

## Supplementary Information 1

### Development of ZF locus

For individuals of the clade identified as belonging to the focal species, we sequenced additionally a nuclear locus. Primers for this locus were developed from a low coverage (<10%) genomic shotgun on a 454 platform (for a principle account on the proceeding see Feldmeyer et al. 2010). Briefly, we x-blasted singleton reads larger than 500 bp against the non-redundant protein data base of NCBI and retained six reads with e-values below e-5. For these reads, we developed primers with the online tool Primer3 (Rozen and Skaletsky, 1998; [http://biotools.umassmed.edu/bioapps/primer3\\_www.cgi](http://biotools.umassmed.edu/bioapps/primer3_www.cgi)). Of the six reads, four yielded PCR products of the expected length. Sequencing the products as described above, a single locus, with a significant BLAST hit against a zinc-finger protein, appeared to amplify a single locus and proved to be polymorphic.

Table 1. Primer for the amplification of COI, ZF and ND1

Primer	5'-3' Sequence	Reference
LCOI (forward)	GGTCAACAAATCATAAAGATATTGG	Folmer et al. 1994
HCOI (reverse)	TAAACTTCAGGGTGACCAAAAAATCA	
ZF (forward)	TGTTTGGGAATCTAGAGCCTGA	results of own shot gun
ZF (reverse)	TTTGTTGCTGTCCTGCTTGT	sequencing
MOL_NAD1F (forward)	CGRAARGGMCCTAACAARGTTGG	Quinteiro et al. 2005
MOL_NAD1R (reverse)	GGRGCACGATTWTGCTCNGCA	

Table2. Concentrations of chemicals for PCRs (total volume 25µl)

Locus	Chemical	Volume (µl)
COI and ZF	10x buffer	2.5
	100 mM MgCl <sub>2</sub>	2.0
	20 mM dNTP-Mix	0.3
	10 pmol LCOI	1.0
	10 pmol HCOI	1.0
	Taq-Polymerase	0.3
	ddH <sub>2</sub> O	11.4
	Bovine Serum Albumin (10 mg/mL)	1.5
Template DNA	5.0	
ND1	10x buffer	2.5
	100 mM MgCl <sub>2</sub>	2.0
	20 mM dNTP-Mix	0.3
	10 pmol LCOI	1.0
	10 pmol HCOI	1.0
	Taq-Polymerase	0.3
	ddH <sub>2</sub> O	11.15
	Bovine Serum Albumin (10 mg/mL)	1.5
	Dimethylsulfoxid (DMSO)	0.25
Template DNA	5.0	

Table 3. Protocols for PCR reactions

COI:

Step	Temperature (°C)	Time (min.)	Iterations
Denaturation	95	01:00	
Denaturation	95	00:30	30
Annealing	52 / 45	00:30	30
Elongation	72	00:30	30
Final elongation	72	03:00	
Storage	8	∞	∞

ZF and ND1:

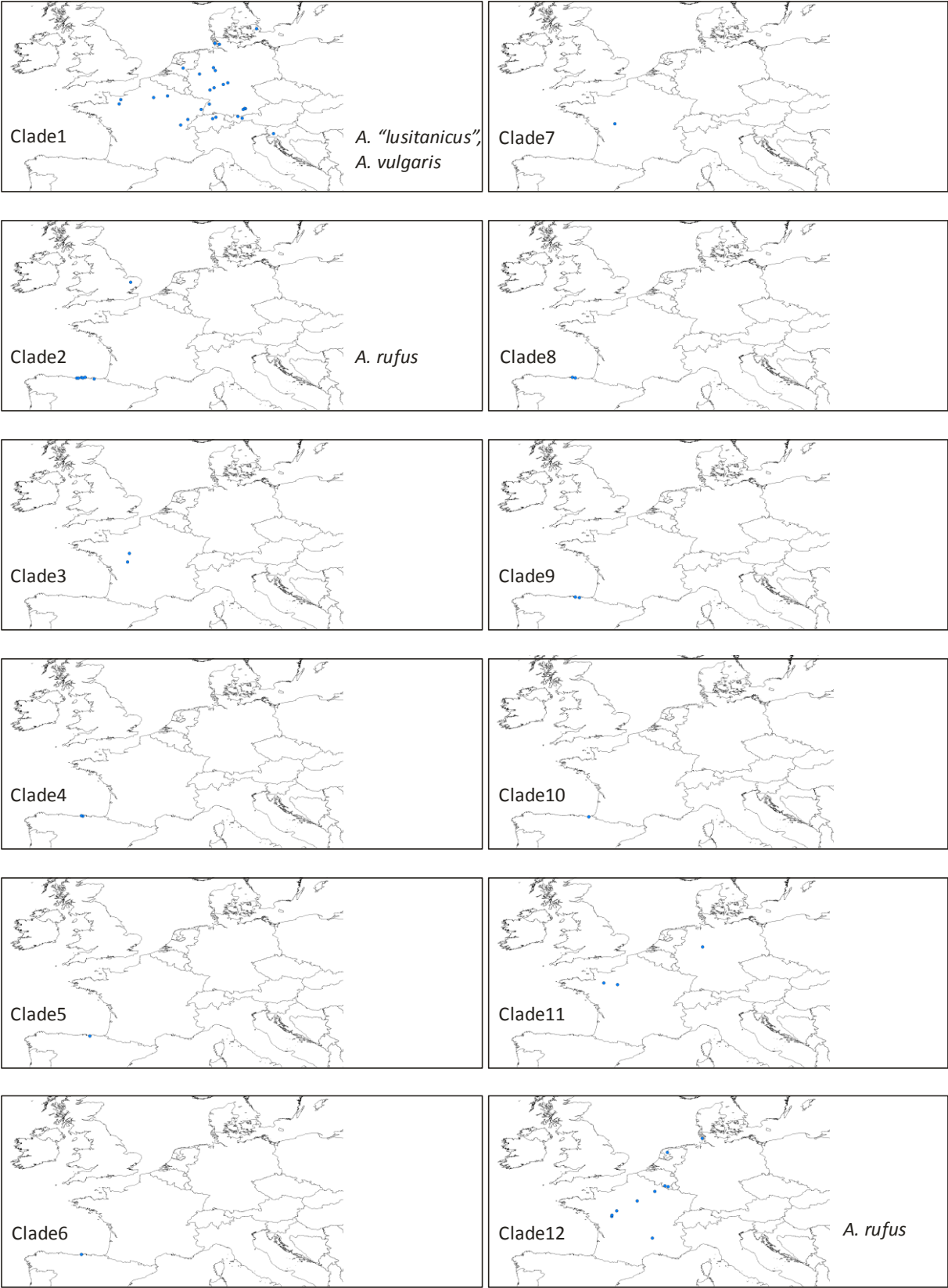
Step	Temperature (°C)	Time (min.)	Iterations
Denaturation	95	01:00	
Denaturation	95	00:30	30
Annealing	52	00:30	30
Elongation	72	00:30	30
Final elongation	72	03:00	
Storage	8	∞	∞

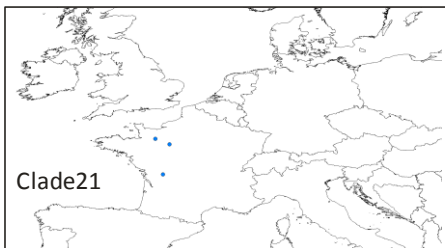
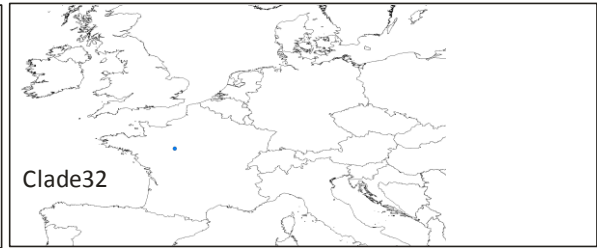
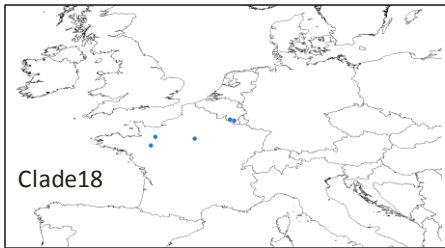
## Supplementary Information 2

Figure 1. ML phylogeny of ND1 locus. The topotypic *A. lusitanicus* does not cluster with the *A. lusitanicus* from the invasion area. The ND1 locus does not resolve *A. rufus* from the latter. Numbers on nodes present bootstraps > 90%.

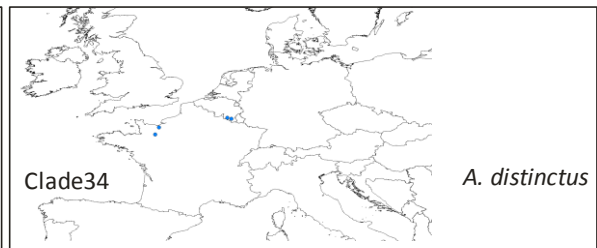


Figure 2. Distribution of clades sampled in the study. The numbering follows Figure 2 of the article.





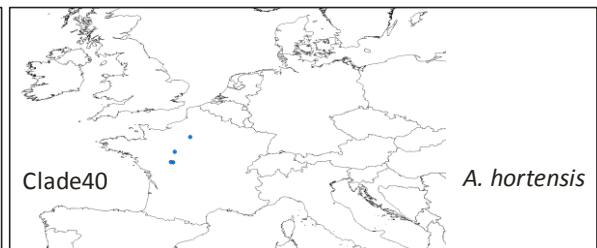
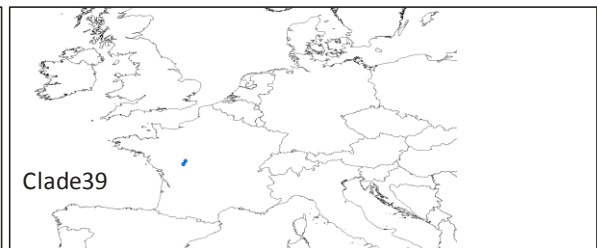
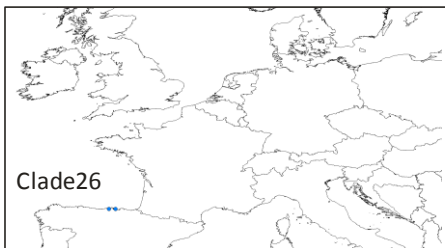
*A. subfuscus*



*A. distinctus*

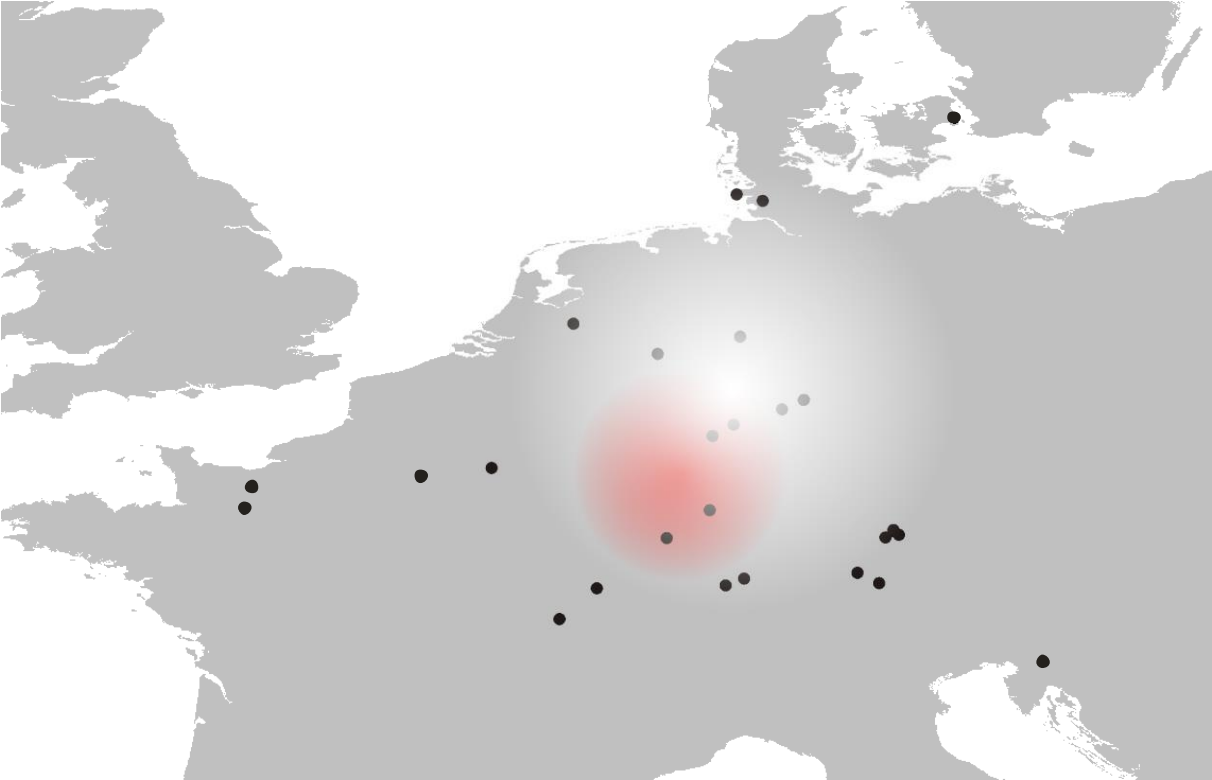


*A. sylvaticus*



*A. hortensis*

Figure 3. Estimated 80% HDP of location of origin of COI haplotypes MRCA (white shade) and ZF haplotypes (red shade) for Clade 1. The estimated age of the MRCA is 294,000 years (90-780 ka 95% HPD) for COI and 920,000 years (210-3600 ka) for ZF.











1 List of 166 GenBank Accession nos. with attributed species for COI used for comparative purposes.

2	EF520640	Arion lusitanicus	58	AJ809440	Arion fuscus	114	EU382753	Arion intermedius
3	EF520641	Arion lusitanicus	59	AY987886	Arion fuscus	115	EU382750	Arion intermedius
4	EF520642	Arion lusitanicus	60	AJ809411	Arion fuscus	116	AY423705	Arion intermedius
5	EF520643	Arion lusitanicus	61	AJ809420	Arion fuscus	117	EU382745	Arion intermedius
6	EU734823	Arion lusitanicus	62	AJ809436	Arion fuscus	118	EU382752	Arion intermedius
7	EU734824	Arion lusitanicus	63	AJ809432	Arion fuscus	119	EU382754	Arion intermedius
8	EU734825	Arion lusitanicus	64	AJ809444	Arion fuscus	120	EU382749	Arion intermedius
9	EU734826	Arion lusitanicus	65	AJ809439	Arion fuscus	121	EU382743	Arion intermedius
10	GQ166169	Arion lusitanicus	66	AJ809412	Arion fuscus	122	EU382747	Arion intermedius
11	AY987903	Arion rufus	67	AJ809427	Arion fuscus	123	AY987892	Arion iratii
12	AY987902	Arion rufus	68	AJ809433	Arion fuscus	124	AY987893	Arion lizarrustii
13	EF520647	Arion rufus	69	AJ809417	Arion fuscus	125	AY987896	Arion molinae
14	FJ481178	Arion rufus	70	AJ809425	Arion fuscus	126	FJ348255	Arion occultus
15	AY987901	Arion rufus	71	AJ809441	Arion fuscus	127	FJ348254	Arion occultus
16	EF520644	Arion rufus	72	AJ809426	Arion fuscus	128	AY423702	Arion owenii
17	AY987900	Arion rufus	73	AJ809418	Arion fuscus	129	AY423704	Arion owenii
18	EF520646	Arion rufus	74	AJ809431	Arion fuscus	130	AY423703	Arion owenii
19	EF520645	Arion rufus	75	AJ809435	Arion fuscus	131	AY987897	Arion owenii
20	AY987874	Arion distinctus	76	AJ809419	Arion fuscus	132	AY987898	Arion owenii
21	AY987875	Arion distinctus	77	AJ809446	Arion fuscus	133	AY987899	Arion paularensis
22	AY423698	Arion distinctus	78	AJ809424	Arion fuscus	134	AF513018	Arion silvaticus
23	AY423700	Arion distinctus	79	AJ809428	Arion fuscus	135	AY987917	Arion silvaticus
24	EF128218	Arion distinctus	80	AJ809438	Arion fuscus	136	AY987918	Arion silvaticus
25	AY423697	Arion distinctus	81	EU382742	Arion hortensis	137	AY987916	Arion subfuscus
26	AY423694	Arion distinctus	82	AY423670	Arion hortensis	138	AY987909	Arion subfuscus
27	AY423692	Arion distinctus	83	AY423671	Arion hortensis	139	AY987911	Arion subfuscus
28	AY423696	Arion distinctus	84	AY423680	Arion hortensis	140	GU249577	Arion subfuscus
29	AY423701	Arion distinctus	85	AY423681	Arion hortensis	141	GU249582	Arion subfuscus
30	AY423699	Arion distinctus	86	AY423673	Arion hortensis	142	GU249580	Arion subfuscus
31	AY987879	Arion fasciatus	87	AY423687	Arion hortensis	143	GU249578	Arion subfuscus
32	AY987877	Arion fasciatus	88	AY423678	Arion hortensis	144	AY987904	Arion subfuscus
33	AY987878	Arion fasciatus	89	AY423679	Arion hortensis	145	GU249579	Arion subfuscus
34	AY987880	Arion flagellus	90	AY423675	Arion hortensis	146	AY987914	Arion subfuscus
35	AY987881	Arion flagellus	91	AY423674	Arion hortensis	147	GU249583	Arion subfuscus
36	AY987882	Arion flagellus	92	AY423688	Arion hortensis	148	GU249581	Arion subfuscus
37	AY987883	Arion franciscocoli	93	AY423683	Arion hortensis	149	GU249584	Arion subfuscus
38	AY987884	Arion franciscocoli	94	AY423682	Arion hortensis	150	GU249586	Arion subfuscus
39	AJ809429	Arion fuscus	95	AY423677	Arion hortensis	151	AY987912	Arion subfuscus
40	AJ809414	Arion fuscus	96	AY423685	Arion hortensis	152	AY987906	Arion subfuscus
41	AJ809423	Arion fuscus	97	AY423691	Arion hortensis	153	AY987905	Arion subfuscus
42	AJ809421	Arion fuscus	98	AY423684	Arion hortensis	154	AY987907	Arion subfuscus
43	AJ809434	Arion fuscus	99	AY423672	Arion hortensis	155	AY987908	Arion subfuscus
44	AJ809416	Arion fuscus	100	AY423690	Arion hortensis	156	AY987910	Arion subfuscus
45	AJ809410	Arion fuscus	101	AY423676	Arion hortensis	157	GU249576	Arion subfuscus
46	AJ809409	Arion fuscus	102	AY987889	Arion hortensis	158	GU249587	Arion subfuscus
47	AJ809430	Arion fuscus	103	AY987888	Arion hortensis	159	AY987913	Arion subfuscus
48	AJ809445	Arion fuscus	104	AY423686	Arion hortensis	160	GU249585	Arion subfuscus
49	AJ809408	Arion fuscus	105	AY423689	Arion hortensis	161	AY987915	Arion subfuscus
50	AY987885	Arion fuscus	106	AY987891	Arion intermedius	162	AY943858	Arion transsylvanus
51	AJ809415	Arion fuscus	107	EU382755	Arion intermedius	163	AY943860	Arion transsylvanus
52	AJ809442	Arion fuscus	108	EU382756	Arion intermedius	164	AY943859	Arion transsylvanus
53	AJ809443	Arion fuscus	109	AY987890	Arion intermedius	165	AY987919	Arion urbiae
54	AJ809437	Arion fuscus	110	EU382748	Arion intermedius	166	AY987921	Arion wiktori
55	AY987887	Arion fuscus	111	EU382746	Arion intermedius	167	AY987920	Arion wiktori
56	AJ809422	Arion fuscus	112	EU382751	Arion intermedius			
57	AJ809413	Arion fuscus	113	EU382744	Arion intermedius			

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1 List of 40 ND1 accession nos. used.

- 2 AY316292
- 3 AY316261
- 4 AY316262
- 5 AY316250
- 6 AY316265
- 7 AY316249
- 8 AY316263
- 9 AY316264
- 10 AY316251
- 11 AY316247
- 12 AY316246
- 13 AY316245
- 14 AY316254
- 15 AY316267
- 16 AY316252
- 17 AY316253
- 18 AY316256
- 19 AY316255
- 20 AY316266
- 21 AY316260
- 22 AY316259
- 23 AY316257
- 24 AY316258
- 25 AY316243
- 26 AY316244
- 27 AY316232
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- 29 AY316234
- 30 AY316235
- 31 AY316248
- 32 AY316230
- 33 AY316238
- 34 AY316239
- 35 AY316240
- 36 AY316242
- 37 AY316241
- 38 AY316228
- 39 AY316229
- 40 AY316231
- 41 AY316237

1 Supplementary Information 3

2 List of taxonomic treatises, determination keys, field guides, etc. consulted.

- 3 • Geyer D (1909) Die Weichtiere Deutschlands. Strecker & Schröder, Stuttgart.
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5 Braun'sche Hofbuchhandlung, Karlsruhe.
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18 Heidelberg.
- 19 • Stresemann E (1976) Exkursionsfauna für die Gebiete der DDR und der BRD - Wirbellose 1.  
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