

A Molecular Assessment of the Taxonomy of Iranian *Sylvia* Warblers (Aves; Sylviidae)

Raziyeh Abdilzadeh¹, Mansour Aliabadian^{1,2*} and Urban Olsson^{3,4}

¹ Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

² Research Department of Zoological Innovations (RDZI), Institute of Applied Zoology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

³ Department of Biology and Environmental Sciences, University of Gothenburg, Sweden

⁴ Gothenburg Global Biodiversity Centre, Göteborg, Sweden

ARTICLE INFO

Article history:

Received 25 April 2019

Accepted 02 July 2019

Available online 22 July 2019

Keywords:

cox1

cytb

phylogeny

songbirds

*Corresponding author:

✉ M. Aliabadian
aliabadi@um.ac.ir

p-ISSN 2423-4257

e-ISSN 2588-2589

ABSTRACT

The largest genus in Sylviidae family is *Sylvia*, which is the archetype for warblers. It contains up to 28 species that are distributed in the Old World. Here, we study Iranian *Sylvia* using cytochrome *c* oxidase subunit 1 (*cox1*) and cytochrome *b* (*cytb*) mitochondrial markers to identify them and place in a phylogenetic context using all available GenBank samples of sufficient length. Bayesian trees for both *cytb* and *cox1* mitochondrial markers are compatible. Previous results suggesting that the *Sylvia curruca* complex is separated into two major clades with high support is corroborated, as our results show two main clades, one that includes *Sylvia atricapilla* and *Sylvia borin* plus *Pseudalcippe atriceps* and *Lioptilus nigricapillus*, and the remainder of the *Sylvia* species in the other one. Our study did not support the geographical groups: The West-Mediterranean group, the Central-Mediterranean group, and the East-Mediterranean or the African-European species, the African-Asian species, and the Mediterranean species inferred on molecular evidenced by previous studies. *Sylvia cantillans* is divided into three clades, corroborating previous studies. We confirmed the following taxa to occur in Iran: *Sylvia borin*, *Sylvia atricapilla*, *Sylvia curruca curruca*, *Sylvia curruca halimodendri*, *Sylvia althaea*, *Sylvia crassirostris*, *Sylvia mystacea* and *Sylvia nisoria*. All Iranian samples were conclusively identified to species level, and none of them give rise to any further taxonomic inquiry.

© 2019 UMZ. All rights reserved.

Please cite this paper as: Abdilzadeh R, Aliabadian M, Olsson U. 2019. A molecular assessment of the taxonomy of Iranian *Sylvia* warblers (Aves; Sylviidae). *J Genet Resour* 5(2): 149-162. doi: 10.22080/jgr.2019.2434

Introduction

The largest genus in the Sylviidae family is *Sylvia*, which is the archetype of warblers (Aves; Sylviidae, *Sylvia*). *Sylvia* has been used extensively as a model system in a variety of ecological, genetic, morphological evolution, the genetics of migration, the effects of climate change on migration, the evolution of range size and morphological studies (Böhning-Gaese and Bauer, 1996; Gwinner and Wiltschko, 1978; Doswald *et al.*, 2009). The genus is distributed from central Eurasia to South Africa, and about 70% of *Sylvia* species have ranges bordering the

Mediterranean Sea and this region is reconstructed as the ancestral area for *Sylvia* (Voelker and Light, 2011). The genus *Sylvia* is comprised of about 28 species (Gill and Donsker, 2019), depending on taxonomy often including species which were until recently placed in other genera: *Parisoma*, *Pseudalcippe* and *Horizhorinus*, respectively (Blondel *et al.*, 1996; Böhning-Gaese *et al.*, 2003; Voelker *et al.*, 2009; Sibley and Monroe, 1990; Sibley and Ahlquist 1990). According to Clement *et al.*, (2016), this genus has 25 species, whereas Dickinson and Christidis (2014) divides the genus into several smaller genera and only includes 4 species in



Sylvia sensu stricto. In this study, we followed the IOC world bird list (Gill and Donsker, 2019). Most of the early studies related to this genus are based on morphology (Hall and Moreau, 1970; Glutz and Bauer, 1991; Jordano, 1987). But, more recent studies have relied on molecular phylogenies as the basis for understanding evolutionary patterns in the genus (Böhning-Gaese and Bauer, 1996; Böhning-Gaese *et al.*, 2006; Voelker and Light, 2011). Based on a combination of morphological characteristics, dispersal patterns, general ecology and behavior of species, six groups are defined in the genus *Sylvia*: *Undata*, *Melanocephala*, *Hortensis*, *Curruca*, *Articapilla* and *Communis* (Glutz and Bauer, 1991). Five of these groups occur in Iran, the exception being *Undata*. A molecular phylogeny based on DNA-DNA hybridization revealed that the *Sylvia-Parisoma* complex is monophyletic and includes three main groups of species, the “mid-European” warblers, the genus *Parisoma*, and the “eu-Mediterranean” *Sylvia* species *sensu stricto*. The latter can be assigned to three main clusters, a “West-Mediterranean” group, a “Central-Mediterranean group”, and an “East-Mediterranean” group.

The phylogeny results were consistent with the DNA-DNA hybridization results that included 20 species (Blondel *et al.*, 1996). All species of *Sylvia* and species of the formerly recognized genus *Parisoma* were included in a study using the mitochondrial cytochrome b gene (*cytb*) (Böhning-Gaese *et al.*, 2003). The main reason the number of species of this genus is uncertain, is because of the presence of two not yet taxonomically evaluated species complexes, *Sylvia curruca* and *Sylvia cantillans* (Voelker and Light, 2011).

The Lesser Whitethroat *Sylvia curruca* complex is a group of warblers divided into at least six clades (*althea*, *blythi*, *halimodendri*, *margelanica*, *curruca* and *minula*) according to Olsson *et al.*, (2013) and Votier *et al.*, (2016). Some authors treat the complex as a single polymorphic species (*e.g.* Shirihai *et al.*, 2001; Cramp, 1992; Mayr and Cottrell, 1986; Portenko, 1960; Ripley, 1982; Svensson, 1992; Williamson, 1976), two species (Loskot, 2005), while others divide it into three different species (Martens and Steil, 1997; Vaurie, 1954; Gill and Donsker, 2019). *Sylvia cantillans* is formally

considered a polytypic species, with four subspecies, European *S. c. cantillans*, *albistriata*, *moltonii* and North African *S. c. inornata* (Gargallo 1994; Shirihai *et al.*, 2001; Brambilla *et al.*, 2008) widely distributed around the Mediterranean. They are very similar in external morphology, and both taxonomic status and phylogeographic relationships among populations is uncertain. For many, differences in their vocalizations are useful for identification. Based on the sequences of the two mitochondrial genes *cytb* and *ND2* (NADH dehydrogenase subunit 2), the relationships of this genus were investigated and biological, geographic history reconstructed in 26 species of the genus *Sylvia* (including species formerly found in *Parisoma* and *Horizorhinus*) (Voelker and Light, 2011). They classified the genus into 3 main clades: African-European species, African and Asian species, species with Mediterranean dispersal (Voelker and Light, 2011). However, several important nodes in the tree had low support, and consequently a well-resolved phylogeny of *Sylvia* is still lacking.

Concerning Iranian taxa, only one sequence belonging to the species *Sylvia mystacea* was used by Voelker and Light (2011), while in a general study of songbirds, only the two species *Sylvia communis* and *S. mystacea* originating from Iran were included in the analyzes (Aliabadian *et al.*, 2009). These are the only studies that have included specimens of the genus *Sylvia* originating in Iran. Our aims in this paper are to identify newly obtained Iranian *Sylvia* samples by molecular methods and assess their phylogenetic position compared to *Sylvia* samples present in public depositories.

We here present the first phylogenetic and taxonomic evaluation of Iranian samples of the genus *Sylvia*, based on part of the mitochondrial cytochrome b and cytochrome c oxidase subunit 1 (*cox1*) genes sampled throughout Iran and compared with the complete dataset of sufficient length sequences from GenBank.

Materials and Methods

Sampling and GenBank dataset

Blood samples were collected from 43 *Sylvia* in 12 sites from Iran (Fig. 1), comprising Arasbaran (three samples), Khoy (four samples), Khalkhal

(four samples), Zanjan (one sample), Kurdistan (five samples), Tandoureh (one sample), Fars (seven samples), Yasuj (four samples), Kerman (eight samples), Golestan (three samples) and

Minab (three samples) between July 2016 and July 2017 under permit No. 96.9101 of the Iranian Department of Environment.



Fig.1. Sampling sites of *Sylvia* Warblers in Iran: 1=Khoy; 2=Arasbaran; 3=Khalkhal; 4=Zanjan; 5=Kurdistan; 6=Golestan; 7=Tandoureh; 8=Yasuj; 9 and 10=Fars; 11=Kerman; 12=Minab.

Much of the sampling was done in Important Bird Areas (IBA). Details of origin and GenBank accession numbers are given in Table 1 and Table 2. Birds were captured using mist nets and approximately 50µl of whole blood was drawn from a brachial vein by insulin needles and was preserved into Queens's buffer (Seutin

et al., 1991). *Acrocephalus scirpaceus* was used as an outgroup for rooting the phylogeny. We used all available GenBank samples for both *cox1* and *cytb* genes for *Sylvia* genus, *Pseudoalcippe* and *Lioptilus*. The included sequences were longer than 600 bp for *cox1* and 800 bp for *cytb*.

Table 1. The information of Iranian *Sylvia* Warblers samples.

Scientific name	Sample ID	Sample sites	Coordinate	Status
<i>S. borin</i>	FUM 160920225	Khoy	38° 55' N, 44° 93' E	Breeding season
<i>S. borin</i>	FUM 161020225	Khoy	38° 55' N, 44° 93' E	Breeding season
<i>S. curruca</i>	FUM 160920218	Khoy	38° 55' N, 44° 93' E	Breeding season
<i>S. curruca</i>	FUM 160920222	Khoy	38° 55' N, 44° 93' E	Breeding season
<i>S. curruca</i>	FUM 170701209	*Arasbaran	38° 98' N, 46° 88' E	Breeding season
<i>S. curruca</i>	FUM 170701210	*Arasbaran	38° 98' N, 46° 88' E	Breeding season
<i>S. curruca</i>	FUM 170701213	*Arasbaran	38° 98' N, 46° 88' E	Breeding season
<i>S. curruca</i>	FUM 170522173	*Kurdistan	35° 29' N, 46° 82' E	Breeding season
<i>S. curruca</i>	FUM 170522177	*Kurdistan	35° 29' N, 46° 82' E	Breeding season
<i>S. curruca</i>	FUM 170522181	*Kurdistan	35° 29' N, 46° 82' E	Breeding season
<i>S. curruca</i>	FUM 170617183	Fars	29° 57' N, 51° 94' E	Breeding season
<i>S. curruca</i>	FUM 170617189	Fars	29° 57' N, 51° 94' E	Breeding season
<i>S. curruca</i>	FUM 170617192	Fars	29° 57' N, 51° 94' E	Breeding season
<i>S. curruca</i>	FUM 170617193	Fars	29° 57' N, 51° 94' E	Breeding season
<i>S. curruca</i>	FUM 170617194	Fars	29° 57' N, 51° 94' E	Breeding season
<i>S. curruca</i>	FUM 170618201	Yasuj	۳۰° 3۰' N, ۵۱° ۴۱' E	Breeding season
<i>S. curruca</i>	FUM 170618204	Yasuj	۳۰° 3۰' N, ۵۱° ۴۱' E	Breeding season
<i>S. curruca</i>	FUM 170618205	Yasuj	۳۰° 3۰' N, ۵۱° ۴۱' E	Breeding season
<i>S. curruca</i>	FUM 170618206	Yasuj	۳۰° 3۰' N, ۵۱° ۴۱' E	Breeding season
<i>S. curruca</i>	FUM 150806093	Zanjan	36° 57' N, 47° 64' E	Breeding season
<i>S. curruca</i>	FUM 140802299	Khalkhal	37° 37' N, 48° 32' E	Breeding season
<i>S. curruca</i>	FUM 140802278	Khalkhal	37° 37' N, 48° 32' E	Breeding season
<i>S. curruca</i>	FUM 140802285	Khalkhal	37° 37' N, 48° 32' E	Breeding season
<i>S. curruca</i>	FUM 140802282	Khalkhal	37° 37' N, 48° 32' E	Breeding season
<i>S. mystacea</i>	FUM 170522180	*Kurdistan	35° 29' N, 46° 82' E	Breeding season
<i>S. althaea</i>	FUM 170814232	*Kerman	29° 05' N, 57° 53' E	Breeding season
<i>S. althaea</i>	FUM 170814233	*Kerman	29° 05' N, 57° 53' E	Breeding season
<i>S. althaea</i>	FUM 170814234	*Kerman	29° 05' N, 57° 53' E	Breeding season
<i>S. althaea</i>	FUM 170814238	*Kerman	29° 05' N, 57° 53' E	Breeding season
<i>S. althaea</i>	FUM 170814239	*Kerman	29° 05' N, 57° 53' E	Breeding season
<i>S. althaea</i>	FUM 170814241	*Kerman	29° 05' N, 57° 53' E	Breeding season
<i>S. althaea</i>	FUM 160512187	*Tandoureh	37° 58' N, 58° 66' E	Breeding season
<i>S. halimodendri</i>	FUM 170319153	Hormozgan	27° 08' N, 57° 01' E	Wintering
<i>S. halimodendri</i>	FUM 170319154	Hormozgan	27° 08' N, 57° 01' E	Wintering
<i>S. halimodendri</i>	FUM 170319155	Hormozgan	27° 08' N, 57° 01' E	Wintering
<i>S. crassirostris</i>	FUM 170617185	Fars	29° 57' N, 51° 94' E	Breeding season
<i>S. crassirostris</i>	FUM 170617190	Fars	29° 57' N, 51° 94' E	Breeding season
<i>S. atricapila</i>	FUM 170808217	Golestan	36° 79' N, 54° 45' E	Breeding season
<i>S. atricapila</i>	FUM 170808221	Golestan	36° 79' N, 54° 45' E	Breeding season
<i>S. communis</i>	FUM 170808218	Golestan	36° 79' N, 54° 45' E	Breeding season
<i>S. communis</i>	FUM 170814240	*Kerman	29° 05' N, 57° 53' E	Breeding season
<i>S. communis</i>	FUM 170814242	*Kerman	29° 05' N, 57° 53' E	Breeding season
<i>S. communis</i>	FUM 170522156	*Kurdistan	35° 29' N, 46° 82' E	Breeding season

*Important Bird Area (<http://www.birdlife.org>)

DNA extraction and sequencing

DNA was extracted from blood using a standard salt extraction method (Bruford *et al.*, 1992), after incubation overnight at 40°C immersed in an extraction buffer (2% sodium dodecyl sulfate (SDS) and 0.5 mg/ml proteinase K). The mtDNA markers, *cox1*, and *cytb* were amplified using published primers that *cox1* primers and PCR

cycling procedure were based on Patel *et al.*, (2010). The oligomers of the forward and reverse primers were for AWCF: AWCF1-f: 5'- CGCYTWAACAYTCYGCCATCTTACC-3'; and AWCF6-r: 5'- ATTCCATGTAGCCGAATGGTTCTTT-3'; and for *cytb* following L-14995: 5'- CTCCCAGCCCCATCCAACATCTCAGCATG

ATGAAACTTCG-3'; H-16065: 5'-CTAAGAAGGGTGGAGTCTTCAGTTTTGGTTACAAGAC-3' (Olsson *et al.*, 2005).

Total PCR reaction volumes were 25 μ l, containing 12.5 μ l Taq DNA Polymerase Master Mix RED (Ampliqon), 1 μ l of each primer with concentration 10 μ M, 3 μ l DNA, and 7.5 μ l ddH₂O. PCR products were screened on 2% agarose gels to confirm amplification of the target fragments. The purified PCR products were sequenced by Macrogen Inc. (Seoul, South Korea).

Data analysis

Sequences were edited in Bioedit version 7.0.1 (Hall, 1999). Sequences were aligned using ClustalW as implemented in <https://www.ebi.ac.uk/Tools/msa/clustalw2/>. The substitution model for the Bayesian trees was determined by jModelTest 2.1.10 (Darriba *et al.*, 2012).

Molecular phylogenies were estimated by Bayesian inference using MrBayes 3.2.7 (Ronquist *et al.*, 2012) by The CIPRES Science Gateway V.3.3 (Miller *et al.*, 2010) (<http://www.phylo.org/index.php/>). The preferred model for *cytb* was HKY+I+G (Hasegawa *et al.*, 1985) and the best-fitting model of nucleotide substitution for *cox1* was GTR+I+G (Lanave *et al.*, 1984; Rodríguez *et al.*, 1990; Tavaré, 1986). A contiguous 651 bp stretch of the *cox1* and 842 bp portion of the *cytb* gene were obtained from 156 and 184 taxa respectively and visualized in FigTree 1.4.0 (Rambaut, 2008).

Results

Bayesian trees estimated under the HKY+I+G and GTR+I+G model, respectively, for both *cytb* and *cox1* mitochondrial markers, are compatible. The *cytb* Bayesian tree (Fig. 2) shows two main clades (clade A and B) with high support (100%). Clade A includes *Pseudoalcippe atriceps*, *Lioptilus nigricapillus*, *Sylvia borin* and *Sylvia atricapilla*. All other species are in Clade B, which is further divided into two main subclades. The first subclade (clade XII) consists of the *Sylvia curruca* complex, and its sister clade (clade XI) consists of *Sylvia crassirostris*, *Sylvia leucomelaena*, *Sylvia buryi*, *Sylvia lugens*. Moreover, *Sylvia hortensis*. *Sylvia cantillans*

(clade V) is sister to clade IV, which contains *Sylvia melanocephala* and *Sylvia mystacea*. Also, *Sylvia layardi*, *Sylvia boehmi*, *Sylvia subcaeruleum* and *Sylvia nana* constitute the sister clades. *Sylvia nisoria* is a separate clade in the clade B. The *cox1* Bayesian tree (Fig. 3) shows two main clades. *S. borin* and *S. atricapilla* (clade B) constitute a sister group to the other taxa (clade A), both with 100% support. In both trees, *S. cantillans* (clade VI) is sister to *S. melanocephala* and *S. mystacea* (clade V) and the *S. curruca* complex (clade IX) is sister to *S. crassirostris* (clade VII). Iranian *S. borin* (FUM 160920225 and FUM 161020225) are part of the same clade as *S. borin* from Europe and Cyprus in the Eastern Mediterranean, with no signs of significant divergence (clade I).

S. atricapilla from North of Iran (FUM 170808217 and FUM 170808221) are in the same clade as European and African (Djibouti) *S. atricapilla*, with no signs of significant divergence (clade I).

All *S. communis* from Iran (FUM 170522156, FUM 170814242, FUM 170814240 and FUM 170808218) are in the same clade as African, European and Kazakh samples, two of them (FUM 170814240, FUM 170814242) possibly diverging slightly (clade IV). Two samples of *S. mystacea* from Iran are correctly in the same clade as a Russian *S. mystacea*. *S. crassirostris* from Fars in Iran are in the same clade with Greece and Norway samples, without signs of significant divergence (clade V). Of course, the Norway sample represents a vagrant and is not biogeographically interesting.

Discussion

Our analysis can be compared with the Glutz and Bauer (1991) study. They proposed 6 groups: *Undata* (*S. sarda*, *S. undata*, *S. deserticola*), *Melanocephala* (*S. cantillans*, *S. mystacea*, *S. melanothorax*, *S. ruppelli*, *S. melanocephala*), *Hortensis* (*S. leucomelaena*, *S. nisoria*, *S. hortensis*), *Curruca* (*S. curruca*, *S. minula*, *S. althaea*), *Atricapilla* (*S. atricapilla*, *S. borin*) and *Communis* (*S. communis*, *S. conspiciolata*, *S. nana*) based on morphological characteristics, dispersal patterns, general ecology and behavior of species.

Table 2. The information of GenBank dataset for Iranian *Sylvia* Warblers.

<i>Scientific name</i>	Origin	GenBank AC NO.		<i>Scientific name</i>	Origin	GenBank AC NO.		<i>Scientific name</i>	Origin	GenBank AC NO.	
		<i>cytb</i>	<i>cox1</i>			<i>cytb</i>	<i>cox1</i>			<i>cytb</i>	<i>cox1</i>
<i>Acrocephalus scirpaceus</i>	-	KF614612.1	-	<i>S. layardi</i>	South Africa	JF502296.1	-	<i>S. mystacea</i>	Iran	JF502302.1	-
<i>S. borin</i>	Uganda	AY124540.1	-	<i>S. minula</i>	China	KC512637.1	-	<i>S. melanocephala</i>	Spain	AJ534544.1	-
<i>S. borin</i>	Malawi	JF502280.1	-	<i>S. minula</i>	China	KC512635.1	-	<i>S. melanocephala</i>	Morocco	KY378798.1	-
<i>S. borin</i>	Russia	JF502281.1	-	<i>S. minula</i>	China	KC512634.1	-	<i>S. melanocephala</i>	Spain	KY378799.1	-
<i>S. borin</i>	Germany	AJ534549.1	-	<i>S. minula</i>	China	KC512636.1	-	<i>S. melanocephala</i>	Spain	KY378797.1	-
<i>P. atriceps</i>	Uganda	JN827158.1	-	<i>S. minula</i>	China	KC512611.1	-	<i>S. sarda</i>	-	FN908758.1	-
<i>P. atriceps</i>	Burundi	JN827159.1	-	<i>S. minula</i>	China	KC512610.1	-	<i>S. sarda</i>	Corsica	JF502309.1	-
<i>L. nigricapillus</i>	South Africa	JN827092.1	-	<i>S. minula</i>	China	KC512609.1	-	<i>S. undata</i>	France	AJ534542.1	-
<i>L. nigricapillus</i>	South Africa	JN827093.1	-	<i>S. minula</i>	China	KC512608.1	-	<i>S. deserticola</i>	Morocco	AJ534540.1	-
<i>S. atricapilla</i>	Russia	JF502273.1	-	<i>S. minula</i>	China	KC512607.1	-	<i>S. balearica</i>	Spain	AJ534541.1	-
<i>S. atricapilla</i>	Russia	JF502276.1	-	<i>S. minula</i>	China	KC512605.1	-	<i>S. melanothorax</i>	Mediterian area	AJ534546.1	-
<i>S. atricapilla</i>	Greece	JF502274.1	-	<i>S. minula</i>	China	KC512604.1	-	<i>S. melanothorax</i>	-	KJ456482.1	-
<i>S. atricapilla</i>	Greece	AF074596.1	-	<i>S. minula</i>	China	KC512612.1	-	<i>S. melanothorax</i>	-	JF502301.1	-
<i>S. atricapilla</i>	Greece	AY308735.1	-	<i>S. minula</i>	China	KC512606.1	-	<i>S. rueppelli</i>	Mediterian area	AJ534547.1	-
<i>S. atricapilla</i>	Mediterian area	AM889140.1	-	<i>S. curruca</i>	England	KC512659.1	-	<i>S. rueppelli</i>	-	JF502307.1	-
<i>S. atricapilla</i>	Mediterian area	NC010228.1	-	<i>S. curruca</i>	Sweden	KC512658.1	-	<i>S. rueppelli</i>	-	JF502308.1	-
<i>S. atricapilla</i>	Greece	JF502275.1	-	<i>S. curruca</i>	Sweden	KC512655.1	-	<i>S. conspicillata</i>	Senegal	AJ534539.1	-
<i>S. cantillans</i>	Sweden	LN650644.1	-	<i>S. curruca</i>	Sweden	KC512656.1	-	<i>S. conspicillata</i>	Spain	KF517355.1	-
<i>S. cantillans</i>	UK	HF562847.1	-	<i>S. curruca</i>	Sweden	KC512652.1	-	<i>S. conspicillata</i>	Spain	KF517358.1	-
<i>S. cantillans</i>	Italy	JN048682.1	-	<i>S. curruca</i>	Sweden	KC512654.1	-	<i>S. conspicillata</i>	Spain	KF517367.1	-
<i>S. cantillans</i>	France	JN048676.1	-	<i>S. curruca</i>	Germany	AJ534536.1	-	<i>S. conspicillata</i>	Spain	KF517357.1	-
<i>S. cantillans</i>	France	JN048679.1	-	<i>S. curruca</i>	Sweden	KC512653.1	-	<i>S. conspicillata</i>	Spain	KF517353.1	-
<i>S. cantillans</i>	France	JN048677.1	-	<i>S. curruca</i>	Sweden	KC512657.1	-	<i>S. conspicillata</i>	Spain	KF517352.1	-
<i>S. cantillans</i>	Spain	AJ534543.1	-	<i>S. margelanica</i>	Mongolia	KC512603.1	-	<i>S. conspicillata</i>	Cape Verde	KF517361.1	-
<i>S. cantillans</i>	Senegal	JF502283.1	-	<i>S. margelanica</i>	China	KC512591.1	-	<i>S. conspicillata</i>	Morocco	KF517360.1	-
<i>S. mystacea</i>	Kuwait	AJ534545.1	-	<i>S. margelanica</i>	China	KC512590.1	-	<i>S. conspicillata</i>	Morocco	KF517359.1	-

Table 2: Continuous

Scientific name	Origin	GenBank AC NO.		Scientific name	Origin	GenBank AC NO.		Scientific name	Origin	GenBank AC NO.	
		<i>cytb</i>	<i>cox1</i>			<i>cytb</i>	<i>cox1</i>			<i>cytb</i>	<i>cox1</i>
<i>S. margelanica</i>	China	KC512585.1	-	<i>S. conspicillata</i>	Spain	KF517356.1	-	<i>S. althaea</i>	Kazakhstan	KC512557.1	-
<i>S. margelanica</i>	China	KC512584.1	-	<i>S. conspicillata</i>	Spain	KF517354.1	-	<i>S. althaea</i>	Kyrgyzstan	KC512566.1	-
<i>S. margelanica</i>	China	KC512588.1	-	<i>S. conspicillata</i>	Portugal	KF517365.1	-	<i>S. althaea</i>	Kyrgyzstan	KC512565.1	-
<i>S. margelanica</i>	China	KC512582.1	-	<i>S. conspicillata</i>	Cape Verde	KF517363.1	-	<i>S. althaea</i>	Pakistan	KC512563.1	-
<i>S. margelanica</i>	China	KC512580.1	-	<i>S. conspicillata</i>	Cape Verde	KF517362.1	-	<i>S. althaea</i>	Kazakhstan	KC512570.1	-
<i>S. margelanica</i>	China	KC512577.1	-	<i>S. conspicillata</i>	Spain	KF517366.1	-	<i>S. crassirostris</i>	Mediterranean area	AJ534535.1	-
<i>S. margelanica</i>	China	KC512576.1	-	<i>S. conspicillata</i>	Cape Verde	KF517364.1	-	<i>S. crassirostris</i>	Mediterranean area	NC010229.1	-
<i>S. margelanica</i>	Kazakhstan	KC512575.1	-	<i>S. communis</i>	Germany	AJ534538.1	-	<i>S. crassirostris</i>	Mediterranean area	AM889141.1	-
<i>S. margelanica</i>	China	KC512589.1	-	<i>S. communis</i>	Russia	JF502288.1	-	<i>S. crassirostris</i>	Greece	JF502290.1	-
<i>S. margelanica</i>	China	KC512581.1	-	<i>S. communis</i>	Germany	JF502285.1	-	<i>S. crassirostris</i>	Greece	JF502295.1	-
<i>S. margelanica</i>	China	KC512578.1	-	<i>S. communis</i>	Russia	JF502287.1	-	<i>S. lugens</i>	Ethiopia	AJ534532.1	-
<i>S. margelanica</i>	China	KC512579.1	-	<i>S. communis</i>	Slovakia	HQ608840.1	-	<i>S. lugens</i>	Tanzania	JF502298.1	-
<i>S. margelanica</i>	China	KC512574.1	-	<i>S. communis</i>	Greece	JF502286.1	-	<i>S. buryi</i>	Saudi Arabia	AJ534531.1	-
<i>S. margelanica</i>	China	KC512573.1	-	<i>S. nisoria</i>	Austria	AJ534527.1	-	<i>S. buryi</i>	-	JF502282.1	-
<i>S. margelanica</i>	China	KC512587.1	-	<i>S. nisoria</i>	Moldova	JF502305.1	-	<i>S. hortensis</i>	Spain	AJ534534.1	-
<i>S. margelanica</i>	China	KC512586.1	-	<i>S. nisoria</i>	Russia	JF502306.1	-	<i>S. leucomelaena</i>	Saudi Arabia	AJ534533.1	-
<i>S. margelanica</i>	China	KC512583.1	-	<i>S. layardi</i>	South Africa	AJ534528.1	-	<i>S. boehmi</i>	Ethiopia	AJ534530.1	-
<i>S. blythi</i>	Kazakhstan	KC512452.1	-	<i>S. layardi</i>	South Africa	JF502297.1	-	<i>S. boehmi</i>	Kenya	JF502279.1	-
<i>S. blythi</i>	Kazakhstan	KC512451.1	-	<i>S. althaea</i>	Kyrgyzstan	KC512568.1	-	<i>S. boehmi</i>	Kenya	JF502278.1	-
<i>S. blythi</i>	Kazakhstan	KC512450.1	-	<i>S. althaea</i>	Kazakhstan	KC512567.1	-	<i>S. subcaeruleum</i>	South Africa	AJ534529.1	-
<i>S. blythi</i>	Kazakhstan	KC512449.1	-	<i>S. althaea</i>	Kyrgyzstan	KC512564.1	-	<i>S. subcaeruleum</i>	South Africa	JF502311.1	-
<i>S. blythi</i>	Kazakhstan	KC512448.1	-	<i>S. althaea</i>	Kyrgyzstan	KC512562.1	-	<i>S. subcaeruleum</i>	South Africa	KY273786.1	-
<i>S. halimodendri</i>	Oman	KC512542.1	-	<i>S. althaea</i>	Kyrgyzstan	KC512561.1	-	<i>S. subcaeruleum</i>	South Africa	JF502310.1	-
<i>S. halimodendri</i>	Oman	KC512541.1	-	<i>S. althaea</i>	Kyrgyzstan	KC512560.1	-	<i>S. nana</i>	Mediterranean area	AJ534537.1	-
<i>S. althaea</i>	Afghanistan	KC512572.1	-	<i>S. althaea</i>	Kyrgyzstan	KC512559.1	-	<i>S. nana</i>	South Africa	JF502303.1	-
<i>S. althaea</i>	Kazakhstan	KC512569.1	-	<i>S. althaea</i>	Kyrgyzstan	KC512558.1	-	<i>S. atricapilla</i>	Russia	-	GQ482701

Table 2: Continuous

Scientific name	Origin	GenBank AC NO.		Scientific name	Origin	GenBank AC NO.		Scientific name	Origin	GenBank AC NO.	
		cytb	cox1			cytb	cox1			cytb	cox1
<i>S. atricapilla</i>	Netherlands	-	KF946870.1	<i>S. communis</i>	Russia	-	GQ482719.1	<i>S. curruca</i>	Mongolia	-	GQ482724.1
<i>S. atricapilla</i>	Russia	-	GQ482707.1	<i>S. communis</i>	Netherlands	-	KF946877.1	<i>S. nisoria</i>	Kazakhstan	-	GQ482733.1
<i>S. atricapilla</i>	Netherlands	-	KF946866.1	<i>S. communis</i>	Netherlands	-	KF946878.1	<i>S. nisoria</i>	Norway	-	GU571647.1
<i>S. atricapilla</i>	Netherlands	-	KF946871.1	<i>S. communis</i>	Netherlands	-	KF946879.1	<i>S. nisoria</i>	Norway	-	GU571648.1
<i>S. atricapilla</i>	Norway	-	GU571641.1	<i>S. communis</i>	Norway	-	GU571643.1	<i>S. nisoria</i>	Kazakhstan	-	GQ482736.1
<i>S. atricapilla</i>	Sweden	-	GU572115.1	<i>S. curruca</i>	Netherlands	-	KF946887.1	<i>S. nisoria</i>	Russia	-	GQ482735.1
<i>S. atricapilla</i>	Cyprus	-	KT803684.1	<i>S. curruca</i>	Netherlands	-	KF946888.1	<i>S. nisoria</i>	Russia	-	GQ482734.1
<i>S. atricapilla</i>	Djibou	-	MF580223.1	<i>S. curruca</i>	Netherlands	-	KF946889.1	<i>S. nisoria</i>	Netherlands	-	KF946891.1
<i>S. atricapilla</i>	Sweden	-	GU572116.1	<i>S. curruca</i>	Netherlands	-	KF946890.1	<i>S. nisoria</i>	Sweden	-	GU572122.1
<i>S. atricapilla</i>	Russia	-	GQ482709.1	<i>S. curruca</i>	Norway	-	GU571645.1	<i>S. nisoria</i>	Sweden	-	GU572123.1
<i>S. atricapilla</i>	Russia	-	GQ482705.1	<i>S. curruca</i>	Norway	-	GU571646.1	<i>S. mystacea</i>	Russia	-	GQ482730.1
<i>S. atricapilla</i>	Russia	-	GQ482702.1	<i>S. curruca</i>	Russia	-	GQ482729.1	<i>S. mystacea</i>	Russia	-	JQ176321.1
<i>S. atricapilla</i>	Russia	-	GQ482703.1	<i>S. curruca</i>	Sweden	-	GU572121.1	<i>S. melanocephala</i>	Cyprus	-	KT803679.1
<i>S. atricapilla</i>	Russia	-	GQ482704.1	<i>S. curruca</i>	Cyprus	-	KT803683.1	<i>S. melanocephala</i>	Cyprus	-	FJ465368.1
<i>S. atricapilla</i>	Russia	-	GQ482706.1	<i>S. curruca</i>	Turkey	-	KX283164.1	<i>S. melanocephala</i>	Cyprus	-	FJ465369.1
<i>S. atricapilla</i>	Netherlands	-	KF946868.1	<i>S. curruca</i>	Turkey	-	KX283165.1	<i>S. cantillans</i>	Greece	-	JQ176318.1
<i>S. atricapilla</i>	Netherlands	-	KF946869.1	<i>S. curruca</i>	Russia	-	GQ482722.1	<i>S. conspicillata</i>	Cyprus	-	KT803681.1
<i>S. atricapilla</i>	Russia	-	GQ482708.1	<i>S. curruca</i>	Russia	-	GQ482728.1	<i>S. undata</i>	-	-	KP780415.1
<i>S. atricapilla</i>	Turkey	-	KX283161.1	<i>S. curruca</i>	Russia	-	GQ482721.1	<i>S. melanothorax</i>	Cyprus	-	KT803682.1
<i>S. crassirostris</i>	Greece	-	JQ176320.1	<i>S. curruca</i>	Russia	-	GQ482727.1	<i>S. communis</i>	Norway	-	GU571642.1
<i>S. crassirostris</i>	Norway	-	GU571644.1	<i>S. curruca</i>	Mongolia	-	GQ482723.1	<i>S. communis</i>	Russia	-	GQ482714.1
<i>S. curruca</i>	Netherlands	-	KF946883.1	<i>S. curruca</i>	Kazakhstan	-	GQ482725.1	<i>S. communis</i>	Kazakhstan	-	GQ482717.1
<i>S. curruca</i>	Netherlands	-	KF946884.1	<i>S. curruca</i>	Russia	-	GQ482726.1	<i>S. communis</i>	Russia	-	GQ482720.1
<i>S. curruca</i>	Netherlands	-	KF946885.1	<i>S. curruca</i>	Netherlands	-	KF946881.1	<i>S. communis</i>	Netherlands	-	KF946876.1
<i>S. curruca</i>	Netherlands	-	KF946886.1	<i>S. curruca</i>	Netherlands	-	KF946882.1	<i>S. communis</i>	Netherlands	-	KF946880.1
<i>S. communis</i>	Russia	-	GQ482718.1	<i>S. curruca</i>	Russia	-	JQ176319.1	<i>S. communis</i>	Russia	-	GQ482715.1

Table 2: Continuous

Scientific name	Origin	GenBank AC NO.		Scientific name	Origin	GenBank AC NO.		Scientific name	Origin	GenBank AC NO.	
		cytb	cox1			cytb	cox1			cytb	cox1
<i>S. communis</i>	Russia	-	GQ482716.1	<i>S. borin</i>	Netherlands	-	KF946875.1	<i>S. cantillans</i>	-	-	KX224439.1
<i>S. communis</i>	Sweden	-	GU572119.1	<i>S. borin</i>	Russia	-	GQ482713.1	<i>S. cantillans</i>	-	-	KX224433.1
<i>S. communis</i>	Sweden	-	GU572120.1	<i>S. borin</i>	Sweden	-	GU572117.1	<i>S. cantillans</i>	-	-	KX224432.1
<i>S. communis</i>	Cyprus	-	KT803680.1	<i>S. borin</i>	Cyprus	-	KT803685.1	<i>S. cantillans</i>	-	-	KX224431.1
<i>S. nana</i>	Mongolia	-	GQ482731.1	<i>S. borin</i>	Sweden	-	GU572118.1	<i>S. cantillans</i>	-	-	KX224430.1
<i>S. nana</i>	Mongolia	-	GQ482732.1	<i>S. cantillans</i>	-	-	KX224442.1	<i>S. cantillans</i>	-	-	KX224429.1
<i>S. nana</i>	Mongolia	-	JQ176322.1	<i>S. cantillans</i>	-	-	KX224441.1	<i>S. cantillans</i>	-	-	KX224428.1
<i>S. borin</i>	Russia	-	GQ482710.1	<i>S. cantillans</i>	-	-	KX224440.1	<i>S. cantillans</i>	-	-	KX224427.1
<i>S. borin</i>	Russia	-	GQ482711.1	<i>S. cantillans</i>	-	-	KX224438.1	<i>S. cantillans</i>	-	-	KX224426.1
<i>S. borin</i>	Russia	-	GQ482712.1	<i>S. cantillans</i>	-	-	KX224437.1	<i>S. cantillans</i>	-	-	KX224425.1
<i>S. borin</i>	Netherlands	-	KF946873.1	<i>S. cantillans</i>	-	-	KX224436.1	<i>S. cantillans</i>	-	-	KX224424.1
<i>S. borin</i>	Netherlands	-	KF946872.1	<i>S. cantillans</i>	-	-	KX224435.1	<i>Acrocephalus scirpaceus</i>	-	-	KC755399.1
<i>S. borin</i>	Netherlands	-	KF946874.1	<i>S. cantillans</i>	-	-	KX224434.1	-	-	-	-

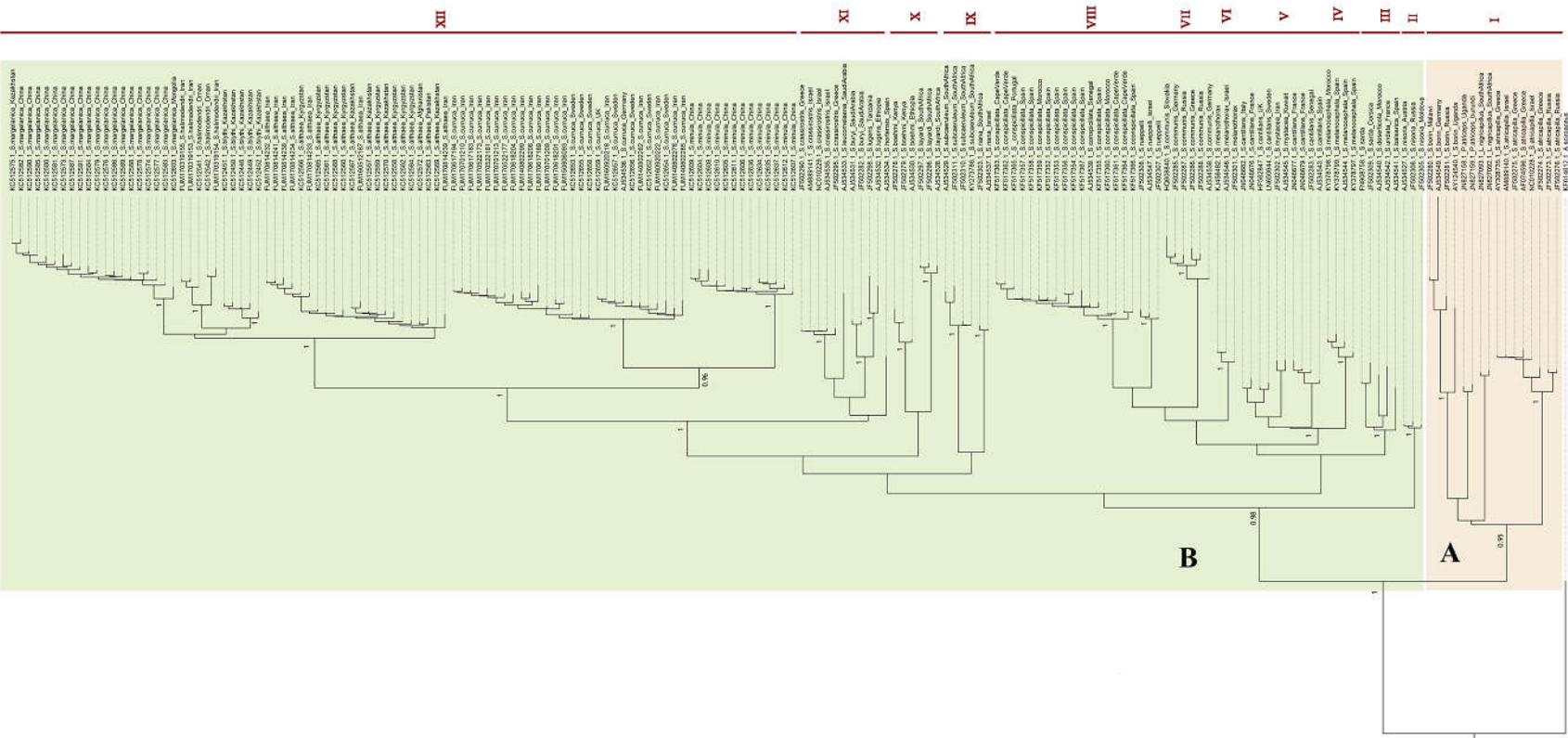


Fig. 2. Phylogeny of *Sylvia* warblers, based on Bayesian analyses of mitochondrial *cytb*: Clade A) *P. atriceps*, *L. nigricapillus*, *S. borin* and *S. atricapilla*; Clade B) All other species in *Sylvia* genus. The *cytb* analysis recovers eight groups (Greek numbers).

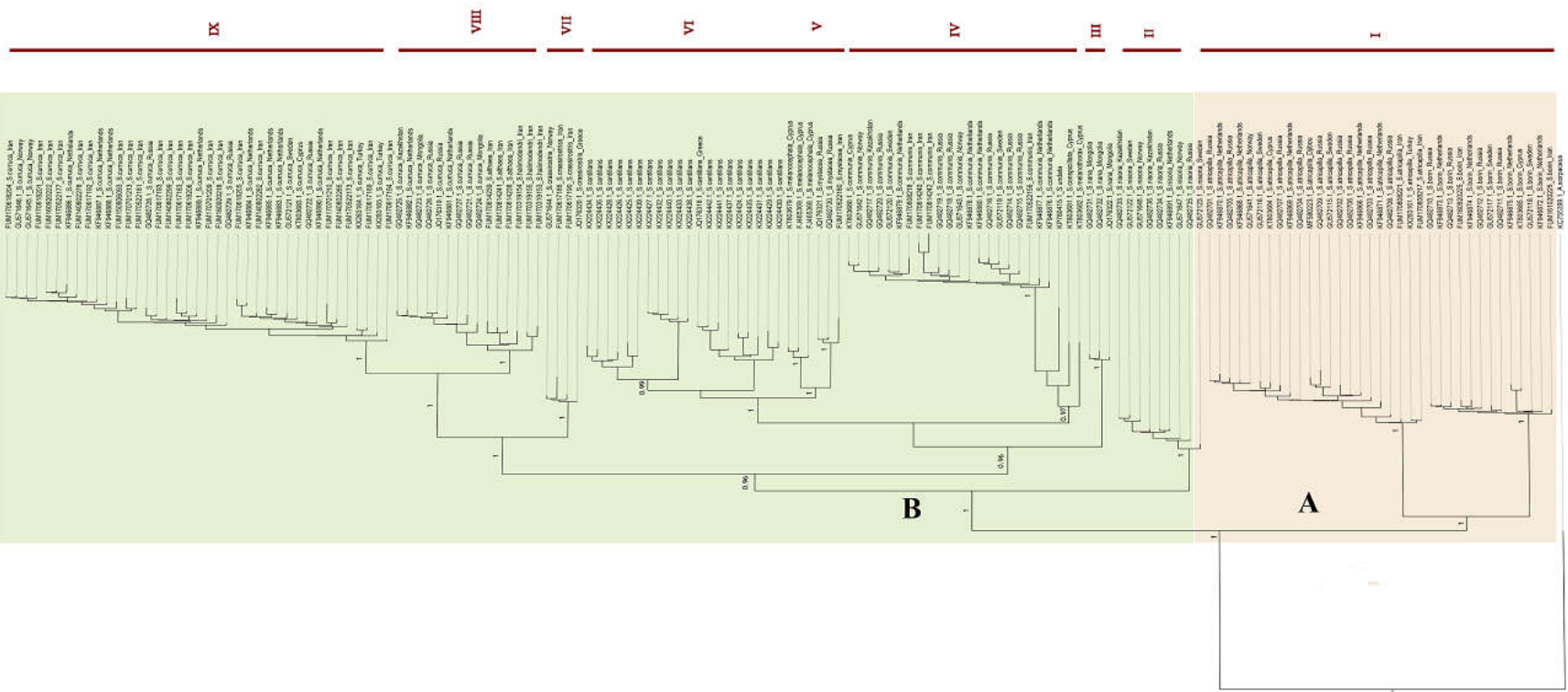


Fig. 3. Phylogeny of *Sylvia* warblers, based on Bayesian analyses of mitochondrial *cox1*: Clade A) *S. borin* and *S. atricapilla*; Clade B) All other species in *Sylvia* genus. The *cytb* analysis recovers eight groups (Greek numbers).

In our *cytb* analysis we recovered two main clades and eight groups compatible with this taxonomy (Fig. 3), *I*: *Atricapilla* (*S. atricapilla*, *S. borin*), *II*: *Nisoria* (*S. nisoria*), *III*: *Undata* (*S. undata*, *S. balearica*, *S. deserticola*, *S. sarda*), *IV*, *V*, *VI*, *VII*, *VIII*: *Melanocephala* (*S. melanocephala*, *S. melanothorax*, *S. rueppelli*, *S. mystacea*, *S. cantillans*, *S. communis*, *S. conspicillata*), *IX*: *Nana* (*S. nana*, *S. subcaeruleum*), *X*: *Layardi* (*S. layardi*, *S. boehmi*), *XI*: *Hortensis* (*S. hortensis*, *S. crassirostris*, *S. leucomelaena*, *S. lugens*, *S. buryi*), *XII*: *Curruca* (*S. margelanica*, *S. blythi*, *S. althaea*, *S. curruca*, *S. minula*, *S. halimodendri*).

According to the *cytb* tree (Fig. 2), *S. atricapilla* and *S. borin* belong in the same clade as *Pseudoalcippe atriceps* and *Lioptilus nigricapillus* (clade B). This clade is so deeply diverged from the rest of the included species (clade A) that treatment as a separate genus may be warranted.

According to Blondel et al., (1996), there were three main groups based on DNA-DNA hybridization: A West-Mediterranean group, a Central-Mediterranean group, and an East-Mediterranean. Also, based on Voelker and Light (2011), based on the two mitochondrial genes *cytb* and *ND2*, this genus was divided into three main clades, the African-European species, the African-Asian species, and the Mediterranean species. Our study doesn't support these results and we didn't recover these groups. All Iranian samples were conclusively identified to species level.

Conclusion

According to the Bayesian trees, *S. atricapilla* and *S. borin* are in the same clade as *Pseudoalcippe atriceps* and *Lioptilus nigricapillus*. This clade is so deeply diverged from the rest of the included species that treatment as a separate genus may be warranted. In our *cytb* analysis we recovered two main clades and eight groups. Our study did not support the geographical groups according the previous studies: The West-Mediterranean, the Central-Mediterranea and the East-Mediterranean groups. We confirmed the following taxa to occur in Iran: *Sylvia borin*, *Sylvia atricapilla*, *Sylvia curruca curruca*, *Sylvia curruca halimodendri*, *Sylvia althaea*, *Sylvia crassirostris*, *Sylvia mystacea* and *Sylvia nisoria*.

All Iranian samples were conclusively identified to species level, and none of them give rise to any further taxonomic inquiry.

Acknowledgements

This research funded by Ferdowsi University of Mashhad (3.42780). We thankful to the department of Environment (DOE) for providing the permit and logistical support for blood sampling in Iran (No. 96.9101). We appreciate the contributions in the early stages of this research by former supervisor, the late Prof. Jamshid Darvish. We are grateful to Vali Ebadi and Leila Noorani that provided samples from Khalkhal and Tandoureh respectively, Maliheh Pirayesh helped in lab works and Hassan Abdilizadeh, Mehri Ahmadi, Mehdi Elahi, Vajiheh Ghaemi, Somayyeh Pourebrahimi, Mohammad Safrang, Sajjad Jafari, Babak Mousavi, Dr. Arya Shafayipour, Dr. Reza Moussavi-Harami, the Galeshi family, Mohammad Ebrahim Sehat, Hossein Barahoyi, Yahya Tarahhomi and also, not least, all the environment guards from the sampling areas for their helping in both sampling and a variety of different ways.

Conflicts of interest

The authors have no conflict of interest to declare.

References

- Aliabadian M, Kaboli M, Nijman V, Vences M. 2009. Molecular identification of birds: performance of distance-based DNA barcoding in three genes to delimit parapatric species. *Plos One* 4(1): e4119.
- Blondel J, Catzeflis F, Perret P. 1996. Molecular phylogeny and the historical biogeography of the warblers of the genus *Sylvia* (Aves). *J Evol Biol* 9: 871–891.
- Böhning-Gaese K, Bauer HG. 1996. Changes in species abundance, distribution, and diversity in a central European bird community. *Conserv Biol* 10(1): 175-187.
- Böhning-Gaese K, Caprano T, Van Ewijk K, Veith M. 2006. Range Size: Disentangling current traits and phylogenetic and biogeographic factors. *Am Nat* 167: 555-567
- Böhning-Gaese K, Schuda MD, Helbig AJ. 2003. Weak phylogenetic effects on

- ecological niches of *Sylvia* warblers. *J Evol Biol* 16: 956-965.
- Brambilla M, Vitulano S, Spina F, Baccetti N, Gargallo G, Fabbri E, Guidali F, Randi E. 2008. A molecular phylogeny of the *Sylvia cantillans* complex: cryptic species within the Mediterranean basin. *Mol Phylogenet Evol* 48(2): 461-472.
- Bruford MW, Hanotte O, Brookfield JFY, Burke T. 1992. Single-locus and multilocus DNA fingerprint. In AR Hoelzel (Ed.), *Molecular genetic analysis of populations: a practical approach* (pp. 225-270) Oxford, IRL Press, UK.
- Clements JF, Schulenberg TS, Iliff MJ, Roberson D, Fredericks TA, Sullivan BL, Wood CL. 2016. The eBird/Clements checklist of birds of the world. *The Cornell Lab Ornithology Ithaca*, New York.
- Cramp S. 1992. The Birds of the Western Palearctic, Vol. 7. Oxford University Press, Oxford.
- Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 9(8), 772.
- Dickinson EC, Christidis L. (Eds.) 2014. The Howard and Moore complete checklist of the birds of the world. 4th ed., Vol. 2- passerines, Aves Press, Eastbourne, UK.
- Doswald N, Willis SG, Collingham YC, Pain DJ, Green RE, Huntley B. 2009. Potential impacts of climatic change on the breeding and non-breeding ranges and migration distance of European *Sylvia* warblers. *J Biogeogr* 36:1194-1208
- Gargallo G, 1994. On the taxonomy of the western Mediterranean islands populations of Subalpine Warbler *Sylvia cantillans*. *Bull. Br. Ornithol. Club* 114: 31-36
- Gill F, Donsker D. 2019. IOC world bird list (Vol 9.1). <https://doi.org/Doi10.14344/IOC.ML.9.1>.
- Glutz VBU, Bauer KM. 1991. Handbuch der vögel mitteleuropas, band 12/I, Passeriformes (3. Teil). Aula, Wiesbaden.
- Gwinner E, Wiltschko W. 1978. Endogenously controlled changes in migratory direction of the Garden Warbler, *Sylvia borin*. *J Comp Physiol* 125:267-273.
- Hall BP, Moreau RE. 1970. An atlas of speciation in African passerine birds. Trustees of the British Museum (Natural History), London.
- Hall T. 1999. BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Res* 41: 95-98.
- Hasegawa M, Kishino K, Yano T. 1985. Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *J Mol Evol* 22:160-174.
- Jordano P. 1987. Frugivory, external morphology and digestive system in Mediterranean sylviid warblers *Sylvia* spp. *Ibis* 129: 175-189.
- Lanave C, Preparata C, Saccone C, Serio G. 1984. A new method for calculating evolutionary substitution rates. *J Mol Evol* 20: 86-93.
- Loskot VM. 2005. Morphological variation and taxonomic revision of five south-eastern subspecies of Lesser Whitethroat *Sylvia curruca* (L.) (Aves: Sylviidae). *Zool Mededelingen Leiden* 79: 157-165.
- Martens J, Steil B. 1997. Reviergesänge und Speziesdifferenzierung in der Klappergrasmücken-Gruppe *Sylvia* [curruca]. *J Ornithol* 138: 1-23.
- Mayr E, Cottrell GW. 1986. Checklist to the Birds of the World, vol. XI. Museum of Comparative Zoology, Cambridge, MA.
- Miller MA, Pfeiffer W, Schwartz T. 2010 November. Creating the CIPRES science gateway for inference of large phylogenetic trees. In 2010 Gateway Computing Environments Workshop (GCE). pp. 1-8. IEEE.
- Olsson U, Alström P, Ericson PG, Sundberg P. 2005. Non-monophyletic taxa and cryptic species-evidence from a molecular phylogeny of leaf-warblers (*Phylloscopus*, Aves). *Mol Phylogenet Evol* 36: 261-276.
- Olsson U, Leader P J, Carey G J, Khan AA, Svensson L, Alström P. 2013. New insights into the intricate taxonomy and phylogeny of the *Sylvia curruca* complex. *Mol Phylogenet Evol* 67(1): 72-85.
- Patel S, Waugh J, Millar CD, Lambert DM. 2010. Conserved primers for DNA barcoding historical and modern samples from New

- Zealand and Antarctic birds. *Mol Ecol Resour* 10(3): 431-438.
- Portenko LA. 1960. The birds of the USSR. Part IV. Acad Sci, USSR, Russian.
- Rambaut A. 2008. FigTree, a graphical viewer of phylogenetic trees. Retrieved from <http://tree.bio.ed.ac.uk/software/figtree/>
- Ripley SD. 1982. A synopsis of the birds of India and Pakistan: together with those of Nepal, Bhutan, Bangladesh, and Sri Lanka /
- Rodríguez J, Oliver L, Marín A, Medina R. 1990. The general stochastic model of nucleotide substitution. *J Theor Biol* 142: 485-501.
- Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck, JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* 61(3): 539-542.
- Seutin G, White BN, Boag PT. 1991. Preservation of avian blood and tissue samples for DNA analyses. *Can J Zool* 69(1): 82-90.
- Shirihai H, Gargallo G, Helbig A, Harris A, Cottridge D, Roselaar D. 2001. *Sylvia* warblers: identification, taxonomy and phylogeny of the Genus *Sylvia*. Christopher Helm, AC Black, London.
- Sibley CG, Ahlquist, JE. 1990. Phylogeny and classification of birds: a study in molecular evolution. Yale University Press.
- Sibley CG, Monroe BL. 1990. Distribution and taxonomy of birds of the world. Yale University Press.
- Svensson L. 1992. Identification guide to european passerines, Fourth ed. Stockholm.
- Tavaré S. 1986. Some probabilistic and statistical problems on the analysis of DNA sequences. *Lec Math Life Sci* 17: 57-86
- Vaurie C. 1954. Systematic notes on Palearctic birds, No 11. Sylviinae: the genus *Sylvia*. *Am Mus Novit* 1692: 1-17.
- Voelker G, Light JE. 2011. Palaeoclimatic events, dispersal and migratory losses along the Afro-European axis as drivers of biogeographic distribution in *Sylvia* warblers. *Bmc Evol Biol* 11(1): 163.
- Voelker G, Melo M, Bowie RCK. 2009. A gulf of Guinea island endemic is a member of a Mediterranean-centered bird genus. *Ibis* 151(3): 580-583
- Votier SC, Aspinall S, Bearhop S, Bilton D, Newton J, Alström P, Olsson U. 2016. Stable isotopes and mtDNA reveal niche segregation but no evidence of intergradation along a habitat gradient in the Lesser Whitethroat complex (*Sylvia curruca*; Passeriformes; Aves). *J Ornithol* 157(4): 1017-1027.
- Williamson, K. 1976. Identification for Ringers No. 3: The Genus *Sylvia*, Second Reprinted. British Trust for Ornithology, Tring.