SUPLEMENTARY MATERIAL

Table S1 Name and acronyms of each locality, as well as the basin and biogeographic province where are located. Samples 13 to 17 were obtained from García-Morales and Elías-Gutiérrez (2013).

Locality	Code	Basin	Biogeographic province
1. La Colorada	COL	Ameca River	Trans-Mexican Volcanic Belt (TMVB)
2. La Vega	VEG		
3. Atotonilco	ATO	Sayula River	
4. Yuriria	YUR	Middle Lerma River	
5. La Joya	JOY		
6. Cuitzeo	CUI	Cuitzeo Lake	
7. La Zarcita	ZAR	Zacapu Lake	
8. Teremendo	TER		
9. Uranden	URA	Patzcuaro Lake	
10. La Magdalena	MAG	Tacambaro-Balsas River	
11. Ixtlahuaca	IXT	Upper Lerma River	
12. Lerma	LER		
13. Escondida	ESC	Papaloapan River	Gulf of Mexico (GM)
14. Vernet	VER	Grijalva-Usumacinta Rivers	
15. Charco 1	CH1		
16. Parque Ecologico	PEC	Chetumal Bay	Yucatan Peninsula (YP)
17. El Rosario	ROS	Caracol-Candelaria Stream	Baja California (BC)

Table S2 PCR reaction mix and cycle profile for 18S gene.

PCR reagents		PCR profile
Volumes for one reaction (12 µL)		Initial step: 95 °C for 5 min
DNA template	4 µL	
Trehalose 10%	5.25 µL	38 cycles of the following profile:
ddH$_2$O	1 µL	
10X PCR buffer	1.25 µL	Denaturing step: 95 °C for 1 min
50 mM MgCl$_2$	0.625 µL	Annealing step: 55 °C for 1 min
0.05 mM dNTP	0.0625 µL of each	Extending step: 72 °C for 2 min
0.01 mM Primer 1	0.125 µL	
0.01 mM Primer 2	0.125 µL	Final step: 72 °C for 10 min
Taq polymerase	0.06 µL	

Table S3 Average percent of uncorrected genetic distances of COI gene. In diagonal genetic distances within the seven *B. quadridentatus* lineages and below the diagonal genetic distances among the seven lineages.

Genetic group	A	B	C	D	E	F	G
A	**0.0**						
B	14.8	**2.0**					
C	15.6	16.4	**0.5**				
D	16.4	18.3	14.8	**1.5**			
E	17.3	16.5	17.2	18.1	**0.0**		
F	13.4	16.6	16.5	16.8	15.2	**0.2**	
G	15.3	17.0	16.8	18.1	16.2	12.4	**3.1**

Table S4 Measurements from some specimens belonging to the mitocondrial lineages discriminaed in the phylogenetic analyses. Total length in the lineages B, C, D and E comprises from the tips of the median anterior spines to the tips of the caudal spines. Total length in lineages F and G comprises from the tips of the median anterior spines to the caudal margin of the lorica. Measures correspond to only one individual from each location.

Lineage B				
Specimen	Total length	Body width	Length of median anterior spines	Length of caudal spines
La Colorada (COL)	275 µm	175 µm	62.5 µm	75 µm
La Vega (VEG)	230 µm	162.5 µm	52.5 µm	62.5 µm
Lineage C				
La Zarcita (ZAR)	195 µm	165 µm	37.5 µm	12.5 µm
Lineage D				
Uranden (URA)	232 µm	162.5 µm	45 µm	52.5 µm
La Magdalena (MAG)	225 µm	172.5 µm	35 µm	42.5 µm
Yuriria (YUR)	225 µm	165 µm	42.5 µm	37.5 µm
La Colorada (COL)	230 µm	172.5 µm	35 µm	37.5 µm
La Zarcita (ZAR)	230 µm	175 µm	35 µm	37.5 µm
Lineage E				
La Joya (JOY)	230 µm	180 µm	35 µm	35 µm

Lineage F			
Specimen	Total length	Body width	Length of median anterior spines
Cuitzeo (CUI)	157.5 µm	152.5 µm	25 µm
Lerma (LER)	167.5 µm	152.5 µm	30 µm
Lineage G			
Teremendo (TER)	200 µm	177.5 µm	25 µm
Ixtlahuaca (IXT)	215 µm	187.5 µm	35 µm
Atotonilco (ATO)	172.5 µm	150 µm	30 µm

Table S5 Sample size (N), Number of haplotypes (H), Haplotype diversity (*Hd*) and nucleotide diversity (π) of the seven *Brachionus quadridentatus* lineages based on the COI gene. The standard deviations of the parameters are within parentheses.

Lineages	N	H	Hd	π
A	1	1	NA	NA
B	20	5	0.721 (0.088)	0.01993 (0.00235)
C	8	2	0.429 (0.169)	0.00473 (0.00186)
D	43	13	0.913 (0.016)	0.01495 (0.00077)
E	11	1	0.000	0.000
F	58	10	0.595 (0.070)	0.00250 (0.00051)
G	35	7	0.824 (0.035)	0.03123 (0.00261)

Table S6 Hierarchical analysis of molecular variance of AMOVA for the seven COI lineages of *B. quadridentatus* complex.

Source of variation	df	Sum of squares	Variance components	Percentage of variation	Fixation indices	*P* value
Genetic groups						
Among genetic groups	5	6172.01	42.79	86.03	F_{CT}= 0.8602	0.000
Among populations within genetic groups	13	557.85	6.05	12.17	F_{SC}= 0.8709	0.000
Within genetic groups	156	139.89	0.896	1.80	F_{ST} = 0.9819	0.000
Total	174	6869.76	49.74			

Table S7 GenBank accession numbers of the cytochrome c oxidase subunit 1 (COI gene) sequences. These 23 sequences belong to *B. quadridentatus* from Europe and China.

Authors	Accessions	Individuals	Published data	Place
Gómez et al. (2002)	AF387294, AF387295	2	Yes	Iberian Peninsula
Fontaneto et al.	EU499772-EU499779	8	No	United Kingdom
Jiang	KU720083	1	No	China
Moreno et al. (2017)	KY749403-KY749414	12	Yes	Iberian Peninsula

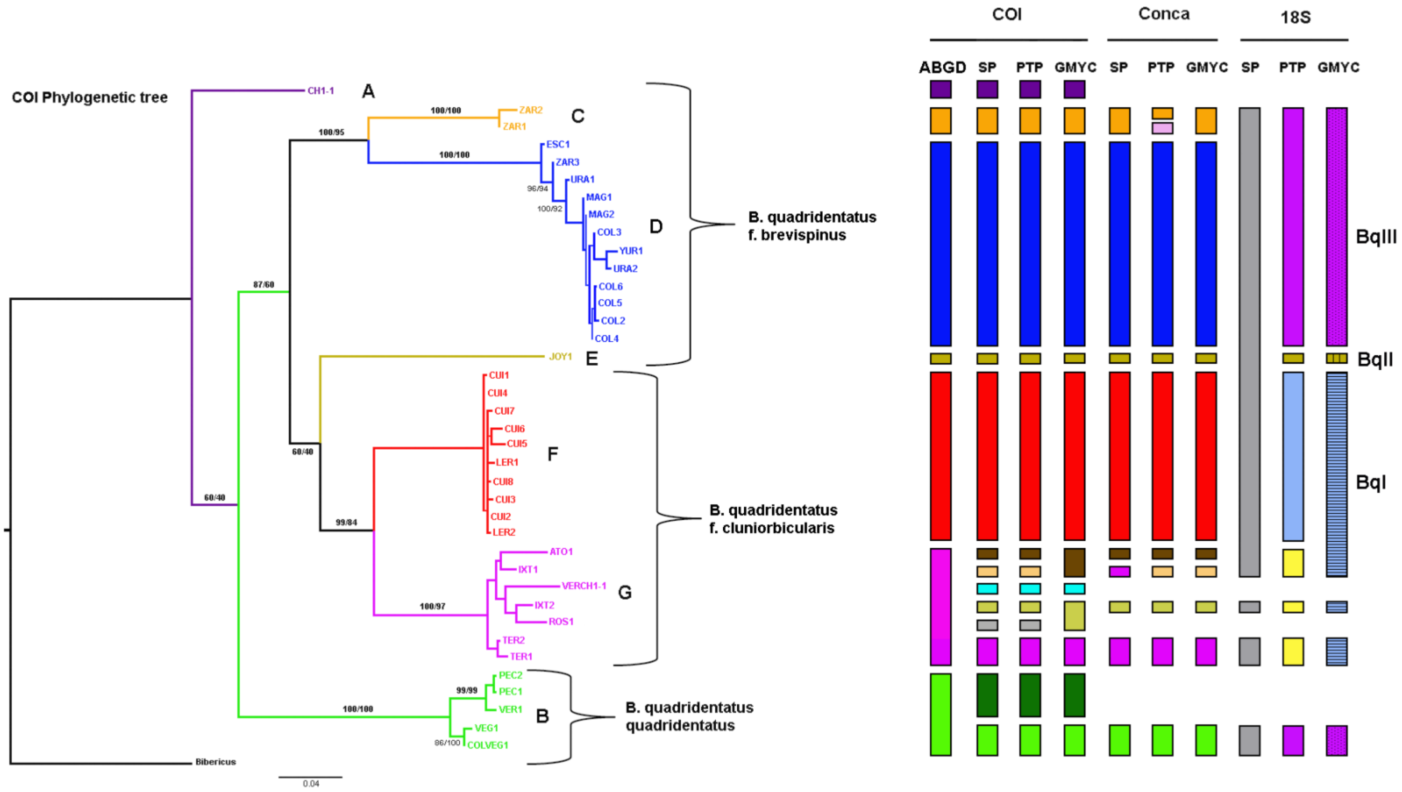

Fig. S1 Molecular based species delineation of *B. quadridentatus*. **A**. Bayesian tree generated with MrBayes from the COI marker. ABGD: Automatic Barcode Gap Discovery, SP: Statistical Parsimony, PTP: Poisson Tree Processes and GMYC: Generalized Mixed Yule-Coalescent analyses performed to COI and 18S genes, as well as to the concatenated data set (Conca).

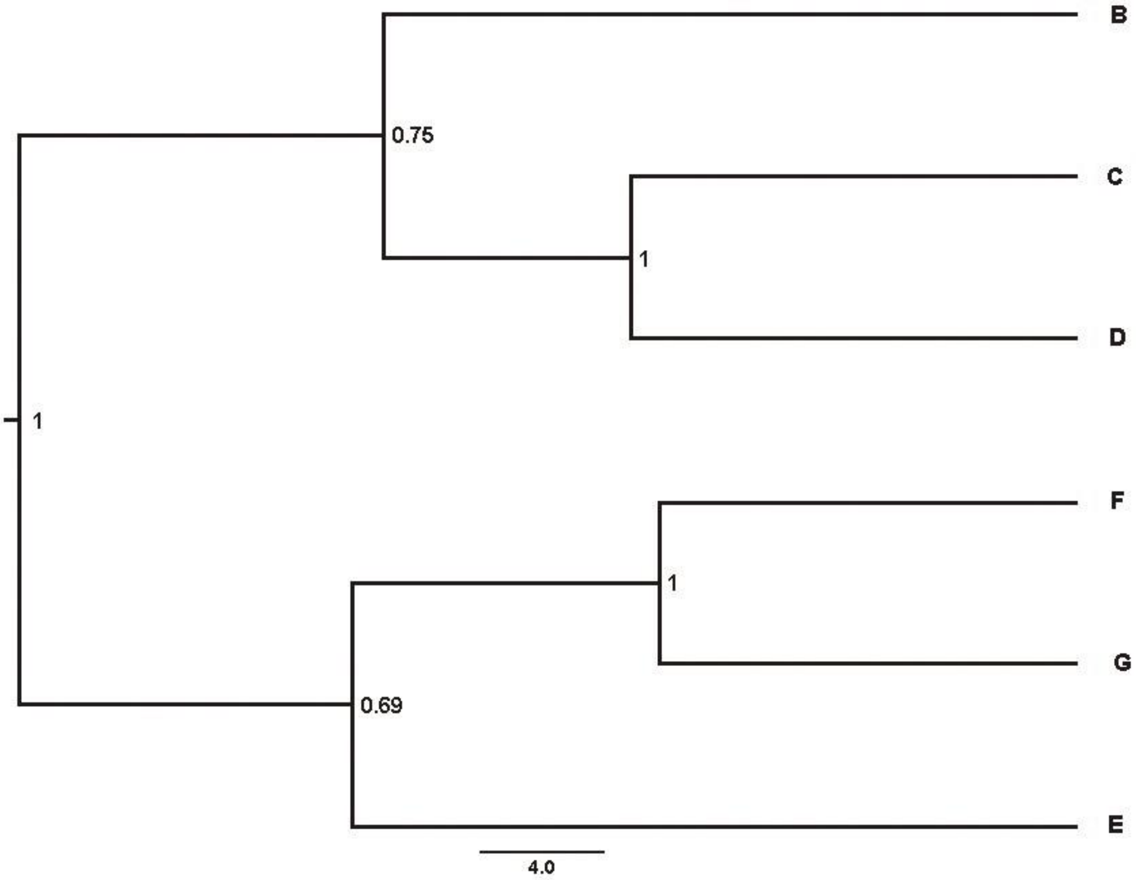

Fig. S2 Coalescent species tree of the concatenated data. Posterior probabilities are shown on branches. Putative species B to G are in accordance to the lineages in the concatenated phylogenetic analysis.

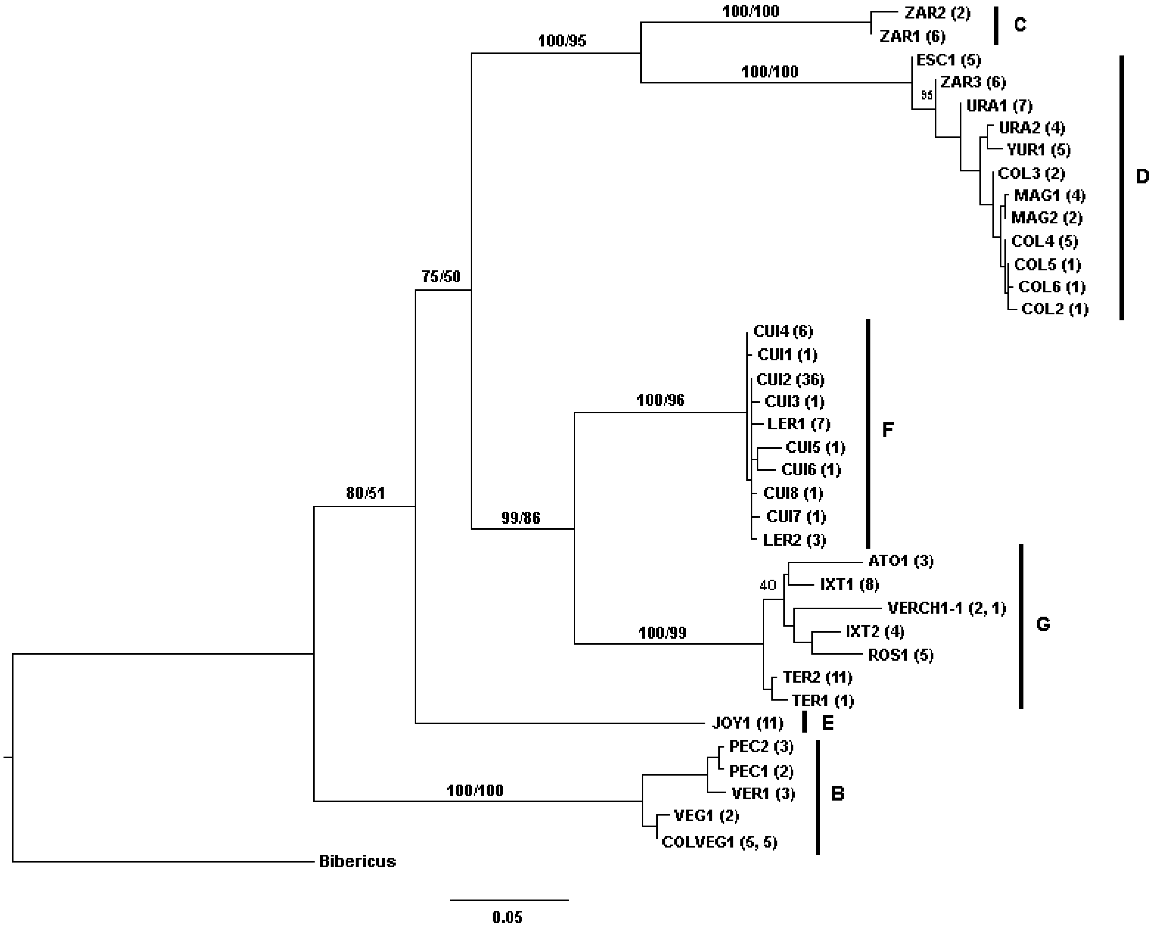

Fig. S3 Bayesian Inference phylogram of the mtDNA showing the relationship of 37 *B. quadridentatus* haplotypes. In this case the sequence corresponding to the lineage A in Figure 3 was not included, in order to see how the absence of lineage A affects the relationships between the other lineages. Haplotype names are indicated by the acronym of the water body in which they were collected, plus a number. Numbers on major branches are the percent of branch support in the Bayesian and Maximun-Likelihood analyses, respectively.

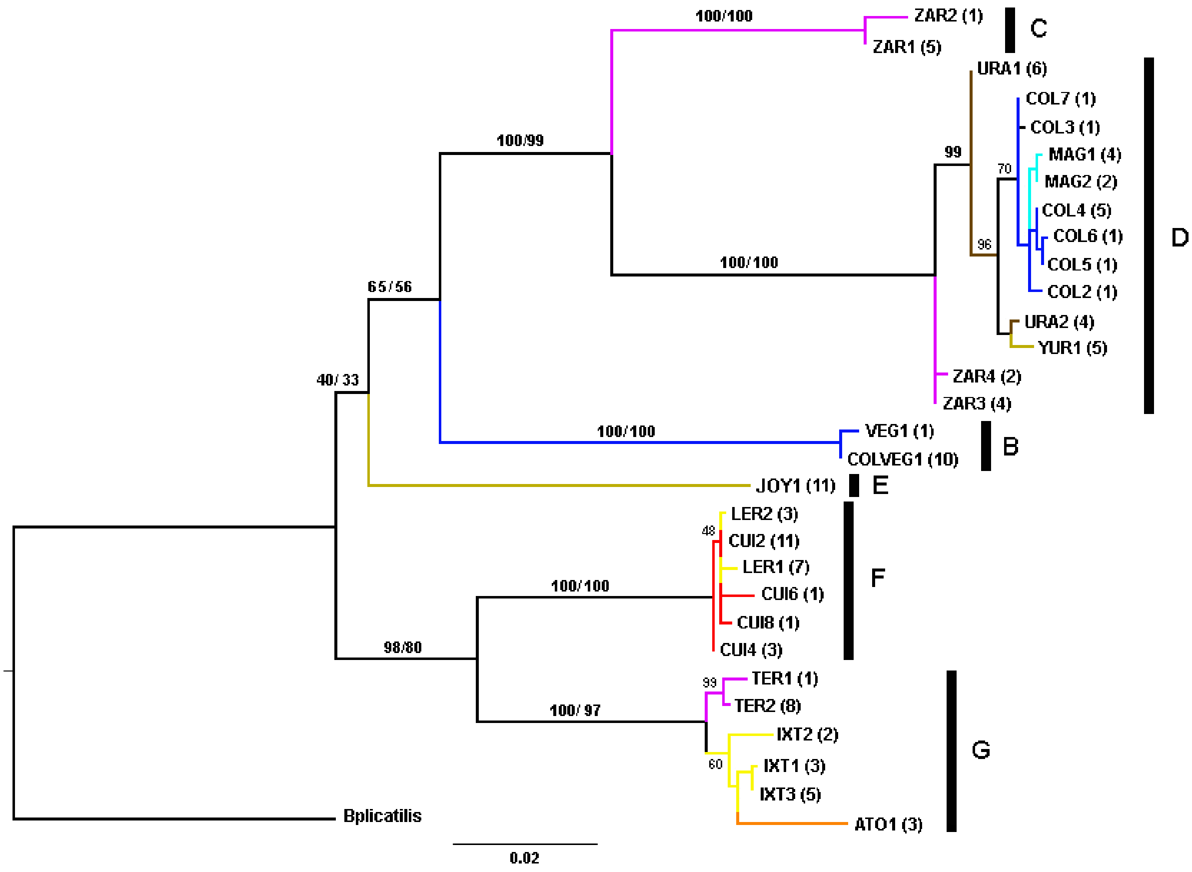

Fig. S4 Bayesian Inference phylogram of the concatenated data, showing the relationship of the 30 *B. quadridentatus* haplotypes. Haplotype names are indicated by the acronym of the water body in which they were collected, plus a number. Numbers on major branches are the percent of branch support in the Bayesian and Maximun-Likelihood analyses, respectively. Lineages B to G correspond to the same lineages in the phylogenetic analysis of the COI gene.

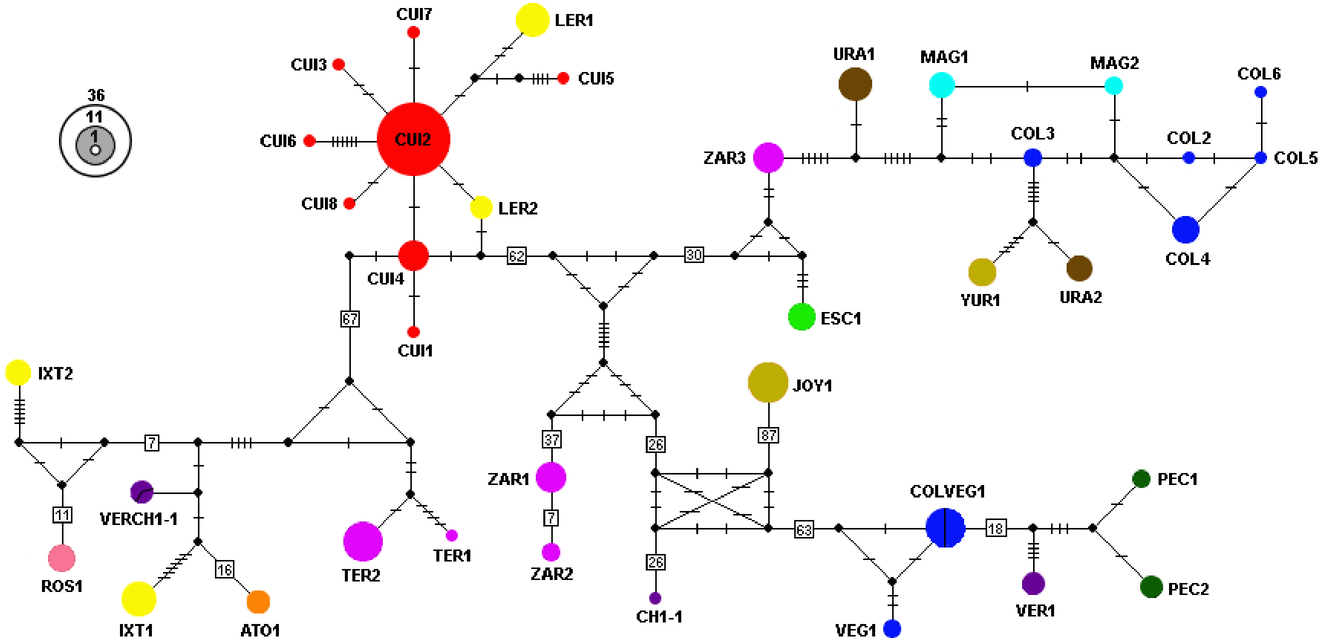

Fig. S5 Median-joining network based on the mitochondrial COI gene. Haplotypes are represented as circles, their size being proportional to the number of individuals possessing the haplotype. Median vectors, which represent either extant unsampled sequences or extinct ancestral sequences are indicated by small black circles. Short black bars and numbers in white squares represent the number of mutational steps. Haplotype names are indicated by the acronym of the water body in which they were collected (see Table 1 and S1).

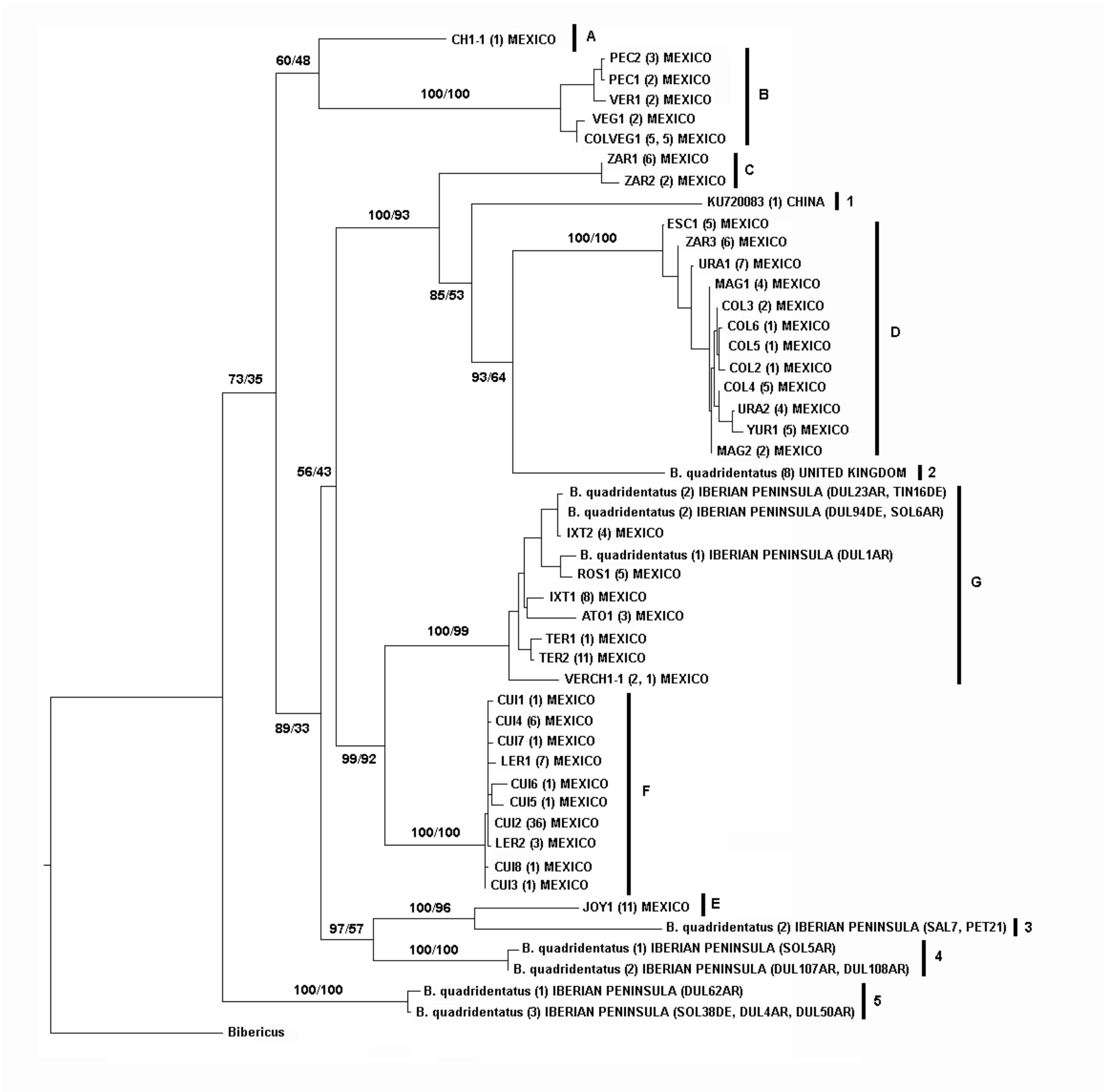
CH1-1 (1) MEXICO
A
60/48
PEC2 (3) MEXICO
PEC1 (2) MEXICO
VER1 (2) MEXICO
VEG1 (2) MEXICO
COLVEG1 (5, 5) MEXICO
100/100
B
ZAR1 (6) MEXICO
ZAR2 (2) MEXICO
C
100/93
KU720083 (1) CHINA
1
ESC1 (5) MEXICO
ZAR3 (6) MEXICO
URA1 (7) MEXICO
MAG1 (4) MEXICO
COL3 (2) MEXICO
COL6 (1) MEXICO
COL5 (1) MEXICO
COL2 (1) MEXICO
COL4 (5) MEXICO
URA2 (4) MEXICO
YUR1 (5) MEXICO
MAG2 (2) MEXICO
100/100
85/53
93/64
D
73/35
B. quadridentatus (8) UNITED KINGDOM
2
56/43
B. quadridentatus (2) IBERIAN PENINSULA (DUL23AR, TIN16DE)
B. quadridentatus (2) IBERIAN PENINSULA (DUL94DE, SOL6AR)
IXT2 (4) MEXICO
B. quadridentatus (1) IBERIAN PENINSULA (DUL1AR)
ROS1 (5) MEXICO
IXT1 (8) MEXICO
ATO1 (3) MEXICO
TER1 (1) MEXICO
TER2 (11) MEXICO
VERCH1-1 (2, 1) MEXICO
100/99
G
CUI1 (1) MEXICO
CUI4 (6) MEXICO
CUI7 (1) MEXICO
LER1 (7) MEXICO
CUI6 (1) MEXICO
CUI5 (1) MEXICO
CUI2 (36) MEXICO
LER2 (3) MEXICO
CUI8 (1) MEXICO
CUI3 (1) MEXICO
89/33
99/92
100/100
F
JOY1 (11) MEXICO
E
100/96
B. quadridentatus (2) IBERIAN PENINSULA (SAL7, PET21)
3
97/57
100/100
B. quadridentatus (1) IBERIAN PENINSULA (SOL5AR)
B. quadridentatus (2) IBERIAN PENINSULA (DUL107AR, DUL108AR)
4
100/100
B. quadridentatus (1) IBERIAN PENINSULA (DUL62AR)
B. quadridentatus (3) IBERIAN PENINSULA (SOL38DE, DUL4AR, DUL50AR)
5
Bibericus

Fig. S6 Bayesian Inference phylogram of the mtDNA COI showing the relationship of 48 *B. quadridentatus* haplotypes from Mexico, Europe and China. Lineages A-G corresponds to the lineages in Figure 3. Lineages 1-5 corresponds to the new lineages found when sequences from Iberian Peninsula, United Kingdom and China were added to our dataset. Haplotypes from Mexico are indicated by the acronym of the water body in which they were collected, plus a number. Number after name corresponds to the number of samples within each haplotype. Numbers on major branches are the percent of branch support in the Bayesian and Maximun-Likelihood analyses, respectively. A sequence of *B. ibericus* (accession GQ894740) was used as outgroup.