Supplementary Information for:

Invasion dynamics of the disease vector *Aedes japonicus* in Spain

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Tables

Supplementary Table S1. Geographic information of *Aedes japonicus* sampling sites, including collection date, sampling method, and life stage. 'NJ label' indicates labels used in the neighbour-joining tree (Fig. 5); 'Lat.' and 'Long.' indicate latitude and longitude in WGS84 coordinates.

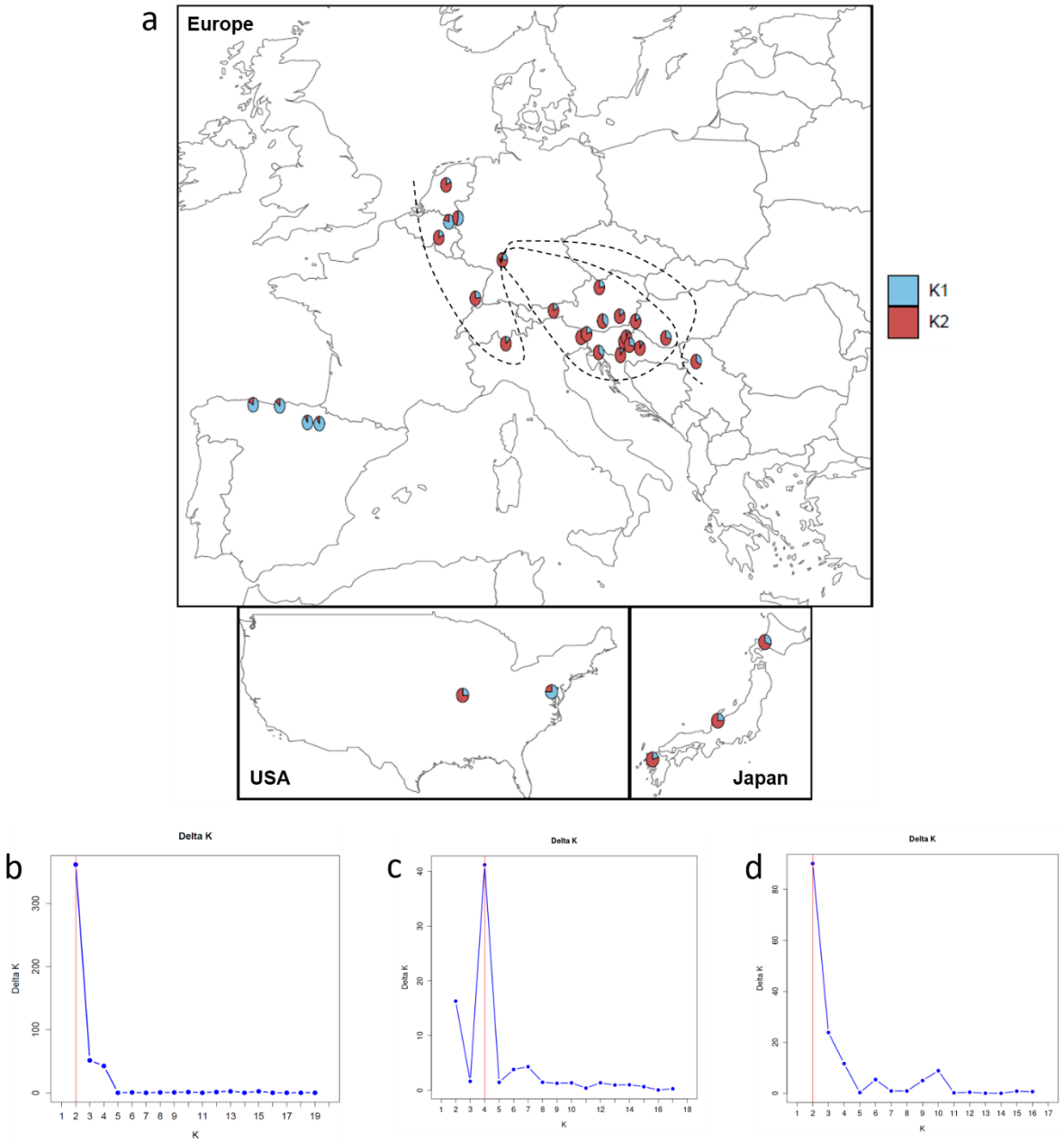
Code	Country	Location	NJ label	Life stage	Collection date(s)	Lat.	Long.	Collection method
1	Austria	Althofen	Austria-1	Larvae	23-28/07/2021	46.87	14.47	Larval dipping
2		Weer	Austria-2	Adults	16-23/08/2023	47.31	11.65	Egg sampling + rearing
3		Güssing	Austria-3	Adults	14-28/08/2023	47.08	16.34	Egg sampling + rearing
4		Graz	Austria-3	Adults	16-23/08/2023	47.08	15.45	Egg sampling + rearing
5		Deutschfeistritz	Austria-3	Adults	08-25/08/2023	47.20	15.32	Egg sampling + rearing
6		Linz	Austria-4	Adults	14-23/08/2023	48.27	14.28	Egg sampling + rearing
7	Belgium	Maasmechelen	Belgium-1	Adults	17/07/2018	51.00	5.62	Gravid traps
8		Natoye	Belgium-2	Adults	05/06/2023	50.34	5.05	Larval dipping + rearing
9	Croatia	Đurmanec	Croatia-1	Larvae	02/09/2022	46.20	15.84	Larval dipping
10		Markuševec, Zagreb	Croatia-2	Larvae	06/08/2021	45.87	16.01	Larval dipping
11		Planina gornja, Zagreb	Croatia-2	Larvae	21/09/2022	45.94	16.08	Larval dipping
12		Donji Dragonožec, Zagreb	Croatia-2	Larvae	25/07/2022	45.66	15.95	Larval dipping
13		Duga Resa	Croatia-3	Larvae	22/07/2021	45.45	15.50	Larval dipping
14		Bjelovar	Croatia-4	Larvae	23/09/2021	45.75	16.62	Larval dipping
15	France	Steinbach	France	Larvae	30/09/2021	47.82	7.15	Larval dipping
16	Germany	Heidelberg	Germany	Larvae	08/2021	49.41	8.69	Larval dipping
17	Hungary	Szalafő	Hungary-1	Larvae	02/09/2021	46.86	16.37	Larval dipping
18		Őriszentpéter	Hungary-1	Larvae	02/09/2021	46.85	16.40	Larval dipping
19		Kovácsszénája	Hungary-2	Adults	27/06/2021 - 25/08/2021	46.17	18.11	Egg sampling + rearing
20	Italy	Attimis	Italy	Adults	10/06/2022	46.20	13.23	Larval dipping + rearing
21		Clauzetto	Italy	Larvae	08/06/2022	46.24	12.91	Larval dipping
22		Travesio	Italy	Larvae	08/06/2022	46.19	12.87	Larval dipping
23	Japan	Saga	Japan-1	Adults	19/05/2018	33.33	130.27	Larval dipping + rearing
24		Ishikawa	Japan-2	Adults	09/01/2021	36.55	136.70	Larval dipping + rearing
25		Sapporo	Japan-3	Adults	09/01/2021	43.07	141.34	Larval dipping + rearing
26	Serbia	Sremska Kamenica	Serbia	Adults	06/03/2023	45.19	19.85	Egg sampling + rearing
27	Slovenia	Bovec	Slovenia-1	Larvae	28/06/2022	46.34	13.55	Larval dipping
28		Velike Žablje	Slovenia-1	Larvae	24/08/2021	45.87	13.85	Larval dipping
29		Verd	Slovenia-2	Larvae	04/07/2022	45.97	14.31	Larval dipping
30		Ilirska Bistrica	Slovenia-2	Larvae	04/07/2022	45.57	14.24	Larval dipping
31		Olmo	Slovenia-2	Larvae	30/06/2022	45.53	13.72	Larval dipping
32		Kočevje	Slovenia-3	Larvae	12/07/2022	45.64	14.85	Larval dipping
33		Bizeljsko	Slovenia-3	Larvae	25/05/2020	46.02	15.70	Larval dipping
34		Novo mesto	Slovenia-3	Larvae	12/06/2020	45.80	15.16	Larval dipping
35	Spain	Siero	Asturias	Adults + larvae	20/07/2018	43.42	-5.70	Larval dipping + rearing
36		Gijón	Asturias	Adults	28/09/2019	43.54	-5.61	Larval dipping + rearing

37		Avín, Onís	Asturias	Adults	27/09/2019	43.33	-4.95	Larval dipping + rearing
38		Cardoso, Llanes	Asturias	Adults	27/09/2019	43.44	-4.92	Larval dipping + rearing
39		Colombres, Pimiango	Asturias	Adults	26/06/2019	43.38	-4.55	Larval dipping + rearing
40		Lieres, Siero	Asturias	Adults	28/09/2019	43.39	-5.58	Larval dipping + rearing
41		Puenteles	Asturias	Adults	26/09/2019	43.39	-4.64	Larval dipping + rearing
42		Robriguero	Asturias	Adults	26/09/2019	43.35	-4.60	Larval dipping + rearing
43		Álava	Basque Country	Adults	07-08/2020	42.66	-2.51	Egg sampling + rearing
44		Orio	Basque Country	Adults	03/08/2021	43.29	-2.11	Larval dipping + rearing
45		Zamudio	Basque Country	Adults	03/05/2021	43.30	-2.87	Larval dipping + rearing
46		Altzola, Elgoibar	Basque Country	Adults	07/06/2020	43.24	-2.40	Larval dipping + rearing
47		Santa Klara, Elgoibar	Basque Country	Adults	14/06/2020	43.22	-2.42	Larval dipping + rearing
48		Polígono industrial, Elgoibar	Basque Country	Adults	07/05/2020	43.21	-2.41	Larval dipping + rearing
49		Alto de Azcarate, Elgoibar	Basque Country	Adults	06/04/2020	43.20	-2.36	Larval dipping + rearing
50		Goiuria, Yurreta	Basque Country	Adults	07/05/2020	43.19	-2.64	Larval dipping + rearing
51		Saja, Los Tojos	Cantabria	Adults	26/09/2019	43.35	-4.10	Larval dipping + rearing
52		Ruente	Cantabria	Adults	26/09/2019	43.26	-4.27	Larval dipping + rearing
53		Bera de Bidasoa	Navarra	Adults	03/07/2023	43.27	-1.70	Larval dipping + rearing
54		Bertiz	Navarra	Adults	03/07/2023	43.14	-1.61	Larval dipping + rearing
55	Switzerland	Morcote	Switzerland	Adults	05/01/2023	45.93	8.90	Egg sampling + rearing
56	The Netherlands	Lelystad	The Netherlands-1	Adults and larvae	24/06/2021 - 14/03/2022	52.53	5.47	Larval dipping + rearing
57		Urk	The Netherlands-1	Larvae	14/03/2022	52.67	5.61	Larval dipping
58		Vlodrop	The Netherlands-2	Larvae	16/03/2022	51.15	6.15	Larval dipping
59		Roermond	The Netherlands-2	Larvae	16/03/2022	51.19	6.06	Larval dipping
60	USA	Saint Louis, MO	USA-1	Adults	16/08/2021	38.53	-90.56	Egg sampling + rearing
61		College Park, MD	USA-2	Adults	09/10/2021	38.99	-76.94	Egg sampling + rearing

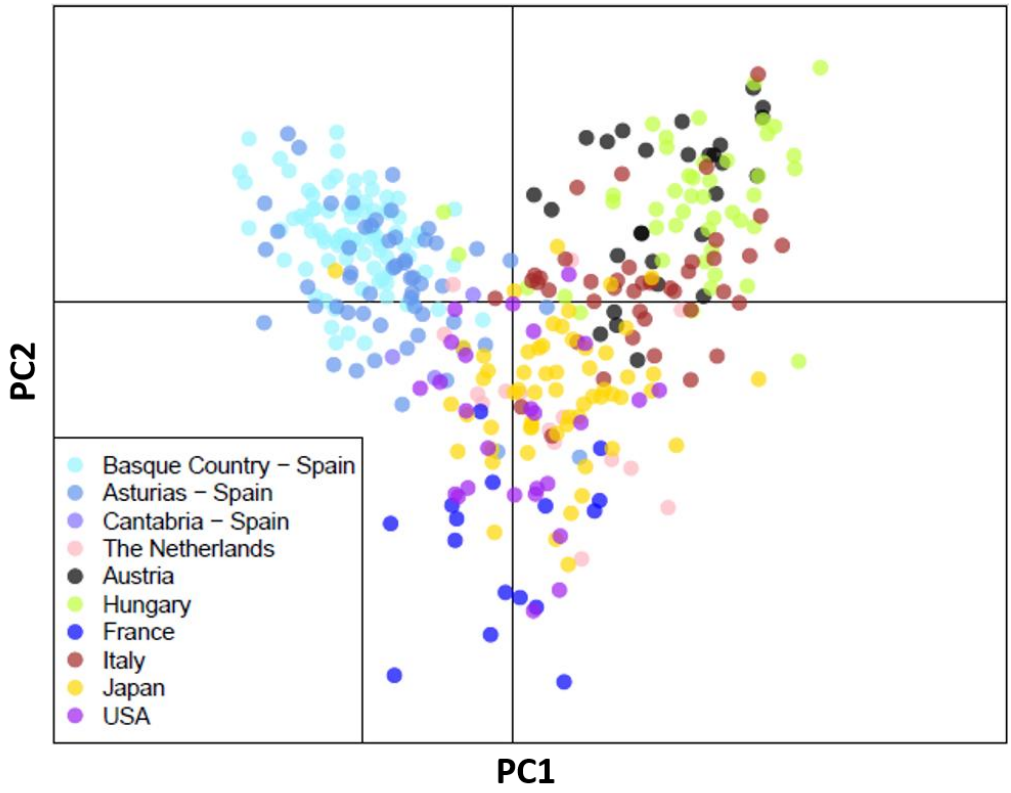
Supplementary Table S2. *Aedes japonicus*/*albopictus* microsatellite set employed in the present study. For each microsatellite, the repeat motif, allele size range, forward (F) and reverse (R) primer sequences, primer concentration (C_0), amplification multiplex panel (MPX) and primer label are shown. [1] *Ae. japonicus* microsatellites from [1]; [2] *Ae. albopictus* microsatellites from [2]; [3]*Ae. albopictus* microsatellites from [3]. Loci Alb-tri-41 and Aealbmic13 were discarded from analyses due to poor amplification.

Locus	Repeat motif	Allele size range (bp)	Primer sequences	C_0 (μM)	MPX	Primer label (fluorescent dye)
OJ10[1]	(GTT)(GTG)(GTT)8	110-139	F: GCTTGTCCTGGCTAAGTACTGC R: CGGTAATGTCCACCTGATTG	0.2	1	6-FAM
OJ70[1]	(GCT)4(GTT)2(GCT)2(GTT) (GCT)2(GTT)2(GCT)7	184-215	F: CGTTGACAAAGCTCATCTGC R: TGATCTCCAACGGAAGTATGC	0.2	1	6-FAM
OJ5[1]	(GTT)6(GCT)3(GTT)	141-157	F: CACGAAGTCTGGAAGATCTGG R: ATTCGTGCAGCGAAATCTG	0.2	1	6-FAM
OJ187[1]	(CGA)11	120-164	F: AAATCAGCTGCCAGTGCAAG R: TGTGTACTTTGCGGTGAAGG	0.2	1	VIC
OJ100[1]	(GT)5	174-202	F: CGCATTCCTCAAACCCTAAC R: TCGGTCCGAGGGAAAAAC	0.2	1	VIC
OJ85[1]	(CAG)6	161-182	F: CATAAAGCAGCAAGCACAGC R: TGTCTTCCGGATTGATTTCC	0.2	1	NED
OJ338[1]	(CAA)10	134-185	F: TCTCCTGATCCTGAAGAAGC R: AGGGAGCAGAGCAACACTTG	0.2	1	PET
Alb-tri-6[2]	(AGC)n	143-188	F: AGCACGAGTACAGAATGTGC R: TGGCCTCCTACCGTTTATCTG	0.3	2	6-FAM
Aealbmic11[3]	(TGT)n	188-230	F: CTCTGCGTTCCGGTTCTATC R: AGGCAACCTCTCGAATGAAA	0.3	2	6-FAM
Alb-tri-33[2]	(GGC)n	118-144	F: GGCTGCTGTTGTTGGTACG R: CACGTTCAATCACCGGTTCC	0.3	2	VIC
Alb-tri-46[2]	[TTC]n	145-182	F: TTCACAACATACGGAATCGC R: GGTCCGGTGTAATAGCCTCC	0.3	2	VIC
Alb-tri-44[2]	(CAC)n	161-197	F: CACTCGCGCGTGTTCTTC R: GACGCACCATCAGCATCATC	0.3	2	NED
Alb-tri-25[2]	(CCAA)n	247-260	F: CCAACCAACAACCCAGGAAC R: TACGATGCGCAACCATCATC	0.3	2	NED
Alb-tri-41[2]	(GAT)n	100-160	F: GATCGATTTGGGAGCTTCTG R: GAACCTCTTCTCGCTTGGCT	0.3	2	PET
Aealbmic5[3]	(TGT)n	136-220	F: AACCCATCGAACACAGAAGG R: GTACGGTTGACTCGCTGTGA	0.3	3	6-FAM
Aealbmic13[3]	(GAT)GAC(GAT)n	123-171	F: TCACACCATGGTCAAAGCAT R: TGCTGAGTTGAATGGAAACG	0.3	3	VIC
Alb-tri-45[2]	(TTT)n	110-136	F: TTTCAGCTCGGTGTTATGGC R: TGATGTTGATGATGATGACTACGA	0.3	3	NED
Alb-tri-20[2]	(GTG)n	145-184	F: GTGCCGTTGATCATCCTGTC R: TCCAGCACCGTGAGTAATCC	0.3	3	NED
Aealbmic3[3]	(AAC)n	200-239	F: ACCATACAGCCTGGAGTTCG R: GGGGTTGTGTGAATTGTCGT	0.3	3	NED
Aealbmic7[3]	(TTG)nATG(TTG)n	188-220	F: ATAGACGGGAGTCGGTTCCT R: TCCAACCGCTAGTGTCATCA	0.3	3	PET

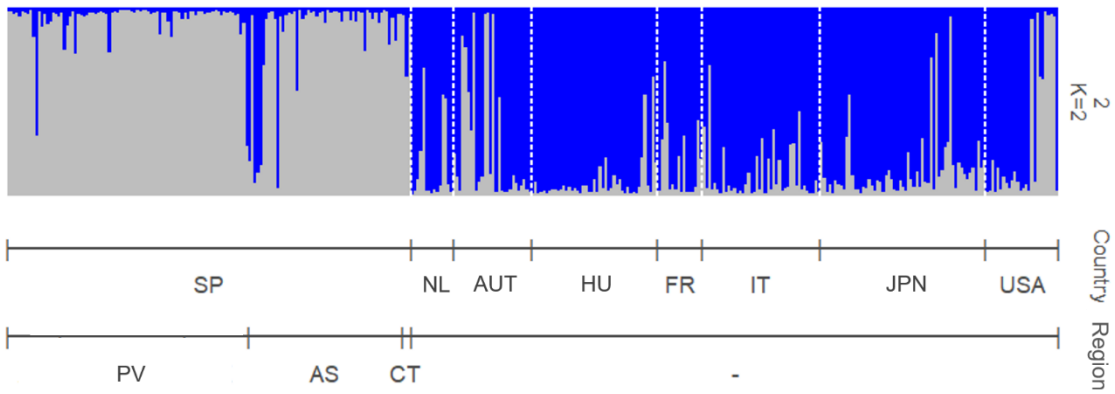
Figures



Supplementary Figure S1. Results of Bayesian clustering analysis (STRUCTURE). (a) Results of STRUCTURE for K = 2 microsatellite groups for *Aedes japonicus*. Sampled populations are represented by pie charts highlighting the population cluster membership obtained in STRUCTURE. Samples were grouped at the regional level to facilitate visualization (see Table S1). Dashed lines separate the two sub-clusters found within the main red group. (b), (c) and (d) ΔK values as a function of K, calculated according to [4], for the total number of sampled sites and for the first (light blue) and second (red) genetic clusters, respectively.



Supplementary Figure S2. Principal Component Analysis (PCA) of a subset of *Aedes japonicus* samples, based on the combination of *Ae. japonicus* and *Ae. albopictus*-specific microsatellites as shown in Table S2. Each point represents a single individual. Samples are coloured according to their geographic origin—by region for Spanish populations and by country for all others.



Supplementary Figure S3. STRUCTURE barplot showing individual membership probabilities for K = 2 in a subset of *Aedes japonicus* samples, based on the combination of *Ae. japonicus* and *Ae. albopictus*-specific microsatellites (see Table S2). Each individual is represented by a vertical bar corresponding to the sum of assignment probabilities to the K cluster. White dashed lines separate countries. Country/Spanish region codes: SP: Spain; NL: The Netherlands; AUT: Austria; HU: Hungary; FR: France; IT: Italy; JPN: Japan; USA: United States; PV: Basque Country; AS: Asturias; CT: Cantabria.

References

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