-eastern Romania, which currently represents the southern distribution limit of this species in Europe. We also detected *P. cardinalis* in sympatry and synchrony with *P. regalis*. Considering the apparent expansion of *P. cardinalis* at least in northern Europe, our findings reinforce the need to gather new field data and to revise material present in collections that will improve knowledge on the distribution dynamics of *P. cardinalis* and the potential causes behind its expansion.

Acknowledgements

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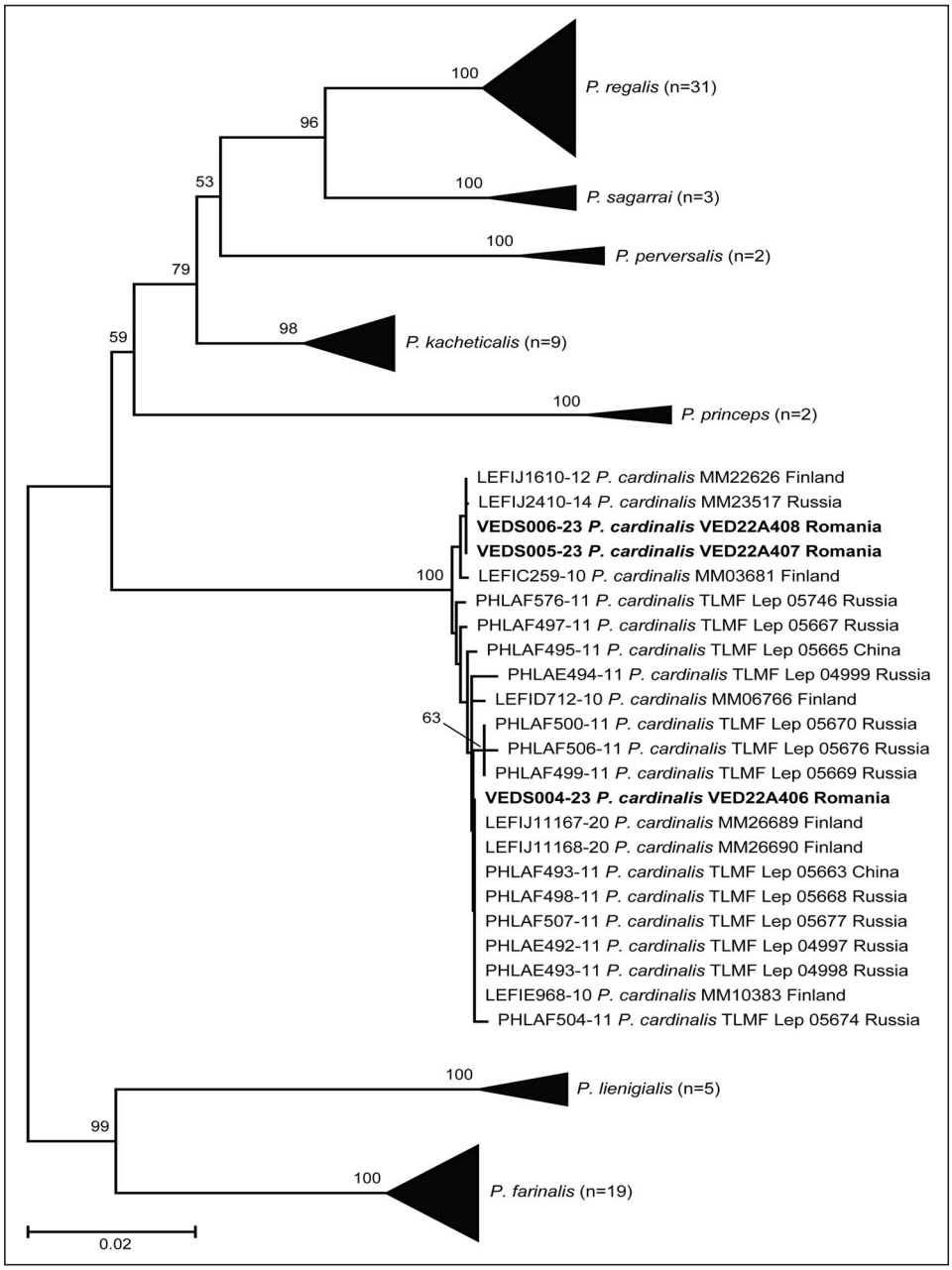

Figure 5. Neighbor-joining tree based on DNA barcodes of European *Pyralis*. Romanian specimens of *P. cardinalis* are bolded. Bootstrap supports (1,000 replicates, >50) are included above the recovered nodes.