



New Record of a Population of *Telestes souffia* (Risso, 1827) (Actinopterygii: Cyprinidae) in Serbia

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Abstract: Western vairone *Telestes souffia* (Risso, 1827) is a small-sized leuciscine species inhabiting rivers in Mediterranean, North and Black Sea drainages. Due to the lack of information on its occurrence in the waters of Serbia, there is a need for renewing knowledge about its geographical range. The new record of a population of *T. souffia* in Gračanica River (tributary of the Lim River, Danube River drainage) is provided based on morphological data and mtDNA cyt b gene analyses. Population belongs to the subspecies *T. s. agassii* (Valeciennes, 1844) (phylogenetic lineage *T. s. agassii* 1). The population from the Gračanica River exhibits moderate values of haplotype and nucleotide diversity. As morphological characteristics distinguishing this population from others are identified (1) the eye diameter in proportion to the head length and (2) the number of rays in the ventral fin.

Key words: *Telestes souffia agassii*, distribution, genetic characterization, Danube drainage

Introduction

For understanding patterns of biodiversity and processes that shape them, it is crucial to have accurate data about geographical and ecological distribution of species (WISZ et al. 2008). In general, information on fish species and trends in their populations are deficient at both local and global scales (REVENGA et al. 2005). Despite that, it is common practice to make conservation decisions at large scales; therefore, the accurate mapping of species distributions is immensely important for both fundamental and applied objectives (WISZ et al. 2008). In the Balkan Peninsula, despite the fact it represents a hotspot of European biodiversity, knowledge about the freshwater fish distribution is still insufficient (OIKONOMOU et al. 2014).

For species such as western vairone *Telestes souffia* (Risso, 1827), which is considered to have a relatively wide distribution (KETMAIER et al. 2004), the lack of information about the exact localities is even higher. According to BUJ et al. (2017), it inhabits rivers belonging to Mediterranean Sea (from Aude to Var drainages in France and Switzerland, Soča drainage in Slovenia and Italy), North Sea (Rhine drainage in Germany and Switzerland) and Black Sea drainages (Danube drainage from Germany downriver to Romania). Despite several overall studies of this species (SALZBURGER et al. 2003, KETMAIER et al. 2004, MÜNDEL et al. 2010, BUJ et al. 2017, VUCIĆ et al. 2017, CURTEAN-BĂNĂDUC et al. 2018), there are no recent data about its occurrence and condition of populations on the territory

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of Serbia. Findings date back to PANČIĆ (1860) who has mentioned the occurrence of this species (if the determination was accurate, see SIMIĆ et al. 2006) in the Crni Timok drainage. According to SIMONOVIĆ (2001), this species has been recorded (as *Leuciscus souffia agassizi* Valenciennes, 1844) in mountainous rivers of the Black Sea basin on the territory of Serbia. The most reliable findings are from the Drina River and upper course of the Serbian part of the Danube River (SIMONOVIĆ 2001). KRIVOKAPIC & MARIĆ (1993) reported a single specimen in lower course of the Tara River in Montenegro (Drina River drainage). DUBUT et al. (2012) recorded *T. s. agassi* in the upper part of the Lim River, a tributary of Drina River (Danube River drainage) in Montenegro.

In general, *T. souffia* occurs in river sections with clear and moderate- to fast-flowing water (CURTEAN-BĂNĂDUC et al. 2018).

Overall, *T. souffia* has been recognised as a threatened species. It is listed in Appendix III of the Bern Convention on the Conservation of European Wildlife and Natural Habitats and Appendix II of the E.U. Directive on the Conservation of Natural Habitats and Wild Fauna and Flora (MÜNDEL et al. 2010). By the International Union for the Conservation of Nature (IUCN), it is categorised as Least Concern (FREYHOF & KOTTELAT 2008). In Serbia, it is on the list of strictly protected species (ANONYMOUS 2010).

The taxonomy of the genus *Telestes* has not been resolved for a long time and there was possibility that several species might have been confused under the name *T. souffia* (see KOTTELAT & FREYHOF 2007). MACHORDOM et al. (1999) and SALZBURGER et al. (2003) subdivided *T. souffia* into two subspecies: the nominotypical *T. s. souffia* (= *Leuciscus souffia souffia*) from the Rhône drainage and *T. s. agassii* (= *Leuciscus souffia agassii*) from the Danube drainage. GILLES et al. (2010) performed phylogenetic reconstructions and multivariate analyses suggesting that *T. souffia* comprises three subspecies (*T. s. souffia*, *T. s. agassii* and *T. s. rysela*). Thus, based on the geographical distribution and on the information based on different genetic marker, GILLES et al. (2010) suggested that *T. s. rysela* corresponded to the lineage *T. s. agassii*-1, and *T. s. agassii* – to the lineage *T. s. agassii*-2.

DUBUT et al. (2012) have discovered that the *Telestes souffia* complex is structured in four populations that display non-overlapping distribution. *Telestes s. souffia* is distributed in the eastern half of France, mainly in the Rhône River drainage (Mediterranean basin), while the lineage *T. s. agassii* 3 is assigned to the Inn River (upper Danube, Black Sea

basin), and *T. s. agassii* 2 – to the Tizsa River system (right tributary of the middle Danube drainage). The fourth lineage, *T. s. agassii* 1, is distributed in both Lim River (Sava River system, left tributary of the middle Danube) and Soča River in the northern Adriatic Sea basin. BUJ et al. (2017) have confirmed that *T. souffia* shows significant intraspecific structuring, which is not characteristic for a single species.

In previous studies (ZACCARA et al. 2009, GILLES et al. 2010, BUJ et al. 2019), sequence data of the complete mtDNA cytochrome b gene were used for the reconstruction of the phylogenetic relationships within the genus *Telestes*. The cytochrome b gene was proved to be a suitable mtDNA marker for studying intraspecific genetic variability (KETMAIER et al. 2004, DUBUT et al. 2012, BUJ et al. 2017).

Taking into consideration the conservation status of the western vairone and the lack of recent data on its distribution, there is a need for renewing the information of its geographical range in Danube River drainage in Serbia. This work provides a new record of the western vairone in aquatic ecosystems of Serbia and genetic characterisation of the newly recorded population.

Materials and Methods

Sample collection

Throughout extensive field work related to fishery management plans in 2017 on the rivers in the Danube River drainage in Serbia, a special focus was put on revealing the potential new data of *T. souffia* distribution. Suitable potential localities (58) for field investigation were selected according to ecology of this species, information gathered from users of fishery waters on this territory, species occurrence based on literature and descriptions of catchment of local fishermen. The only locality where *T. souffia* was recorded (in June 2017) is the Gračanica River (43°18' 8.55" N, 019°40' 7.72" E), a left tributary of the Lim River in the Danube River drainage. Totally, 19 specimens were sampled with standardised electro-fishing method using electrofisher "Aquatech" IG1300 (2.6 kW, 80–470 V).

Determination of specimens

Of a total number of sampled specimens, 15 sexually mature specimens were identified using keys for determination (VUKOVIĆ & IVANOVIĆ 1971, SIMONOVIĆ 2001, KEITH & ALLARDI 2001, KOTTELAT & FREYHOF 2007). For proper identification of analysed specimens, the procedure was repeated twice. In order to avoid misunderstanding of count of the dorsal and anal fin branched rays, the two last

branched dorsal and anal rays, which are articulated on a single pterygiophore, were counted as 1½ (KOTTELAT & FREYHOF 2007).

Genetic characterisation of the population

For genetic studies, approximately 4 mm² of fin from all 19 specimens was sampled and stored in 96% ethanol. Sequence variations of complete mitochondrial cytochrome b gene (cyt b, 1140 bp) were studied. Methods of DNA isolation, amplification of cyt b gene and sequencing at MACROGEN® Europe, using modified Sanger sequencing method, were performed as described in BUJ et al. (2017). Sequences were edited and aligned using programs Chromas 2.6.5© Technelysium Pty. Ltd and Al-iView (LARSSON 2014) and compared using the tool BLAST (Basic Local Alignment Search Tool) to ones already deposited in Gene Bank (<http://www.ncbi.nlm.nih.gov/genbank/>) within NCBI base (National Center for Biotechnology Information) (WHEELER et al. 2006).

Mitochondrial cyt b haplotype network was constructed using all four evolutionary units of *T. souffia* complex (*sensu* DUBUT et al. 2012, BUJ et al. 2017). Available published sequences of cytochrome b (Appendix 1) were retrieved from the NCBI base (National Center for Biotechnology Information) (WHEELER et al. 2006). The haplotype network was constructed using the software PopART 1.7 (LEIGH & BRYANT 2015) following the median-joining network algorithm (BANDELT et al. 1999).

Haplotype (gene) diversity and nucleotide diversity calculations for populations belonging to lineage *T. s. agassii* 1 were performed in DnaSP v5 (LIBRADO & ROZAS 2009).

Results

The new record of *T. souffia* on the territory of Serbia represented a population from the Gračanica River (Fig. 1).

Selected morphological characters were measured based on 15 adult specimens sampled in this work (Table 1). Comparisons of meristic and metrical characteristics of the Gračanica population with data described by several authors (VUKOVIĆ & IVANOVIĆ 1971, SIMONOVIĆ 2001, KEITH & ALLARDI 2001, KOTTELAT & FREYHOF 2007) for other populations of *T. souffia* showed certain inter-population variations. The two key characteristics showing differences compared to literature data were the eye diameter in proportion to the head length and the number of rays in the ventral fin. For the general view of a specimen from the Gračanica River, see Fig. 2.

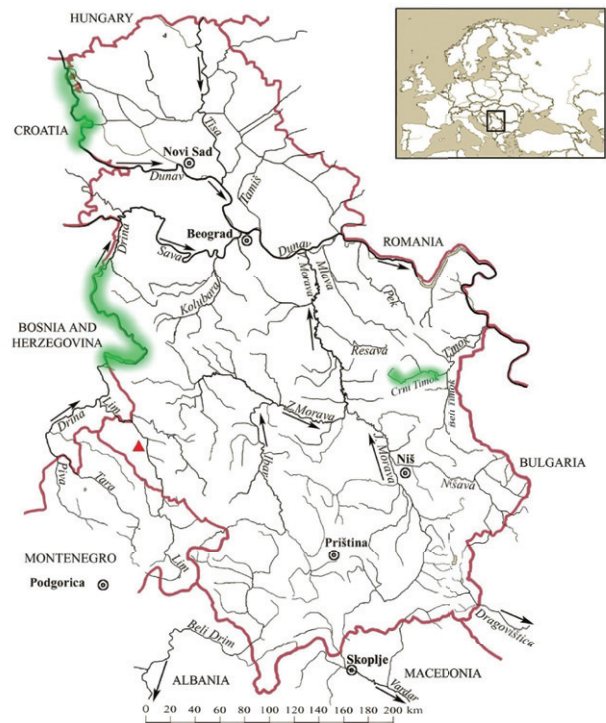


Fig. 1. Distribution of *Telestes souffia* in Serbia. Red triangle represents the newly-recorded population from the Gračanica River. Green areas represent distribution of this species in Serbia based on literature data.

Table 1. Comparative review of the key characters of the sampled specimens in the course of the present study and those described by previous authors (VUKOVIĆ & IVANOVIĆ 1971, SIMONOVIĆ 2001, KEITH & ALLARDI 2001, KOTTELAT & FREYHOF 2007).

Characters	Literature	Present study
Number of lateral line scales	50-57+2-3	50-53+2-3
Number of branched anal rays	9 ^{1/2}	9 ^{1/2}
Eye diameter in HL	4.0-4.6	3.6-3.8
Standard length	≤ 200	≤ 200
Number of rays in caudal fin	17-21	18-20
Scale rows between lateral line and pelvic origin	4 ^{1/2} -5	5
Number of branched rays in dorsal fin	7	7
Number of rays in ventral fin	10	9

Identification of mitochondrial cyt b haplotypes showed that specimens from the Gračanica River represented the lineage *T. s. agassii* 1 *sensu* DUBUT et al. (2012). Detected haplotypes from 19 individuals included in this study were BRN01 (accession number JQ651595) within 10 individuals, BRN02 (accession number JQ651596) within eight individuals and BRN15 (accession number JQ651608)

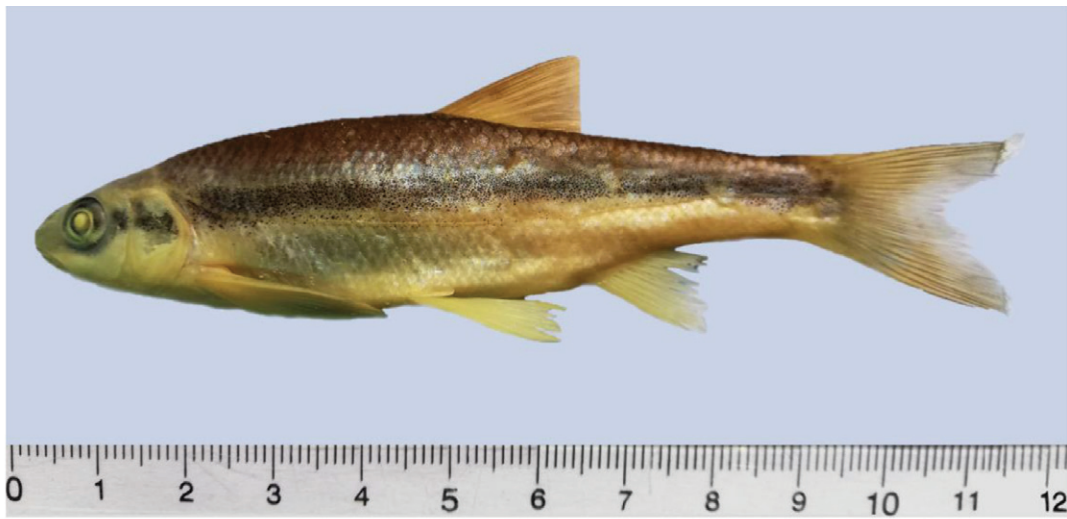


Fig. 2. Specimen of *Telestes souffia* from the Gračanica River (after preservation in 96% ethanol).

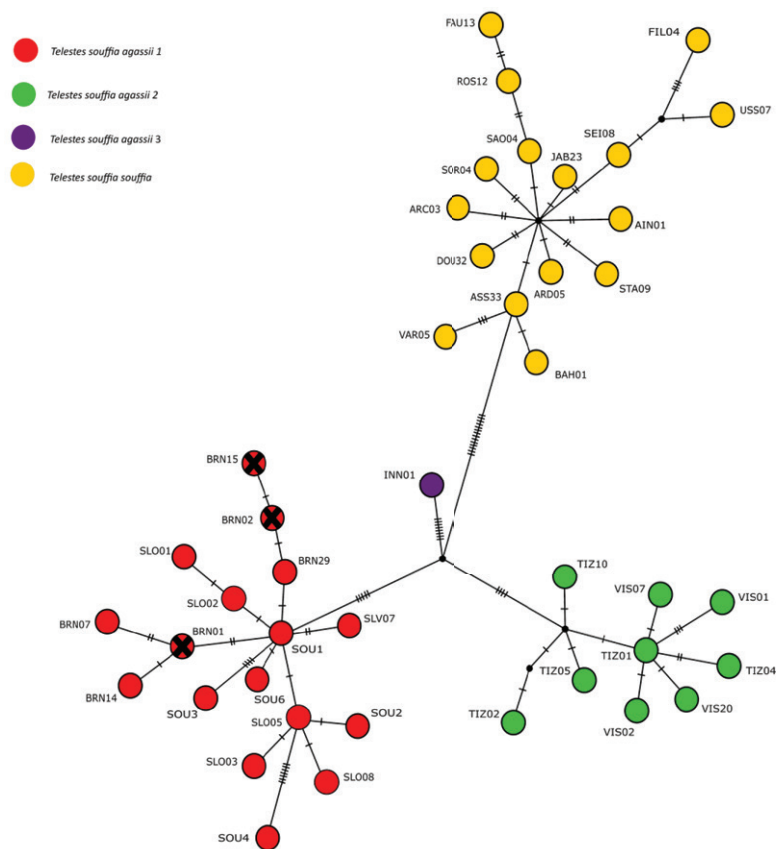


Fig. 3. Median-Joining network of the *T. souffia* complex mitochondrial *cyt b* haplotypes (haplotypes detected in this study are marked). Mutations are represented by slashes crossed with the network branches. The black circle indicates median vector as extinct ancestral or unsampled haplotype.

within one individual (Table 2). These haplotypes were firstly recorded by DUBUT et al. (2012) in the Lim River in Montenegro.

The haplotype network (Fig. 3) illustrated the relationships among the haplotypes detected by the present study and the other lineages belonging to the

T. souffia complex. The network confirmed that three haplotypes from the Lim River drainage recorded in this study belonged to the lineage *T. s. agassii* 1. The same lineage also included Western Balkans samples from the rivers Jevnica, Gradaščica and Bregana from the upper Sava River system (Black

Table 2. *T. souffia agassii* 1 populations (from Soča River, Sava River and Lim River systems), with a summary of mitochondrial cyt b haplotype frequencies and diversity. Abbreviations: Hd – haplotype diversity; Pi – nucleotide diversity.

Population	N	Cyt b haplotype frequency												Cyt b diversity						
		BRN01	BRN02	BRN07	BRN14	BRN15	BRN29	SL001	SL002	SL003	SL005	SL008	SL07	SOU1	SOU2	SOU3	SOU4	SOU6	Hd	Pi
Lim	31	11	12	3	1	3	1	-	-	-	-	-	-	-	-	-	-	-	0.727	0.00234
Gračanica*	19	10	8	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	0.573	0.00194
Soča	43	-	-	-	-	-	1	30	1	9	1	1	1	-	-	-	-	-	0.478	0.00092
Gradaščica	9	-	-	-	-	-	-	-	-	6	1	1	1	-	-	-	-	-	0.583	0.00058
Jevnica	6	-	-	-	-	-	-	-	-	4	-	-	-	1	-	-	-	1	0.600	0.00076
Bregana	5	-	-	-	-	-	-	-	-	-	-	-	-	2	1	1	1	-	0.900	0.00457

*population from this work

Sea basin) and from the Soča River drainage (Adriatic basin). In the network, the lineages *Telestes s. souffia*, *T. s. agassii* 1, *T. s. agassii* 2 and *T. s. agassii* 3 were clearly separated.

All measures of DNA polymorphisms revealed differences among populations compromising the lineage *T. s. agassii* 1 and genetic variability inside the lineage (Table 2). The values of haplotype and nucleotide diversity of the population from the Gračanica River were moderate and reached up to 0.573 and 0.00194, respectively.

Discussion

Updated information about areas, in which species occur, is crucial for the implementation of adequate conservation measures (CORSI et al. 2000). The newly-recorded locality of *T. souffia* is important for knowledge of both its actual distribution in Serbia and its global distribution (FREYHOF & KOTTELAT 2008), especially having in view that the abundance of this fish species is notably decreasing in certain areas in the second half of the 20th Century (CURTEAN-BĂNĂDUC et al. 2018).

The specimens found in the Gračanica River in Serbia have shown certain variations of their morphological features compared to those described in literature (VUKOVIĆ & IVANOVIĆ 1971, SIMONOVIĆ 2001, KEITH & ALLARDI 2001, KOTTELAT & FREYHOF 2007). The detected minor morphological differences can be explained by the species phenotypic plasticity and the geographical isolation of the newly-recorded population (NORTON et al. 1995). DUBUT et al. (2012), while analysing population belonging to the same river system (Lim River in Montenegro, more upstream part of the drainage), have found specimens of the phylogenetic lineage *T. s. agassii* 1, which also includes the population from the Soča River from Slovenia. With the work by BUJ et al. (2017), the known distribution of this lineage has been extended with the records in the rivers Gradaščica, Bregana and Jevnica from the upper Sava River system, which are located between the Soča and the Lim rivers. The present distribution pattern of this lineage has resulted, most likely, from the Late Pleistocene glaciations but the exact age of this event remains unclear (DUBUT et al. 2012). The mean time of the most recent common ancestor of the *T. s. agassii* 1 (for both Danubian and Adriatic populations) is ~120-160 Kyr old, although Danubian and Adriatic population have had expansions ~60 Kyr ago (DUBUT et al. 2012). The populations in the Danube River drainage are highly differentiated and have no sign of

admixture – *T. s. agassii* 1, *T. s. agassii* 2 and *T. s. agassii* 3 are reciprocally monophyletic (DUBUT et al. 2012).

The presence of the phylogenetic lineage *T. s. agassii* 1 is recorded in the Gračanica River. The recorded haplotypes from this work, i.e. BRN01, BRN02 and BRN15, have previously been described by DUBUT et al. (2012) and they are showing a ratio 1/2 of the total haplotype diversity in the drainage of the Lim River (Fig. 3, Table 2). The limited diversity of haplotypes can be explained most likely by the fact that the Gračanica River is a small tributary of the Lim River, which may have smaller population size compared to the main river system. The values of the haplotype diversity and the nucleotide diversity of the population in the Gračanica River are moderate, with only two other populations showing higher values, one of them being that in the Lim River (Table 2).

The record of the phylogenetic lineage *T. s. agassii* 1 is important from the point of view of the phylogeography and phylogenetic relationships of *Telestes* populations from Danube River drainage in Serbia. The finding of this lineage is important as this species occurs in a fragmented range and species boundaries are greatly debated. This work confirms the presence of *T. s. agassii* in waters of Serbia and puts an emphasis on the need of further investigations on its distribution. The identified lineage *T. s. agassii* 1, also found in DUBUT et al. (2012), confirms the genetic complexity of the genus *Telestes*, widening its range in the drainage of the Lim River.

This work also confirms the report by SIMONOVIĆ (2001) suggesting the distribution of this subspecies in mountain rivers in Serbia. Accurate and up-to-date distribution information is highly significant both on local and global perspective. Its deficiency may cause wrong conclusions and decisions in the species conservation and management (VUČIĆ et al. 2017).

For further genetic analyses of the newly-discovered population, it is crucial to perform a population genetics study using microsatellite markers. Additional studies will confirm the geographical range of this genetic lineage on the territory of Serbia and help to implement the appropriate conservation measures.

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Appendix 1. Sequences of *T. souffia* complex mitochondrial cyt b haplotypes retrieved from the NCBI base (National Center for Biotechnology Information) (WHEELER et al. 2006).

Haplotype	Accession number	Reference
BRN07	JQ651600	DUBUT et al. 2012
BRN14	JQ651607	DUBUT et al. 2012
BRN29	JQ651621	DUBUT et al. 2012
SLO01	JQ652058	DUBUT et al. 2012
SLO02	JQ652059	DUBUT et al. 2012
SLO03	JQ652060	DUBUT et al. 2012
SLO05	JQ652061	DUBUT et al. 2012
SLO08	JQ652064	DUBUT et al. 2012
SLV07	JQ652091	DUBUT et al. 2012
TIZ01	JQ652161	DUBUT et al. 2012
TIZ02	JQ652162	DUBUT et al. 2012
TIZ04	JQ652164	DUBUT et al. 2012
TIZ05	JQ652165	DUBUT et al. 2012
TIZ10	JQ652169	DUBUT et al. 2012
VIS01	JQ652341	DUBUT et al. 2012
VIS02	JQ652342	DUBUT et al. 2012
VIS07	JQ652347	DUBUT et al. 2012
VIS20	JQ652360	DUBUT et al. 2012
INN01	JQ651818	DUBUT et al. 2012
AIN01	JQ651395	DUBUT et al. 2012

Appendix 1. Continuation.

Haplotype	Accession number	Reference
ARC03	JQ651426	DUBUT et al. 2012
ARD05	JQ651458	DUBUT et al. 2012
ASS33	JQ651505	DUBUT et al. 2012
BAH01	JQ651551	DUBUT et al. 2012
DOU32	JQ651683	DUBUT et al. 2012
FAU13	JQ651699	DUBUT et al. 2012
FIL04	JQ651719	DUBUT et al. 2012
JAB23	JQ651866	DUBUT et al. 2012
SAO04	JQ652005	DUBUT et al. 2012
SEI08	JQ652039	DUBUT et al. 2012
SOR04	JQ652097	DUBUT et al. 2012
STA09	JQ652133	DUBUT et al. 2012
USS07	JQ652188	DUBUT et al. 2012
VAR05	JQ652220	DUBUT et al. 2012
ROS12	JQ651974	DUBUT et al. 2012
SOU1	MG372548	BUJ et al. 2017
SOU2	MG372549	BUJ et al. 2017
SOU3	MG372550	BUJ et al. 2017
SOU4	MG372551	BUJ et al. 2017
SOU6	MG372579	BUJ et al. 2017