

DNA Barcoding And Species Delimitation Of *Pyrausta* (Lepidoptera: Crambidae, Pyraustinae) With Some Populations In Turkey

Sibel KIZILDAĞ^{1*}

ABSTRACT: *Pyrausta aurata* (Scopoli, 1763), *P. despicata* (Scopoli, 1763), *P. sanguinalis* (Linnaeus, 1767), *P. castalis* (Treitschke, 1829), *P. pavidalis* (Zerny in Osthelder, 1935) and *P. gulpembe* (Kemal & Koçak, 2018) from Turkey were first time barcoded in the present study. Turkish populations and new species *P. tatarica* (Kemal, Kızıldağ & Koçak, 2020) were evaluated the phylogenetic positions with other *Pyrausta* species and populations. In the phylogenetic tree based on the mtCOI gene region delimitation of species and populations constructed with Neighbor-joining, Bayesian inference, and maximum-likelihood algorithms. For understanding the importance of the phylogenetic species concept in species delimitation, was reviewed cladistic topology and genetic distances of *Pyrausta* species with new data.

Keywords: *Pyrausta*, barcoding, phylogeny, Turkey

¹ Sibel KIZILDAĞ ([Orcid ID: 0000-0003-0182-5154](https://orcid.org/0000-0003-0182-5154)), Van Yüzüncü Yıl Üniversitesi, Fen Fakültesi, Biyoloji Bölümü, Van, Türkiye

*Sorumlu Yazar/Corresponding Author: Sibel KIZILDAĞ, e-mail: sibelkizildag@yyu.edu.tr

INTRODUCTION

The genus *Pyrausta* is one of the genera in the subfamily (Pyraustinae) with the largest number of species, it has over 320 species. The members of the genus are abundant in number and diversity and these moths widely distributed in the world. However, although the number of species is well known, a taxonomic revision of this genus has not been performed (Sutrisno, 2002). Most species still haven't detailed genital form and definition. This causing doubts that the numbers of species may be higher than known (Chen et al., 2018). Today, with the development of molecular techniques, species boundaries can be tested with molecular characters, and biodiversity can be determined. A strong molecular character can determine whether known species are valid/invalid, and the new species and closely related species, cryptic species in the genus (Patwardhan et al., 2014). In the last years, much progress has been made in the ability to define moths species through the use of mtCOI data (Yang et al., 2016; Mally et al., 2019). This gene region has identity information for many species and determines species boundaries stably as a DNA barcode. The reliability of species boundaries increases with the molecular data of a large number of different populations containing large geographic distributions (Silva-Brandão et al., 2009).

Currently, almost a third of *Pyrausta* species are barcoded and work is still in progress. The vast majority of barcoded samples are from the USA and Canada, then from European countries and China (other Palearctic realms are limited), very few from South America, Africa, and Australia (Anonymous, 2020a). In Turkey were recorded 14 species of this genus (some of which are new species); the names respectively are *Pyrausta aurata* (Scopoli, 1763), *P. despicata* (Scopoli, 1763), *P. sanguinalis* (Linnaeus, 1767), *P. castalis* (Treitschke, 1829), *P. virginalis* (Duponchel, 1832), *P. limbopunctalis* (Herrich-Schäffer, 1849), *P. falcatalis* (Guenée, 1854), *P. pauperalis* (Staudinger, 1879), *P. ferrealis* (Hampson, 1900), *P. mauretanic* (Rebel, 1907), *P. delicatalis* (Caradja, 1916), *P. pavidalis* (Zerny in Osthelder, 1935), *P. gulpembe* (Kemal & Koçak, 2018), and *P. tatarica* (Kemal, Kızıldağ & Koçak, 2020). But there isn't any molecular data that still belongs to them (except *P. tatarica*) (Kemal and Koçak, 2018; Kemal et al., 2020; Anonymous, 2020b).

In this study, six species of the *Pyrausta* belong to Cesa Collection recorded from Turkey were firstly barcoded. Molecular taxonomic relationships of the present *Pyrausta* species were evaluated with new data.

MATERIALS AND METHODS

Turkish populations of *Pyrausta* were evaluated by keeping materials of the Centre for Entomological Studies Ankara (Cesa) Collection (Table 1).

Table 1. Registration information belong to Turkish populations of *Pyrausta*

No	Species	Province	Accession numbers in GenBank	Cesa Sample ID Numbers
01	<i>Pyrausta despicata</i>	Bitlis	MN630685	Cesa Pyr015
02	<i>Pyrausta despicata</i>	Van	MN630686	Cesa Pyr044
03	<i>Pyrausta castalis</i>	Hakkari	MN630688	Cesa Pyr040
04	<i>Pyrausta pavidalis</i>	Van	MN624144	Cesa Pyr009
05	<i>Pyrausta tatarica</i>	Van	MN640435	Cesa Pyr059
06	<i>Pyrausta sanguinalis</i>	Bitlis	MN630687	Cesa Pyr014
07	<i>Pyrausta aurata</i>	Van	MN630689	Cesa Pyr010
08	<i>Pyrausta aurata</i>	Van	MN630690	Cesa Pyr045
09	<i>Pyrausta gulpembe</i>	Siirt	MN259520	Cesa Pyr002

The legs from the *Pyrausta* specimens were cleaned thoroughly with ethanol and dried. The RED Extract-N-Amp Tissue PCR Kit (Sigma-Aldrich, St. Louis, Missouri, USA) previously used by Kemal et al., (2018) was used to extract the total genomic DNA extraction from the tissue in the first stage and DNA barcode region copied also in the second stage. The PCR products were sent to Macrogen (Macrogen, Amsterdam, Netherlands) with the LepF1/R1 universal primers for purification and bilateral sequencing.

For phylogenetic analysis, barcodes of 533 species/ populations belong to *Pyrausta* were downloaded from GenBank and Boldsystem database, and the data set was prepared by adding barcodes of 9 populations presented in this study (Anonymous, 2020a; 2020c). Genetic distances between populations and species were calculated using the Kimura 2-parameter distance model (Kimura, 1980). The neighbor-joining (NJ) tree was constructed used the Kimura 2-Parameter distance model in MEGA 7.0 software. Maximum-likelihood (ML) bootstrapping analyses were achieved with 1000 replicates using RAxML Blackbox on XSEDE v.8.2.4 (Stamatakis et al., 2008) on the CIPRES Science Gateway. A Bayesian inference (BI) analysis was performed in MrBayes 3.2.6 (Ronquist and Huelsenbeck, 2003) with the Markov chain Monte Carlo algorithm. The program JModeltest v.2.1.7 (Posada, 2008) selected the TIM3+I+G evolutionary model as the best model according to the Akaike information criterion for Bayesian inference. The program was run for 5 000 000 generations, with a sample frequency of 100 and a burn-in of 12 500.

RESULTS AND DISCUSSION

In this study, barcodes of nine Turkish populations of seven species belonging to *Pyrausta* are presented for the first time. Also *P. gulpembe* and *P. pavidalis* were barcoded for the first time on a global scale and recorded in GenBank. *P. tatarica*'s phylogeny estimate is presented for the first time in this study.

Intragenus phylogeny prediction was calculated with three algorithmic trees. Since topologies of the NJ, ML, and BI phylogenetic trees are each other similar, three-support values were shown in a single (NJ) tree (Figure 1, Figure 2, Figure 3). Today, detailed phylogenetic trees of 75 species, whose exact barcode area has already been determined, have been built. Likewise, all of the populations of these species were presented in the form of "clades" by constricting due to taking up space in the consensus phylogenetic tree. Although *Pyrausta* spp. were generally seen as monophyletic taxa, populations of some species are not monophyletic. Different populations of some species were found to be more closely related to populations of other species than congeners. These non-monophyletic populations may have been misdiagnosed morphologically or morphologically apomorphic characters could not be identified between these taxa. In the phylogenetic tree, the Turkish population of *P. castalis* is located in the "castalis clade". Genetic distances between *P. castalis* Turkish population and European (Greece, Italy and Macedonia) populations are between 0.31% and 1.39%, and NJ/BI/ML node values are 88/0.72/62. *P. pavidalis* and *P. gulpembe* located to this clade as a sister group and the *P. generosa* clade were basal to them (Figure 1). The genetic distance of the Turkish population of *P. castalis* to *P. gulpembe* was 9.31%, to *P. pavidalis* to 7.88% and for *P. generosa* is 8.25%. The genetic distance between *P. gulpembe* and *P. pavidalis* is 8.05% and 9.31% with *P. generosa*. In addition, the genetic distance between *P. pavidalis* and *P. generosa* is 9.50%.

The monophyletic *P. despicata* clade formed from the 20 populations (18 from Europe, 2 from Turkey) with the strong support values (NJ /BI /ML; 76/0.83/82) (Figure 1). The genetic distances between these populations were in the range of 0.00-1.56% and were 2.04% with the German population (KX044594). *P. despicata* clade, which also includes the Turkish population, is a monophyletic taxon and was in a closely related position with the clade, mostly North American species. Populations of North American species seemed to be phylogenetically problematic in them.

In the presented phylogenetic tree, *P. tatarica* and *P. aerealis* clade are closely related and have a sister position with almost complete support values (NJ /BI /ML; 99/1.00/100) (Figure 2). Genetic distances between populations of two species are in the range of 2.85-4.49%.

P. sanguinalis clade and *P. andrei* are sister groups and the genetic distance between each population is 11.39% on average (Figure 3). The genetic distance between the *P. sanguinalis* populations and the Turkish population is in the range of 0.15-1.24%.

P. aurata, *P. generosa*, and *P. orphisalis* are closely related species. *P. aurata* clade was separated from the two species (generosa/orphisalis) with strong support values (NJ/BI/ML; 98/1.00/97) (Figure 3). The genetic distance between *P. aurata* and *P. generosa* is 5.67% and 5.50% with *P. orphisalis*. Also, the genetic distance between *P. generosa* and *P. orphisalis* is 2.85%.

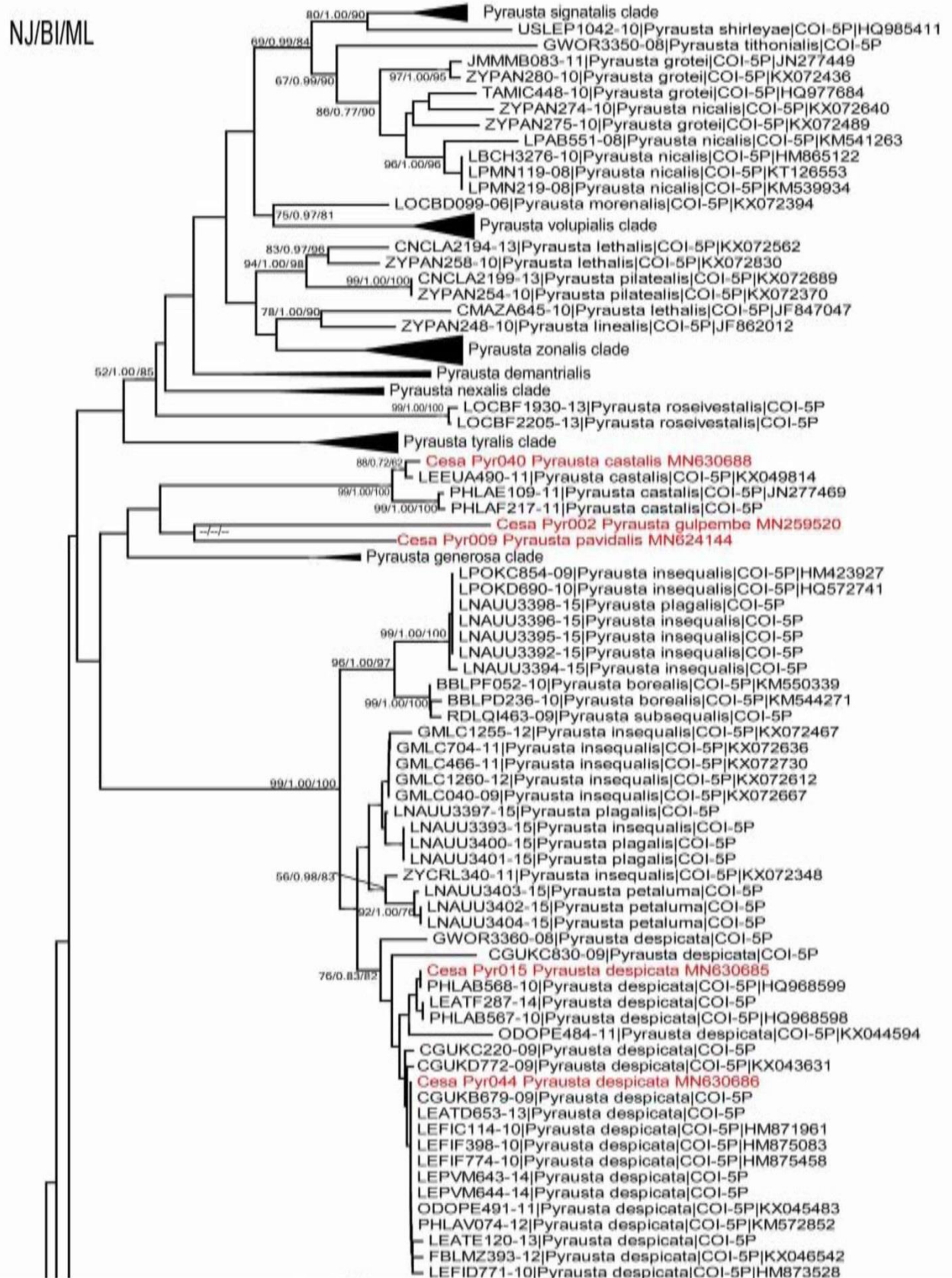


Figure 1. NJ, BI, and ML analyses based on mt COI gene sequences. The sequences of *Pyrausta* populations in the study are indicated in red-bold. Numbers at the nodes indicate the BI posterior probability and the NJ/ML bootstrap values. A dash indicates a value of less than 0.50 (BI) or 50% (NJ/ML). Bar, 1 substitutions per 100 nucleotide positions.

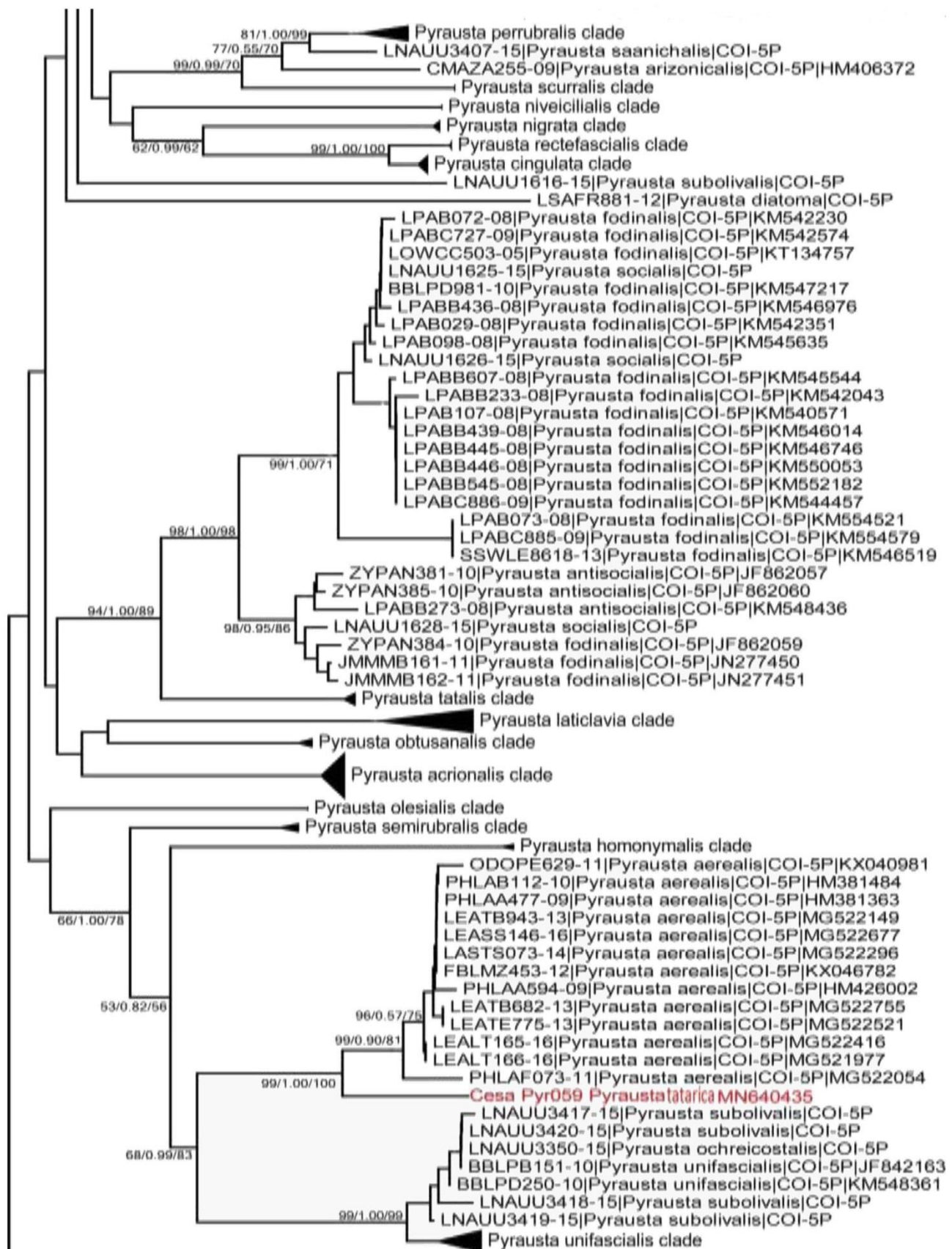


Figure 2. Continuation of the phylogenetic tree.

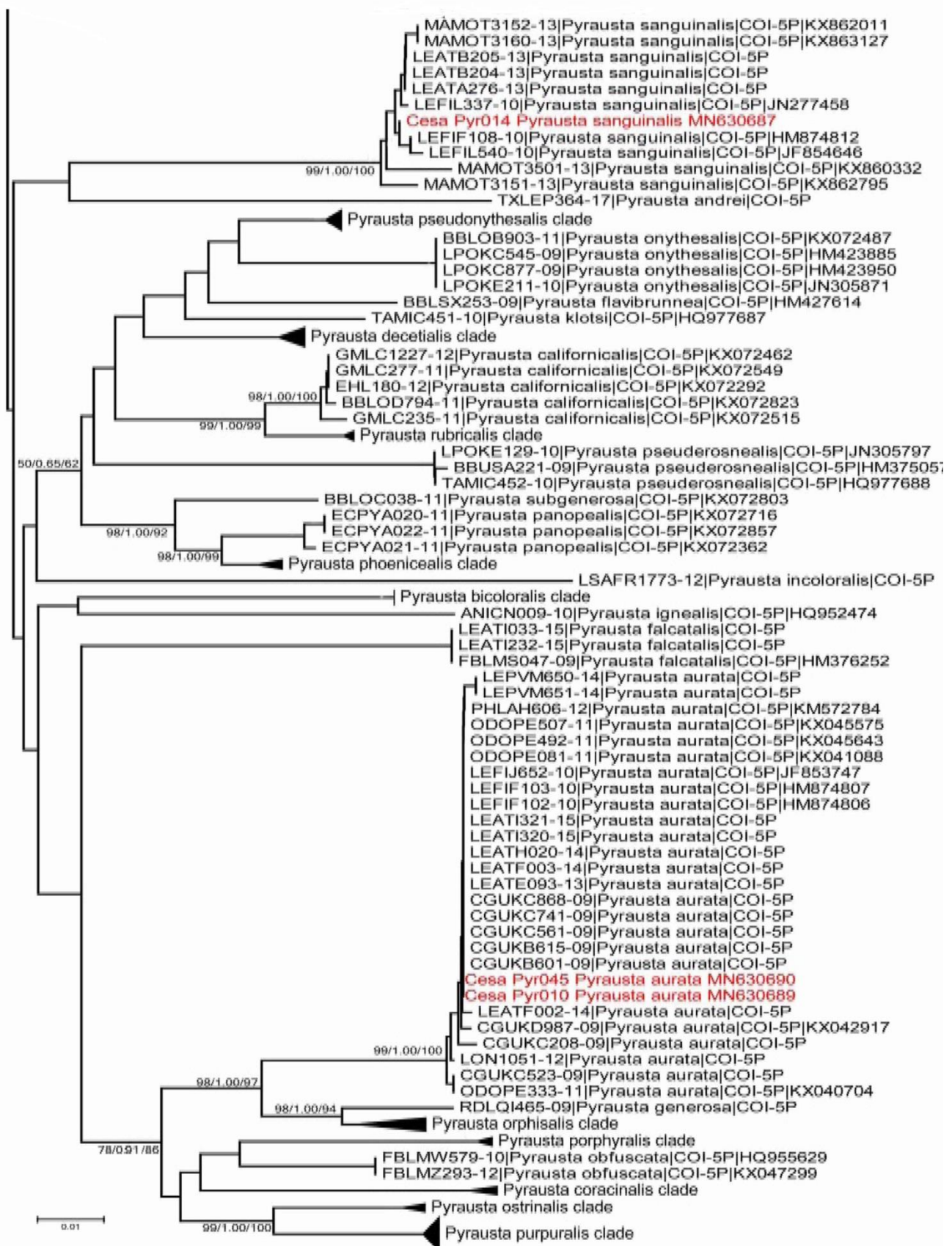


Figure 3. Continuation of the phylogenetic tree.

CONCLUSION

In this study, *Pyrausta aurata* (Scopoli, 1763), *P. despicata* (Scopoli, 1763), *P. sanguinalis* (Linnaeus, 1767), *P. castalis* (Treitschke, 1829), *P. pavidalis* (Zerny in Osthelder, 1935) and *P. gulpembe* (Kemal and Koçak, 2018), molecular barcodes of Turkey's populations were presented for the first time.

The clustering of these populations in the same clade with their congeners in the presented phylogenetic tree indicates that they were diagnosed morphologically correctly by taxonomists. In other words, the molecular taxonomies of these species were compatible with the morphological species distinction. In addition, it has been found that the diagnostic characters of these species represent the species correctly and that the species limits are evident at the molecular level. Kemal and Koçak (2018) defined *P. gulpembe* as the new morphological species, and for the first time it was confirmed by testing phylogenetic analysis in this study that this species was a separate species. For the first time in this study, *P. tatarica* evaluated the phylogenetic position. While defining this species, the authors reported that it was the closest taxon to *P. aerealis* morphologically and molecularly. In the presented phylogenetic tree, *P. tatarica* was positioned as a sister to the *P. aerealis* clade. In other words, the morphological definition and the phylogeny prediction of *P. tatarica* were consistent.

As a result, there is a huge number of deficiencies in the molecular data of *Pyrausta*. Barcodes of a large number of species and populations from different geographies are required to estimate the correct phylogeny. For this reason, in the present study molecular taxonomic evaluation of *Pyrausta* species and populations was done by the results obtained with new data from Turkey. It is aimed to shed light on similar studies in the future.

ACKNOWLEDGEMENTS

Special thanks are given to Prof. Dr. Ahmet Ömer KOÇAK and Dr. Muhabbet KEMAL for their continuous help, comments, and use of the Cesa Collection.

Conflict of Interest

I declare that there is no conflict of interest during the planning, execution and writing of the article.

Author's Contributions

I hereby declare that the planning, execution and writing of the article was done by me as the sole author of the article.

REFERENCES

- Anonymous, 2020a. Barcode Of Life Data System. <http://www.boldsystems.org/index.php>. (Date of access: 9 May 2020).
- Anonymous, 2020b. Global information system on pyraloidea. <http://www.pyraloidea.org>. (Date of access: 9 May 2020).
- Anonymous, 2020c. National Center for Biotechnology Information. <https://www.ncbi.nlm.nih.gov/nuccore/?term=Pyrausta>. (Date of access: 9 May 2020).
- Chen K, Zhang D, Stănescu M. 2018. Revision of the genus *Eumorphobotys* with descriptions of two new species (Lepidoptera, Crambidae, Pyraustinae). *Zootaxa*, 4472 (3): 489–504.
- Kemal M, Yıldız İ, Kızıldağ S, Uçak H, Koçak, AÖ. 2018. Taxonomical and molecular evaluation of *Apochima agassiz* in East Turkey, with a description of a new genus (Lepidoptera, Geometridae, Ennominae). *Miscellaneous Papers*, 169: 1–14.
- Kemal M, Koçak A. 2018. Vernal aspect of the Lepidoptera from Nemrut Mountain and the vicinity of Karadut in the Kahta district with a description of a new species (SE Turkey, Adiyaman Province). *Miscellaneous Papers*, 174:1–22.
- Kemal M, Kızıldağ S, Koçak A. 2020. Description of a new *Pyrausta* species from East Turkey with comments about related species (Lepidoptera, Pyraloidea). *Miscellaneous Papers*, 210: 1–5.
- Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16: 111–120.

- Maes K V N. 2002. On the systematic position of some Palaearctic Pyraustinae (Pyraloidea, Crambidae). *Nota Lepidopterologica*, 24 (4):51 –57.
- Mally R, Hayden JE, Neinhuis C, Jordal BH, Nuss M. 2019. The phylogenetic systematics of Spilomelinae and Pyraustinae (Lepidoptera: Pyraloidea: Crambidae) inferred from DNA and morphology. *Arthropod Systematics & Phylogeny*, 77(1): 141–204.
- Patwardhan A, Ray S, Roy A. 2014. Molecular Markers in Phylogenetic Studies – A Review. *Journal of Phylogenetics & Evolutionary Biology*, 2: 131.
- Posada D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology Evolution*, 25 (7):1253–1256.
- Ronquist F, Huelsenbeck JP. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19: 1572–1574.
- Shodotova AA. 2011. New Species of the Genus *Pyrausta* (Lepidoptera, Crambidae) from Southern Siberia and Transcaucasia. *Zoologicheskii Zhurnal*, 90 (4): 505–512.
- Silva-Brandão KL, Lyra ML, Freitas AVL. 2009. Barcoding Lepidoptera: Current Situation and Perspectives on the Usefulness of a Contentious Technique. *Neotropical Entomology*, 38 (4): 441–451.
- Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology*, 57(5):758–771.
- Sutrisno H. 2002. A Preliminary Study on Relationships among Selected Australian Members of the Tribe Spilomelini (Lepidoptera: Crambidae: Pyraustinae). *Zoological Science*. 19: 915–929.
- Yang Z, Landry J-F, Hebert PDN. 2016. A DNA Barcode Library for North American Pyraustinae (Lepidoptera: Pyraloidea: Crambidae). *PLoS ONE*, 11(10): e0161449.