ORIGINAL ARTICLE

Complete mitochondrial genome of the Spanish toothcarp, *Aphanius iberus* (Valenciennes, 1846) (Actinopterygii, Aphaniidae) and its phylogenetic position within the Cyprinodontiformes order

Alfonso López-Solano[1] · Tessa Lynn Nester[1] · Silvia Perea[1,2] · Ignacio Doadrio[1]

Abstract

Background The Spanish toothcarp (*Aphanius iberus* Valenciennes, 1846) is a small fish endemic to the eastern coastline of the Iberian Peninsula and is currently listed as "Endangered" (category IUCN: EN). It mainly inhabits brackish waters which can exhibit large fluctuations in temperature and salinity throughout the year. The genetics of *A. iberus* are not well-known since most studies have only evaluated the genetic structure of the species under a conservation framework in order to identify its potential conservation units. Different phylogenetic relationships of *Aphanius* have been published based on some particular genes. In the present study, the entire mitochondrial genome of *A. iberus* was obtained for the first time in the context of an *A. iberus* reference genome and a hypothesis regarding its phylogenetic position was considered.

Methods and results The mitogenome (a circular doble-stranded DNA sequence of 16,708 bp) was reconstructed and aligned against 83 Cyprinodontiformes and two outgroup taxa to identify the phylogenetic position of *A. iberus.* PartitionFinder was first used to test for the best evolutionary model and the phylogenetic analyses were performed using two methods: Maximun-Likelihood Approximation (IQ-Tree) and Bayesian inference (MrBayes). Our results show that *A. iberus* forms a sister group with *Orestias ascotanensis,* a cyprinodontiform species native to South America.

Conclusions The results were congruent with the traditional morphometric reconstructed trees and with a geological vicariant hypothesis involving Cyprinodontiformes where Aphaniidae is shown as a monophyletic family separated from the family Cyprinodontidae. The information gathered from this study is not only valuable for improving our understanding of the evolutionary history of *A. iberus*, but for future genomic studies involving the species.

Keywords Mitogenome · *Aphanius iberus* · Cyprinodontiformes · Phylogeny

Introduction

The Spanish toothcarp (*Aphanius iberus* Valenciennes, 1846) is a small fish species endemic to the Mediterranean Coast of the Iberian Peninsula [1–3]. It belongs to the Cyprinodontiformes order [4] within the family Aphaniidae and although it can tolerate varying environmental conditions, including high temperatures and elevated salt concentrations [5], its populations have been in decline due to urbanism and agricultural exploitation which have dramatically transformed the eastern landscape of the Iberian Peninsula. Due to its fragmented distribution and its severe population decline in recent years as a result of habitat destruction, the Spanish toothcarp is currently listed as "Endangered" (IUCN: EN) [3, 6, 7], with about 20 isolated populations along the Iberian Mediterranean slope and it is currently restricted to salt marshes, coastal lagoons and natural springs referred to locally as Ullals [1, 3, 8]. To aid in the recovery of the species, conservation programs including two EU LIFE Projects (LIFE96 NAT/E/003118; LIFE04/NAT/ES/000035) are dedicated to the restoration of the natural habitat of *A. iberus* and the implementation of captive breeding programs. Currently, there is debate regarding the genus assignment of this species where some authors argue its belonging to the genus *Apricaphanius* Freyhof &

✉ Alfonso López-Solano
Alfonso.lopez@mncn.csic.es

[1] Department of Biodiversity and Evolutionary Biology, Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain

[2] Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Tercer Circuito Exterior S/N, C.P. 04510 Ciudad de Mexico, Mexico

YoǦurtÇuoǦlu [9], while others say it belongs to the genus *Aphanius* Nardo, 1827 [10]. Until further evidence arises, we have opted for the more conservative option in order to remain consistent with the International Code of Zoological Nomenclature, and therefore the genus, in this study, is referred to as *Aphanius*. We have also maintained *Aphanius farsicus* over *Esmaeilius persicus* which was recently proposed by Freyhof & YoǦurtÇuoǦlu [9].

Genetic studies involving *A. iberus* have mainly been focused on evaluating the genetic structure of the species under a conservation framework in order to identify its potential conservation units either with allozymes [8], partial gene sequences [11–14] or microsatellites [15]. However, the phylogenetic relationships within the order Cyprinodontiformes are still controversial and not yet clear especially since very few complete mitochondrial genomes are available causing some inconsistencies in relation to the phylogenetic position of *Aphanius* [10, 16, 17]. In this study, the entire mitochondrial genome of *A. iberus* was obtained for the first time in the context of an *A. iberus* reference genome sequence and a phylogenetic tree was constructed within the Cyprinodontiformes order. This new data will not only be valuable for improving our understanding of the evolutionary history of the species and its phylogenetic relationships, but also for future evolutionary and genomic studies.

Methods

Three specimens of *A. iberus* were collected in El Palmar (Valencia, Spain) and euthanized following the appropriate protocols. The DNA was then isolated using the MagAttract HMW DNA isolation kit (Qiagen). The final elution was done in a volume of 100 μL. DNA was quantified using the Qubit High Sensitivity dsDNA Assay (Thermo Fisher Scientific).

Sequencing data within the context of a reference genome was obtained from PacBio and Illumina DNA sequencing in order to obtain the adequate assembly and annotation. Prior to PacBio library preparation, the sample was further purified and size-selected in order to maintain the largest fragments. Next, the recommended SMRTbell Express Template Prep Kit 2.0 (PacBio) was used to prepare the library, following the manufacturer's instructions. The library was sequenced in a Sequel II sequencer (PacBio), using a SMRT Cell 8 M, under the Long-reads mode. A total of 7.3 million long reads (~9500) were obtained and quality-checked using the software SequelTools [18]. For posterior Illumina library preparation, the Illumina DNA Prep kit was used strictly following the manufacturer's instructions. The fragment size distribution and concentration of the library was checked in the Agilent 2100 Bioanalyzer (using the Agilent

HS DNA Kit). Then, the library was sequenced in a fraction of a NovaSeq PE150 flow cell, aiming for a total output of 50 gigabases, that yielded close to 435 million short paired-end reads (~150). The raw fastq files were quality-checked using the software FastQC v0.11.5 [19].

For the mitochondrial genome assembly NOVOPlasty v4.2 [20] was used as a seed-extend based assembler to reconstruct organelle genomes from whole-genome sequencing data, starting from a related or distant seed sequence. The Cytochrome C Oxidase Subunit 1 (COX1) gene of the species *Aphanius vladykovi* (NCBI Reference Sequence: MN702439.1) was selected as the seed, with a k-mer length of 33 bp.

The quality of the resulting mitochondrial assembly was then evaluated with the package QUAST 5.0.2 [21]. The number of contigs and the total length (in base pairs, bp) of the mitochondrial genome assembly are represented in Table 1. The mitogenome assembly generated was queried against the NCBI's (National Center for Biotechnology Information) nr/nt (nucleotide) database using the Basic Local Alignment Search Tool BLAST. The best match found was the cyprinodontiform species *Cyprinodon variegatus* (Accession Number: KT288182.1) with a nucleotide identity percentage of 82% and the closely related *Orestias ascotanensis* exhibited a nucleotide identity percentage of 81.5%.

Table 1 Number of contigs and their total length

Assembly	Mito-chondrial assembly
# contigs (≥ 0 bp)	1
# contigs (≥ 1000 bp)	1
# contigs (≥ 5000 bp)	1
# contigs (≥ 10,000 bp)	1
# contigs (≥ 25,000 bp)	0
# contigs (≥ 50,000 bp)	0
Total length (≥ 0 bp)	16,708
Total length (≥ 1000 bp)	16,708
Total length (≥ 5000 bp)	16,708
Total length (≥ 10,000 bp)	16,708
Total length (≥ 25,000 bp)	0
Total length (≥ 50,000 bp)	0
# contigs	1
Largest contig	16,708
Total length	16,708
GC (%)	45.06
N50	16,708
N75	16,708
L50	1
L75	1
'# N"s per 100 kbp'	0

The Mitochondrial genome annotation Server (MITOS2) [22] was used to automatically annotate the mitochondrial genome selecting RefSeq 63 Metazoa as the reference database and the vertebrate mitochondrial genetic code. The protein prediction method from Al Arab et al. [23] was enabled.

Results

The mitogenome of *Aphanius iberus* is a circular doble-stranded DNA sequence that is 16,708 bp long including 13 protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes, and the putative control region (Table 2, Figs. 1 and 2). The base percentage composition showed smaller G+C content (45.06%) compared to A+T content (54.94%). MITOS2 software annotated several peculiarities and found some OH overlaps in the following genes: (atp8,atp6):10; (nad4l,nad4):7; (nad5,nad6):4; (nad3,trnR):2; (trnI,trnQ):1; (trnQ,trnM):1; (nad2,trnW):1; (atp6,cox3):1; (cox3,trnG):1; (trnT,trnP):1.

The mitochondrial genome was deposited on GenBank with the Accession Number OP884090; (BankIt2647138 Seq1). The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAPXFQ000000000. The version described in this paper is version JAPXFQ010000000. BioProject: PRJNA913687. BioSample: SAMN32303939.

A phylogenetic analysis was performed and included both coding regions (Nad1, Nad2, Cox1, Cox2, Atp8, Atp6, Cox3, Nad3, Nad4L, Nad4, Nad5, Nad6, CytB) and non-coding regions 12s (rrnS) and 16s (rrnL). Eighty-three cyprinodontiform species were selected and the mentioned mitogenome regions were downloaded from GenBank (Table 3) with the aim to evaluate the phylogenetic position of the mitogenome of *Aphanius iberus*. The following outgroup taxa were selected: *Oryzias uwai* (Accession Number: MN832874) and *Bedotia geayi* (Accession Number: AP006770) from the closely related Beloniformes and Atheriniformes orders respectively [25]. Geneious software [26] was used to align sequences using the MUSCLE alignment method including all of the 83 cyprinodontiform species, the two outgroup taxa, and the new mitogenome obtained for *Aphanius iberus* (Table 3). PartitionFinder2 software [27] was used to search for the best evolutionary model for each gene separately. The results revealed GTR+I+G as the best fit model for each gene, therefore, the entire mitogenome alignment was considered to belong to only one partition. Afterwards, two phylogenetic approximations were conducted based on two different methods. First, the Maximum-Likelihood reconstruction analysis was performed using the option MFP+MERGE in the IQ-TREE software [29]. The support for each node was evaluated with

the SH-like approximate likelihood ratio test [30] and 1.000 ultrafast bootstrap (UFBoot2) approximations [31]. Then a Bayesian inference was performed with MrBayes [32]. Two analyses were run for 10,000,000 generations simultaneously, each with two parallel runs and four MCMC chains with a sampling frequency of 1000 generations. We rejected the first 25% of generations as burn-in and obtained the 50% majority rule consensus tree and the posterior probabilities
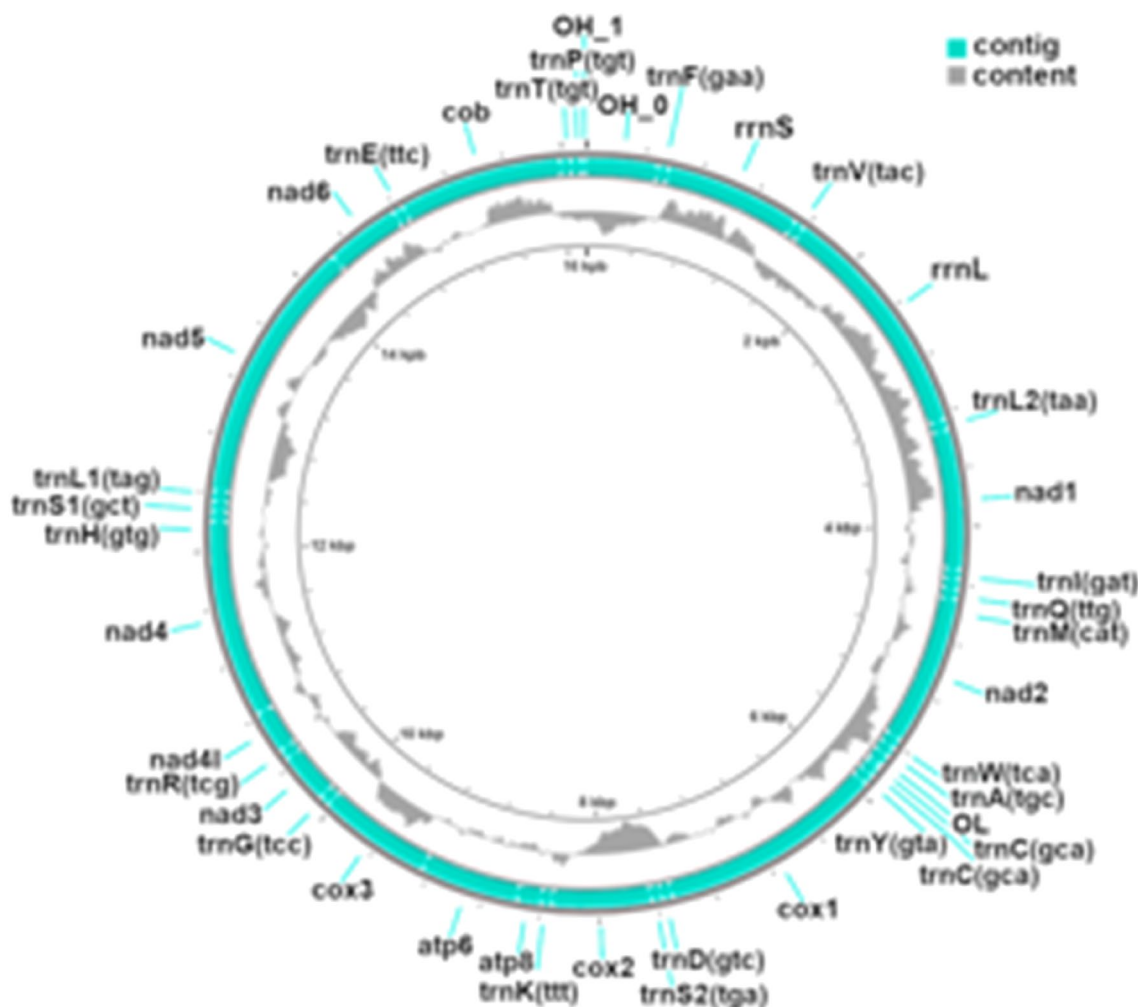
Table 2 Organization of the *Aphanius iberus* mitogenome

Initial bp	Final bp	Type	Gene
223	730	D-loop	Control region
791	859	tRNA	tRNA-PHE
860	1807	rRNA	s-rRNA
1808	1879	tRNA	tRNA-VAL
1903	3565	rRNA	l-rRNA
3566	3639	tRNA	tRNA-LEU2
3640	4611	CDS	NADH dehydrogenase subunit 1
4613	4680	tRNA	tRNA-ILE
4750	4680	tRNA	tRNA-GLN
4750	4818	tRNA	tRNA-MET
4819	5865	CDS	NADH dehydrogenase subunit 2
5865	5935	tRNA	tRNA-TRP
6007	5939	tRNA	tRNA-ALA
6081	6009	tRNA	tRNA-ASN
6086	6119	rep_origin	Origin of L-strand replication
6184	6120	tRNA	tRNA-CYS
6255	6186	tRNA	tRNA-TYR
6257	7816	CDS	Cytochrome c oxidase subunit 1
7873	7943	tRNA	tRNA-ASP
8084	8014	tRNA	tRNA-SER2
8156	8846	CDS	Cytochrome c oxidase subunit 2
8847	8920	tRNA	tRNA-LYS
8922	9089	CDS	ATP synthase F0 subunit 8
9080	9763	CDS	ATP synthase F0 subunit 6
9763	10,548	CDS	Cytochrome c oxidase subunit 3
10,548	10,619	tRNA	tRNA-GLY
10,620	10,970	CDS	NADH dehydrogenase subunit 3
10,969	11,037	tRNA	tRNA-ARG
11,038	11,334	CDS	NADH dehydrogenase subunit 4L
11,328	12,708	CDS	NADH dehydrogenase subunit 4
12,709	12,777	tRNA	tRNA-HIS
12,778	12,845	tRNA	tRNA-SER1
12,856	12,928	tRNA	tRNA-LEU1
12,929	14,767	CDS	NADH dehydrogenase subunit 5
15,285	14,764	CDS	NADH dehydrogenase subunit 6
15,353	15,286	tRNA	tRNA-GLU
15,358	16,497	CDS	Cytochrome b
16,499	16,570	tRNA	tRNA-THR
16,637	16,570	tRNA	tRNA-PRO
16,678	16,708	D-loop	Control region

Fig. 1 A graphical map of the mitochondrial genome of *A. iberus* showing the GC content and annotation results using the CGView server [24] (see Figure)
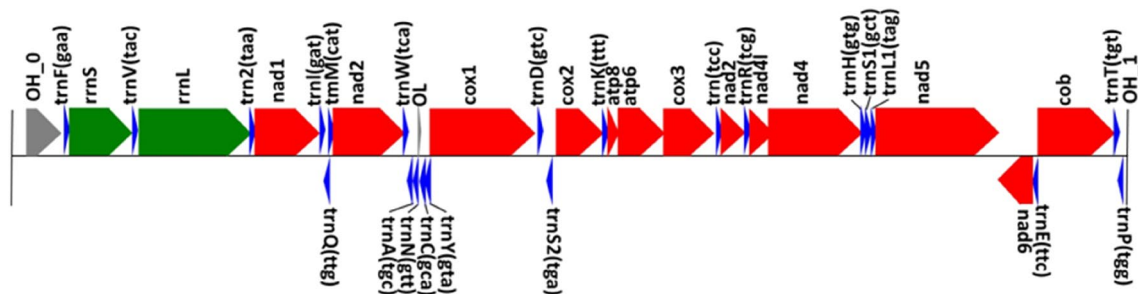

Fig. 2 Linear representation of the *A. iberus* mitochondrial genome

(PP). The convergence of the runs was corroborated using Tracer v1.7.1 [33]. Finally, both phylogenetic trees inferred were imported into the software FigTree [34] and presented together with bootstrap values (over 100) and Bayesian probabilities (over 1) as the branch support (Fig. 3).

Discussion

The phylogenetic analysis suggests the close relationship of *Aphanius iberus* with *Orestias ascotanesis.* The evolutionary

Table 3 Species selected from Genbank and classified by order and family with their respective accession numbers, authors and submission dates

Order, family	Species	Accession number	Author and submission date on GenBank
Cyprinodontiformes			
Rivulidae	*Austrolebias charrua*	KP718940	Gutierrez, V. et al. 28-JAN-2015
	Kryptolebias marmoratus	KT893707	Tatarenkov, A. et al. 08-OCT-2015
	Kryptolebias hermaphroditus	KX268503	Kim, H.-S. et al. 23-MAY-2016
Nothobranchiidae	*Nothobranchius furzeri*	EU650204	Reichwald, K. et al
	Epiplatys dageti	MK784208	Cui, R. et al. 11-APR-2019
	Nothobranchius nubaensis	MK784209	Cui, R. et al. 11-APR-2019
	Aphyosemion kunzi	MK784210	Cui, R. et al. 11-APR-2019
	Scriptaphyosemion guignardi	MK784211	Cui, R. et al. 11-APR-2019
	Aphyosemion coeleste	MK784212	Cui, R. et al. 11-APR-2019
	Scriptaphyosemion bertholdi	MK784213	Cui, R. et al. 11-APR-2019
	Callopanchax toddi	MK784214	Cui, R. et al. 11-APR-2019
	Fundulopanchax amieti	MK784215	Cui, R. et al. 11-APR-2019
	Epiplatys togolensis	MK784217	Cui, R. et al. 11-APR-2019
	Epiplatys multifasciatus	MK784219	Cui, R. et al. 11-APR-2019
	Fundulopanchax scheeli	MK784220	Cui, R. et al. 11-APR-2019
	Scriptaphyosemion schmitti	MK784221	Cui, R. et al. 11-APR-2019
	Nothobranchius vosseleri	MK784222	Cui, R. et al. 11-APR-2019
	Epiplatys guineensis	MK784223	Cui, R. et al. 11-APR-2019
	Epiplatys lamottei	MK784224	Cui, R. et al. 11-APR-2019
	Aphyosemion cognatum	MK784225	Cui, R. et al. 11-APR-2019
	Fundulopanchax gardneri	MK784226	Cui, R. et al. 11-APR-2019
	Aphyosemion kouamense	MK784227	Cui, R. et al. 11-APR-2019
	Fundulopanchax sjostedti	MK784228	Cui, R. et al. 11-APR-2019
	Callopanchax monroviae	MK784229	Cui, R. et al. 11-APR-2019
	Nothobranchius kuhntae	MK784230	Cui, R. et al. 11-APR-2019
	Epiplatys spilargyreius	MK784231	Cui, R. et al. 11-APR-2019
	Nimbapanchax petersi	MK784232	Cui, R. et al. 11-APR-2019
	Nothobranchius ocellatus	MK784233	Cui, R. et al. 11-APR-2019
	Pronothobranchius seymouri	MK784234	Cui, R. et al. 11-APR-2019
	Fundulopanchax filamentosus	MK784236	Cui, R. et al. 11-APR-2019
	Aphyosemion australe	MK784237	Cui, R. et al. 11-APR-2019
	Aphyosemion gabunense	MK784238	Cui, R. et al. 11-APR-2019
	Callopanchax sidibeorum	MK784239	Cui, R. et al. 11-APR-2019
	Archiaphyosemion guineense	MK784240	Cui, R. et al. 11-APR-2019
	Aphyosemion cyanostictum	MK784241	Cui, R. et al. 11-APR-2019
	Nimbapanchax leucopterygius	MK784242	Cui, R. et al. 11-APR-2019
	Epiplatys grahami	MK784243	Cui, R. et al. 11-APR-2019
	Nothobranchius kafuensis	MK784244	Cui, R. et al. 11-APR-2019
	Scriptaphyosemion cauveti	MK784245	Cui, R. et al. 11-APR-2019
	Nothobranchius thierryi	MK784246	Cui, R. et al. 11-APR-2019
	Nothobranchius foerschi	MK784247	Cui, R. et al. 11-APR-2019
	Aphyosemion cameronense	MK784248	Cui, R. et al. 11-APR-2019
Procatopodidae	*Poropanchax normani*	MW354542	Peng, Y. et al. 07-DEC-2020
Aplocheilidae	*Pachypanchax playfairii*	MK784207	Cui, R. et al. 11-APR-2019
	Aplocheilus lineatus	MK784216	Cui, R. et al. 11-APR-2019
	Aplocheilus panchax	NC_011176	Setiamarga, D. H. et al. 27-AUG-2008
Fundulidae	*Xenotoca eiseni*	AP006777	Setiamarga, D. H. et al. 06-APR-2004
	Fundulus olivaceus	AP006776	Setiamarga, D. H. et al. 06-APR-2004

Table 3 (continued)

Order, family	Species	Accession number	Author and submission date on GenBank
	Fundulus diaphanus	FJ445394	Whitehead, A. 07-NOV-2008
	Fundulus grandis	FJ445396	Whitehead, A. 07-NOV-2008
	Fundulus heteroclitus	FJ445398	Whitehead, A. 07-NOV-2008
	Fundulus notatus	KP013106	Renshaw, M. A. et al. 21-OCT-2014
	Fundulus zebrinus	MW300328	Diver, T. A. et al. 27-NOV-2020
Goodeidae	*Empetrichthys latos latos*	KY014102	Jimenez, M. et al. 19-OCT-2016
	Crenichthys baileyi moapae	KY014104	Jimenez, M. et al. 19-OCT-2016
Poeciliinae	*Gambusia affinis*	AP004422	Miya, M. et al. 12-DEC-2001
	Xiphophorus hellerii	FJ226476	Bai, J.J. et al. 20-SEP-2008
	Gambusia holbrooki	KP013085	Renshaw, M.A. et al. 21-OCT-2014
	Poeciliopsis occidentalis	KP013108	Renshaw, M.A. et al. 21-OCT-2014
	Poecilia reticulata	KJ013505	Kong, X.F. et al. 31-DEC-2013
	Poecilia formosa	KT166983	Dang, X. et al. 17-JUN-2015
	Poecilia latipinna	KT175511	Stoeck, M. et al. 17-JUN-2015
	Poecilia mexicana	KT175512	Stoeck, M. et al. 17-JUN-2015
	Xiphophorus couchianus	KT594624	Zhang, K. et al. 25-AUG-2015
	Poeciliopsis monacha	KX229692	Jeon, Y. S. et al. 09-MAY-2016
	Poeciliopsis sonoriensis	MK860197	Mateos, M. et al. 27-APR-2019
	Xiphophorus variatus	MW934558	Eastis, A. N. et al. 15-APR-2021
	Xiphophorus maculatus	NC_011379	Setiamarga, D. H. et al. 17-OCT-2008
Cyprinodontidae	*Jordanella floridae*	AP006778	Setiamarga, D. H. et al. 06-APR-2004
	Cyprinodon rubrofluviatilis	EF442803	Crowl, T. M. et al. 29-MAR-2007
	Cyprinodon tularosa	KP013105	Renshaw, M. A. et al. 21-OCT-2014
	Cyprinodon variegatus variegatus	KR061357	Barcelon, B. R. et al. 04-APR-2015
	Orestias ascotanensis	KR296656	Quezada-Romegialli, C. et al. 28-APR-2015
	Cyprinodon variegatus	KT288182	Sheng, L. 14-JUL-2015
	Cyprinodon nevadensis amargosae	KU883631	Barcelon, B. R. et al. 08-MAR-2016
	Cyprinodon diabolis	KX061747	Lema, S. C. et al. 11-APR-2016
	Cyprinodon julimes	MG727890	Smith, N. L. et al. 24-DEC-2017
	Cyprinodon elegans	MW300326	Diver, T. A. et al. 27-NOV-2020
	Cyprinodon macularius	MW300330	Diver, T. A. et al. 27-NOV-2020
	Cyprinodon bovinus	MW300332	Diver, T. A. et al. 27-NOV-2020
	Cyprinodon pecosensis	MW300337	Diver, T. A. et al. 27-NOV-2020
	Cyprinodon salinus salinus	MW446237	Del Core, A. A. et al. 06-JAN-2021
Aphaniidae	*Aphanius persicus*	MN578038	Teimori, A. et al. 16-OCT-2019
	Aphanius iberus		This study
Beloniformes			
Adrianichthyidae	*Oryzias uwai* (outgroup)	MN832874	Ngamniyom, A. 14-DEC-2019
Atherinomorphae			
Atheriniformes	*Bedotia geayi* (outgroup)	AP006770	Miya, M. et al. 06-APR-2004

relationship between *A. iberus* and *O. ascotanensis* exhibits high branch support with a bootstrap value of 73 and a posterior probability value of 0.99 (Fig. 3). This result was congruent with the traditional morphometric reconstructed trees [35–38] based on recent molecular studies [10, 17, 25] and with a geological vicariant hypothesis involving Cyprinodontiformes [16]. All of them revealed Aphaniidae (*Aphanius iberus*) as a monophyletic family separated from the Cyprinodontidae family (*Cyprinodon* and *Jordanella* genera, both included in our phylogenetic tree) [4] with a close proximity to Valenciidae [39]. Recently the complete mitogenome of *Aphanius* farsicus was published by Teimori & Motamedi, where they show *A. farsicus* positioned within the same evolutionary clade as the *Cyprinodon* and *Jordanella* genera [40]. Despite the genetic proximity of *A. farsicus* with *A. iberus* postulated by some studies [9,
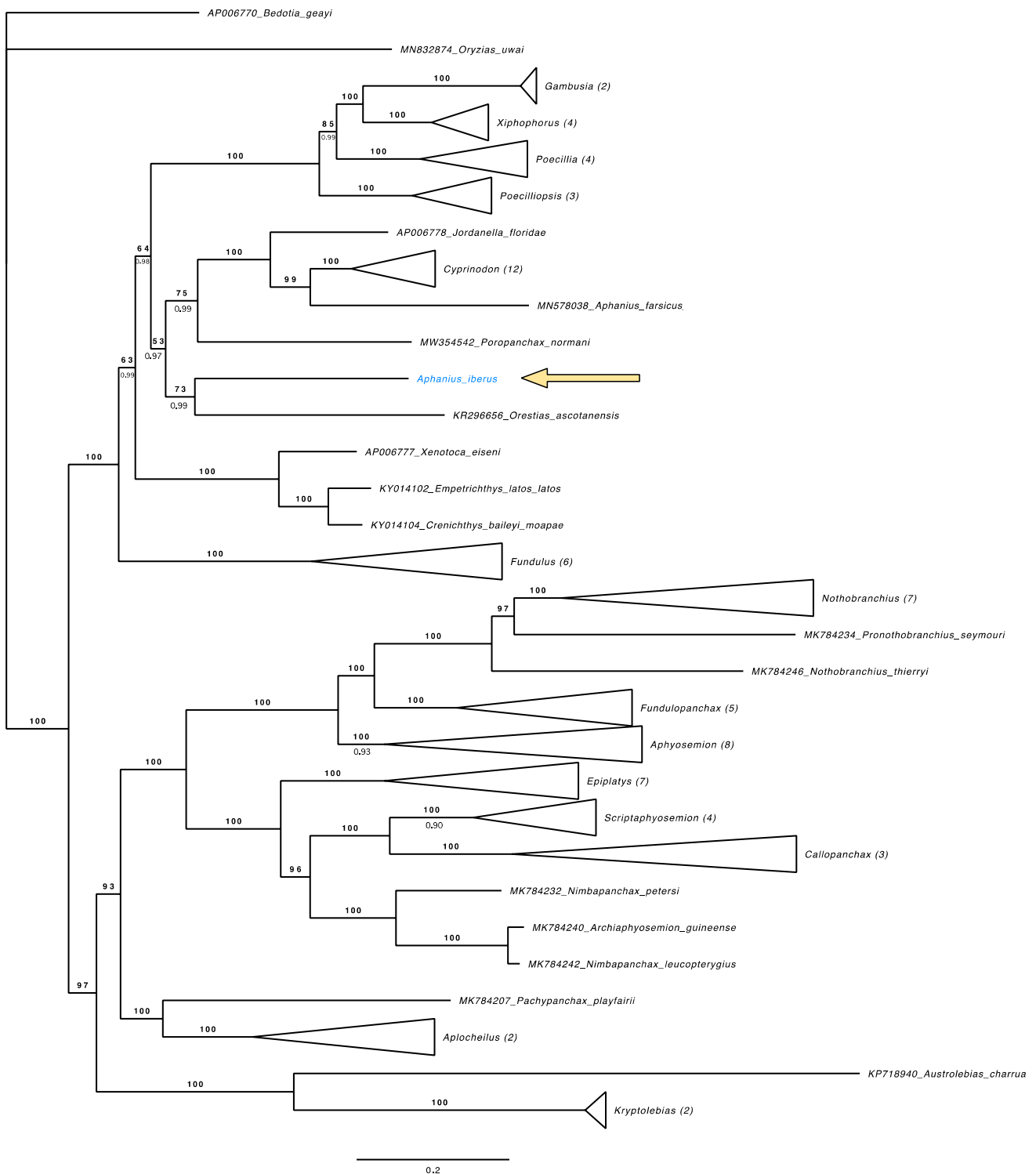
Fig. 3 Phylogenetic tree rendered by Maximum likelihood and Bayesian inference based on the complete mitochondrial genome of 83 cyprinodontiform fishes. The phylogenetic position of *Aphanius iberus* is highlighted in red. The numbers on the branches indicate bootstrap (top of the branch) and posterior probability (bottom of the branch) values. (Color figure online)

10, 17], our analysis corroborated the close and highly supported mitochondrial relationship between *A. farsicus* and the *Cyprinodon-Jordanella* clade instead of with *A. iberus*.

This latter issue questions the monophyly of the Aphaniidae family and poses the need for a further revision of this particular fish family within the order Cyprinodontiformes.

Mitochondrial DNA has proved to be a useful marker for deciphering the phylogenetic relationships at both inter- and intraspecific species levels [41]. On the one hand, its relatively fast evolutionary rate and nucleotide polymorphism has enough resolution to identify species and to even differentiate genetic groups within species. On the other hand, DNA recombination and the lack of complex genomic structures found in nuclear DNA (e.g. repeated elements, pseudogenes or introns) make for straightforward analyses. In fact, one of the mitochondrial genes, the cytochrome c oxidase subunit 1, has been designated as a barcode for species identification in many taxonomic studies [42, 43], but not without debate [44]. Some limitations of its use have been reported in the literature, including discrepancies with nuclear phylogenies [45, 46] or difficulty to identify hybridizations when these evolutionary events are present due to the maternal inheritance of the mitochondrial genome [47]. For these reasons, and due to the fact that only a few complete mitogenomes have ever been published for the genera *Aphanius* (two mitogenomes) and *Orestias* (one mitogenome), more research should be done in order to clarify the phylogenetic relationships of these genera and their species within the Cyprinodontiformes order in an evolutionary context.

Conclusion

In this study, we have revealed that the genus *Aphanius s.l.* is not monophyletic. We have additionally proposed the close relationship between the western Mediterranean species *Aphanius iberus* and *Orestias ascotanensis*, a species from the Andean Region in South America. Our results are congruent with previous phylogenetic and biogeographical vicariant hypotheses involving Cyprinodontiformes. However, due to limitations of the mitochondrial genome and the fact that only a few were analyzed, further studies are required. Nevertheless, the information gained from this study is valuable for improving our understanding of the evolutionary history of *A. iberus* and for future genomic studies.

Declarations

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