

Supplementary Materials

Table S1. Primer pairs and PCR conditions for selected mtDNA fragments amplification of roaches.

mtDNA Region	Primer pairs	PCR conditions	Products, bp
ATP6 partial gene	ATP6_L: 5'-TCCAACCTCCACCATCTCGTT-3' ATP6_R: 5'-GCCTGGATTATGGCTACTGC-3'	2 min at 94°C, 35 cycles (30 s at 94°C, 45 s at 52°C, 45 s at 72°C), 5 min at 72°C.	520
D-loop region fragment	Rut_2F: 5'-GTTTCGGGGTTTGACAAAGA-3' Rut_2R: 5'-AGGTCAGGACCATGCCTTTA-3'	2 min at 95°C, 35 cycles (1 min at 94°C, 45 s at 59°C, 45 s at 72°C), 5 min at 72°C.	590

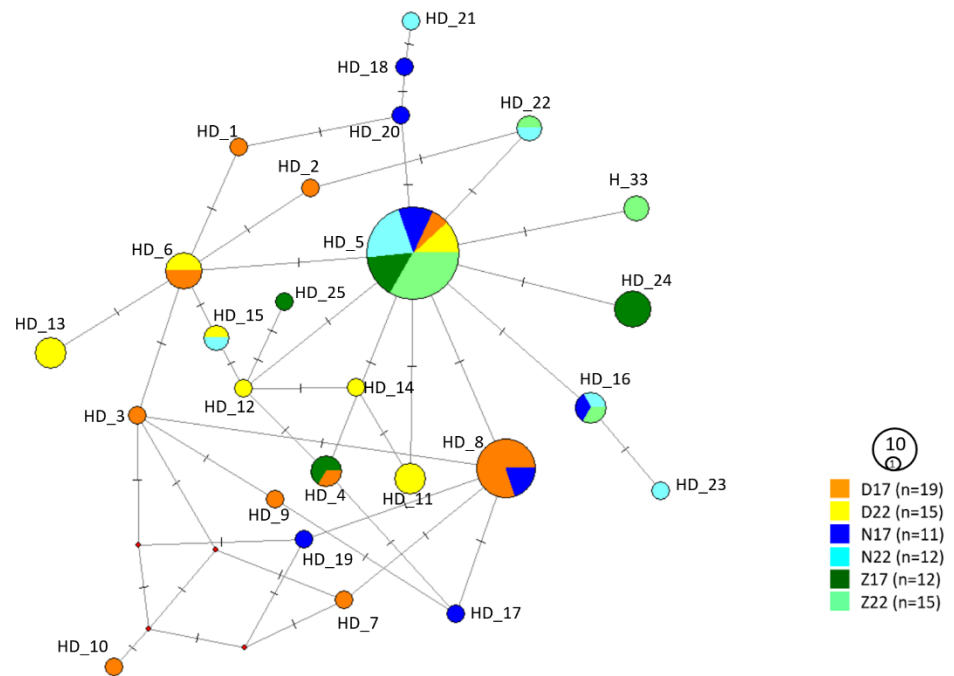


Figure S1. Median-joining haplotype networks of *R. rutilus* using mtDNA D-loop region sequences. Haplotype network of roaches collected from three different locations in 2017 and 2022; Each circle represents a haplotype, and the size of a circle is proportional to the number of sequences assigned to that haplotype. The location from which the sequence was obtained is indicated by color in the legend. The number of hatch marks indicates the number of nucleotide differences that separate the haplotypes.

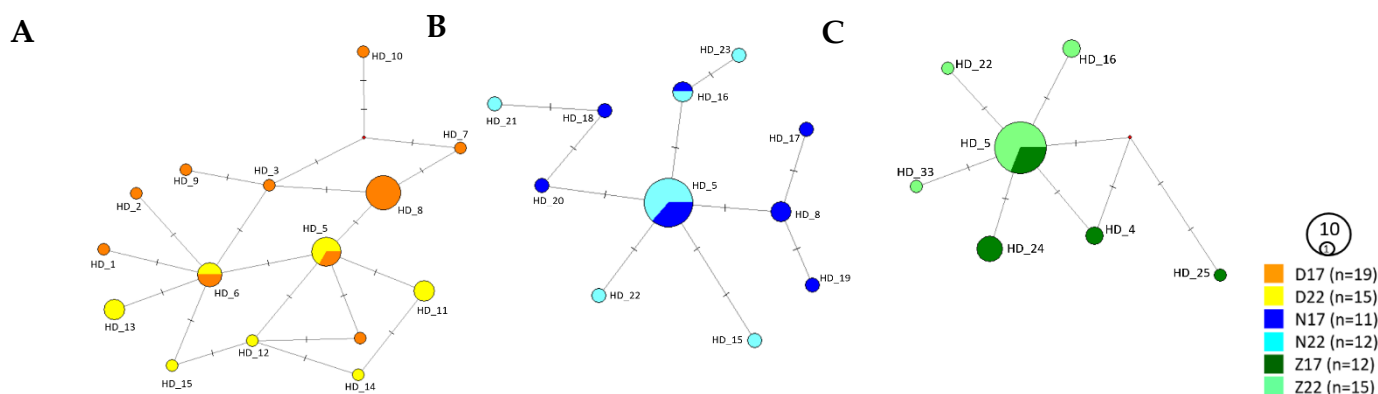


Figure 2S. Median-joining haplotype networks of *R. rutilus* using mtDNA D-loop marker. (A) Haplotype network of roaches collected from Drūkšiai in 2017 and 2022. (B) Haplotype network of roaches collected from Neris in 2017 and 2022. (C) Haplotype network of roaches collected from Žeimena in 2017 and 2022. Each circle represents a haplotype, and the size of a circle is proportional to the number of sequences assigned to that haplotype. The location from which the sequence was obtained is indicated by color in the legend. The number of hatch marks indicate the number of nucleotide differences that separate the haplotypes.

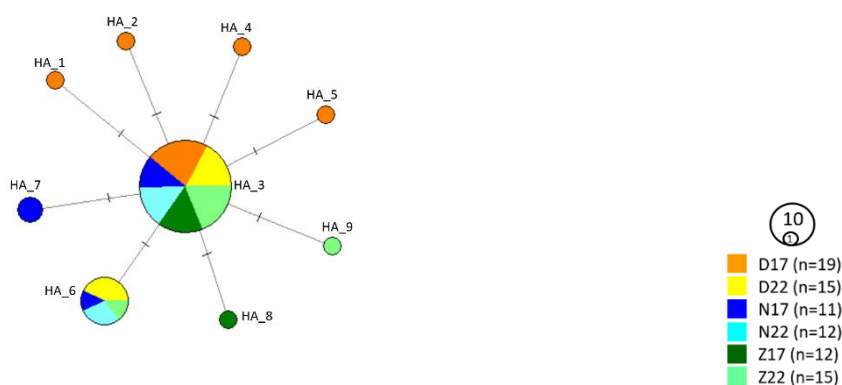


Figure S3. Median-joining haplotype networks of *R. rutilus* using mtDNA ATP6 gene marker. Haplotype network of roaches collected from three different locations in 2017 and 2022. Each circle represents a haplotype, and the size of a circle is proportional to the number of sequences assigned to that haplotype. The location from which the sequence was obtained is indicated by color in the legend. The number of hatch marks indicates the number of nucleotide differences that separate the haplotype.

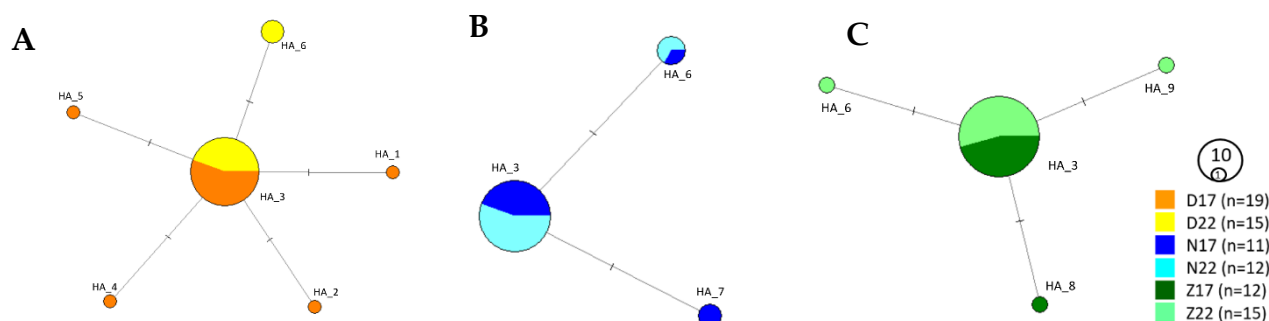


Figure S4. Median-joining haplotype networks of *R. rutilus* using mtDNA ATP6 gene marker. (A) Haplotype network of roaches collected from Drūkšiai in 2017 and 2022. (B) Haplotype network of roaches collected from Neris in 2017 and 2022. (C) Haplotype network of roaches collected from Žeimena in 2017 and 2022. Each circle

represents a haplotype, and the size of a circle is proportional to the number of sequences assigned to that haplotype. The location from which the sequence was obtained is indicated by color in the legend. The number of hatch marks indicates the number of nucleotide differences that separate the haplotype.

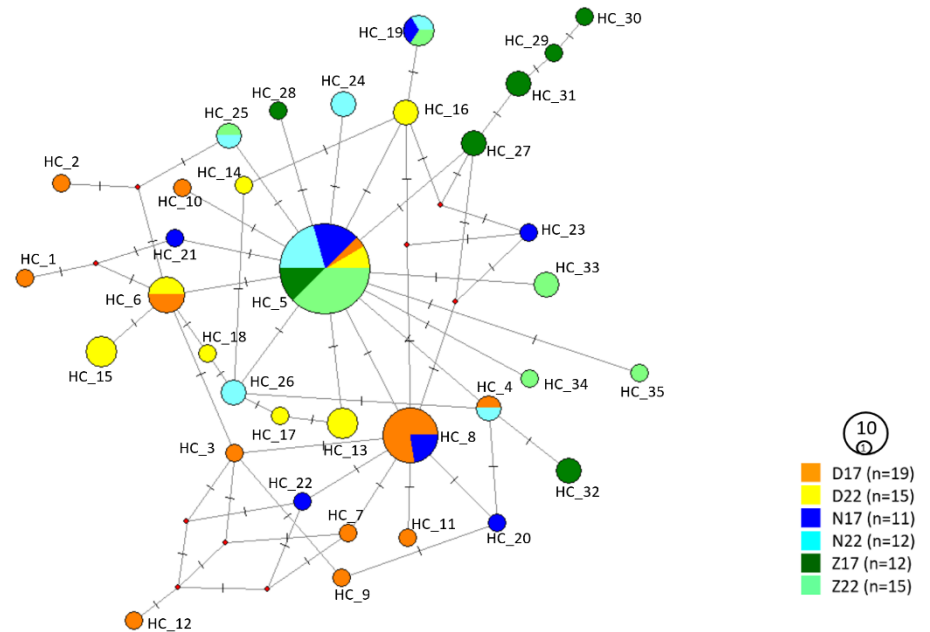
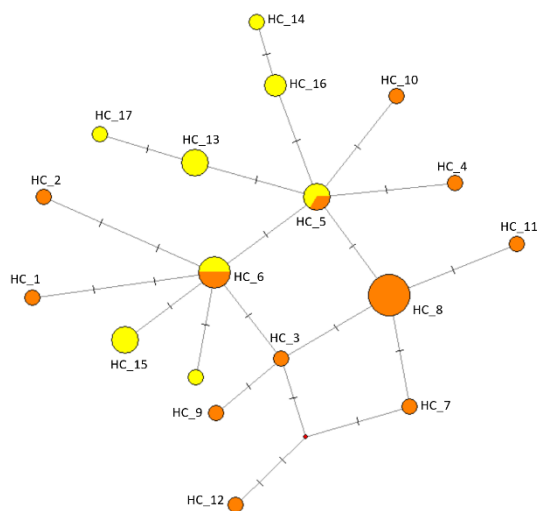
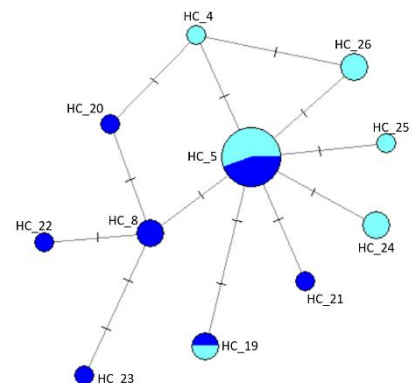


Figure S5. Median-joining haplotype networks of *R. rutilus* using mtDNA ATP6_D-loop marker. Haplotype network of roaches collected from three different locations in 2017 and 2022. Each circle represents a haplotype, and the size of a circle is proportional to the number of sequences assigned to that haplotype. The location from which the sequence was obtained is indicated by color in the legend. The number of hatch marks indicates the number of nucleotide differences that separate the haplotype.

A



B



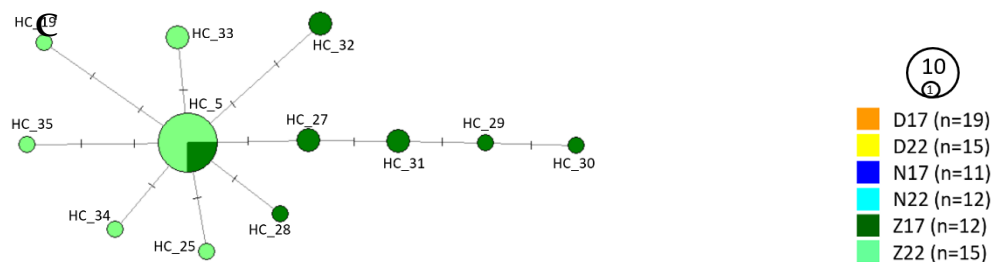


Figure S6. Median-joining haplotype networks of *R. rutilus* using mtDNA ATP6-D-loop marker. (A) Haplotype network of roaches collected from Drūkšiai in 2017 and 2022. (B) Haplotype network of roaches collected from Neris in 2017 and 2022. (C) Haplotype network of roaches collected from Žeimena in 2017 and 2022. Haplotype network of roaches collected from three different locations in 2017 and 2022; Each circle represents a haplotype, and the size of a circle is proportional to the number of sequences assigned to that haplotype. The location from which the sequence was obtained is indicated by color in the legend. The number of hatch marks indicates the number of nucleotide differences that separate the haplotype.

Table S2. Analysis of molecular variance (AMOVA) based on mtDNA D-loop, ATP6 and ATP6-D-loop markers for populations of *R. rutilus*. d.f. degrees of freedom; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Sequence	Source of variation	d.f.	Sum of squares	Percentage of variance, %	PhiPT
D-loop					
Drūkšiai: 2017 and 2022	Among populations	1	4.721	21	0.211***
	Within populations	32	27.544	79	
	Total	33	32.265	100	
Neris: 2017 and 2022	Among populations	1	1.171	5	0.048
	Within populations	21	15.568	95	
	Total	22	16.739	100	
Žeimena: 2017 and 2022	Among populations	1	1.335	14	0.140***
	Within populations	25	10.517	86	
	Total	26	11.852	100	
ATP6					
Drūkšiai: 2017 and 2022	Among populations	1	0.428	7	0.068
	Within populations	32	6.189	93	
	Total	33	6.618	100	
Neris: 2017 and 2022	Among populations	1	0.223	1	0.01
	Within populations	21	4.212	99	
	Total	22	4.435	100	
Žeimena: 2017 and 2022	Among populations	1	0.106	0	-0.004
	Within populations	25	2.783	100	
	Total	26	2.889	100	
ATP6-D-loop					
Drūkšiai: 2017 and 2022	Among populations	5	17.762	18	0,188***
	Within populations	78	69.309	82	
	Total	83	87.071	100	
Neris: 2017 and 2022	Among populations	2	8.615	18	0,103*
	Within populations	39	41.409	82	
	Total	41	50.024	100	
Žeimena: 2017 and 2022	Among populations	2	3.862	11	0,195***
	Within populations	39	27.900	89	
	Total	41	31.762	100	