Arachnology (2018) **17** (7), 337–357

Molecules v. morphology—is *Eratigena atrica* (Araneae: Agelenidae) one species or three?

Geoff S. Oxford
Department of Biology,
University of York,
Heslington, York, YO10 5DD
email: geoff.oxford@york.ac.uk

Angelo Bolzern
Biosciences, Natural History Museum Basel,
Augustinergasse 2,
CH-4001 Basel, Switzerland

Abstract

DNA analyses may reveal groupings of species that are discordant with phylogenies constructed using morphological characters. Here, we examine the taxonomic status of what, historically, was called the *Tegenaria atrica* group of large house spiders: *T. atrica*, *T. saeva*, and *T. duellica* (*gigantea*). A recent phylogenetic analysis, based largely on European material, proposed that this group should be transferred to a new genus, *Eratigena*, and that a lack of morphological and mtDNA distinctiveness between constituent members indicated a single species: *Eratigena atrica*. The new genus is well supported but the synonymy of the three species is inconsistent with information from Britain. Here, we sequence new specimens from Britain, and examine morphologically both this and additional material from Britain, continental Europe, and North America. We test the hypothesis that, although the three species are distinct in Britain, in continental Europe hybridization may have led to their fusion into a single entity. Our mtDNA sequence data confirm the previously demonstrated small genetic distances and complex phylogenetic relationships between taxa. However, we show that three distinct morphological groupings exist across the range of the species and that, as a result, the specific names *atrica s.s.*, *saeva*, and *duellica* should be resurrected.

Keywords: incomplete lineage sorting • introgression • large house spiders • reproductive barriers • *Tegenaria atrica* • *Tegenaria duellica* • *Tegenaria gigantea* • *Tegenaria saeva*

Introduction

DNA sequencing has revolutionized our understanding of the phylogenetic relationships between taxa. In many examples, the groupings within a molecular phylogenetic tree match those based on morphological criteria, and mutually reinforce a single model of speciation and divergence. However, in some situations, there is disparity between morphological and molecular signals, leading to different conclusions about the relatedness of taxa. These cases can result from several evolutionary processes; for example, hybridization leading to the sharing of neutral molecular markers but not morphology (e.g. the house mice *Mus musculus* and *M. domesticus*: Prager *et al.* 1993), convergence of morphological characters in genetically distinct lineages (e.g. Stern 2013), the rapid divergence of key structures among relatively young species (e.g. genitalia in the spiders *Enoplognatha ovata* and *E. latimana*: Lasut, Nentwig & Kropf 2015) and incomplete lineage sorting in recently diverged taxa (e.g. in grasshoppers: Carstens & Knowles 2007). Although of great interest in themselves, such processes can cause problems with the resolution of taxonomic affinities and lead to a conflict as to whether molecules or morphology indicate the correct relationships.

Here, we examine a situation involving three closely related species of agelenid spiders, historically placed in the *Tegenaria atrica* group. Locket (1975) attributed three species to this group: *T. atrica* C. L. Koch, 1843, *T. saeva* Blackwall, 1844, and *T. propinqua* Locket, 1975. The last species was found to be a junior synonym of *T. gigantea* Chamberlin & Ivie, 1935 (Crawford & Locket 1976), which itself was proposed as a junior synonym of *T. duellica* Simon, 1875 (Brignoli 1978). This last change is arguable since no holotype exists and, in the current check list of British spiders (Merrett, Russell-Smith & Harvey 2014), the name *gigantea* is retained. However, given the previous acceptance of *duellica* by the World Spider Catalog (2018), that is the name used here. Recently, Bolzern, Burckhardt & Hänggi (2013) proposed, on the basis of both molecular (mtDNA) and morphological evidence, that members of the *Tegenaria atrica* group belong to a different, newly-erected genus, *Eratigena*, and that the three species should be regarded as one species, *E. atrica*. This nomenclatorial change is now accepted by the World Spider Catalog (2018) and has been quickly adopted elsewhere.

The placement of the species in a genus different to *Tegenaria* is well supported by the evidence presented by Bolzern, Burckhardt & Hänggi (2013). They showed that there are deep divisions in both morphological and molecular phylogenies between *Tegenaria* and the new genus *Eratigena* and that the species considered here fall well within *Eratigena*.

The synonymy of the three historical species as *Eratigena atrica*, however, poses problems. Bolzern, Burckhardt & Hänggi (2013: 761) gave two main reasons for their judgement: '… the three species are not recovered with CO1 and NADH1 gene sequences (the genetic distances are very small)', and '… seemingly major morphological differences arise when structures are not observed in the same perspective', a point made previously by Barrientos & Ribera (1988). In Britain, the three species are readily differentiated morphologically when pedipalps and epigynes are consistently oriented (Merrett 1980; Oxford 2008) and, over much of Britain, have distinct distributions (Merrett 1980; Croucher *et al.* 2007; Oxford 2009, 2011; Oxford & Smith 2014). In addition, Oxford & Croucher (2014) showed that there was a considerable mechanical barrier to hybridization between *T. saeva* and *T. duellica* taken from both allopatric and parapatric populations. How might the lack of consistency in species identification between the studies be explained?

There seem to be three possibilities. First, Bolzern, Burckhardt & Hänggi (2013) and Barrientos & Ribera (1988) may have confused species because they did not appreciate fully the precise orientation of the genitalia required to separate them reliably and, as a result, failed to find either morphological and, in the case of Bolzern, Burckhardt & Hänggi (2013), mtDNA differences between them. Interpretation of the latter depends critically on correct identification. Most of the material examined by Bolzern, Burckhardt & Hänggi (2013) and Barrientos & Ribera (1988) came from continental Europe and so a second, and much more interesting, hypotheses is that continental species might have undergone

hybridization paralleling, but to a more extreme degree, that currently observed in northern England and Scotland (Oxford & Smith 1987; Oxford & Plowman 1991; Croucher *et al.* 2007; Oxford 2011) and now represent essentially a single taxon. If this is the case, the species in southern Britain, which are easily separable morphologically, may represent important 'ark' populations that preserve intact historical taxonomic distinctions. If so, one might also expect more pronounced differences in mtDNA sequences between species in British material. Finally, if mtDNA differences are not found between the clearly differentiated species in southern Britain, this would provide a further example of discordance between morphological and molecular signals, and could be the result of a number of past and current evolutionary processes, as outlined above.

To examine these possibilities, we collected new material from England and Wales for mtDNA sequencing and examined the morphology of these and other samples from Britain, continental Europe and North America.

Material and methods

Morphological analyses

As well as the specimens used for DNA extraction (see below) additional British material was selected from GSO's personal collection to boost the numbers of males and/or females examined morphologically. All British material is retained by GSO at the University of York. Specimens were also loaned from the following institutions: Denver Museum of Nature and Science, Denver, USA (Paula Cushing and Jeff Stephenson); Finnish Museum of Natural History, Helsinki, Finland (Timo Pajunen); Manchester Museum, Manchester, UK (Dmitri Logunov); Naturhistorisches Museum Basel, Basel, Switzerland (Ambros Hänggi); Natural History Museum, London, UK (Janet Beccaloni); Royal British Columbia Museum, Victoria, Canada (Claudia Copley). Robert Bosmans (Gent, Belgium), Julien Pétillon (University of Rennes, France), and Rick West (Vancouver Island, Canada) kindly made additional specimens available. Known F1 hybrids from an *E. saeva* × *E. duellica* cross were generated in a previous study (Oxford & Croucher 2014). The overall distribution of specimens examined, but excluding synthesized hybrids, is shown in Fig. 1.

All material used for morphological analyses was examined in 70% ethanol. Measurements were made by GSO using a Reichert binocular microscope fitted with an eyepiece graticule and fixed objective lenses, and are expressed here in millimetres. It was decided from the start only to measure characters that had been shown to be taxonomically informative for the *E. atrica* group (Merrett 1980; Oxford & Plowman 1991; Croucher 1998; Croucher *et al.* 2007). Eight characters were measured on males: maximum prosoma length (PROL), maximum prosoma width (PROW), maximum tegulum + conductor length (TCL), tegulum width (TW) and maximum tegulum width (TWMX) (for details of these measurements see Croucher *et al.* 2007). Conductor length (CL), conductor width (CW) and a new character, conductor angle (CANG), are shown in Fig. 2A. CANG was not quantified as an angle *per se*, but as a linear deviation from a baseline, which is a function of the conductor angle. CANG was measured by aligning a major eyepiece graticule mark with the ventral surface of the tegulum and adjusting the horizontal rotation of the palp such that when the mechanical stage was moved from side to side the graticule mark exactly traced the red lines shown in Fig. 2A. The mark was moved out until the graticule scale just coincided with the tip of the conductor, when the conductor angle was measured as a linear distance. Left or right palps were removed at random; where a palp had previously been excised, that was the one used. In females, 12 characters were measured, eight external and four internal. The external features were: PROL and PROW (see above), tip of apophysis to the posterior edge of the receptacle (spermathecal) opening (APR), maximum width between receptacle openings (RWMX), minimum width between receptacle openings (RWMN), receptacle opening long width (RLW) (see Croucher *et al.* 2007). RWMN and RLW, together with two new measurements, receptacle opening short width (RSW) and top of receptacle opening to epigastric furrow (RH) are shown in Fig. 2B. The term receptacle is retained here so as to be consistent with the usage and acronyms of Croucher *et al.* (2007). Internal measurements were maximum spermatheca width (SWMX), total length of a spermatheca (SMX), distance from the epigastric furrow to the major constriction in the spermatheca width (CSW) and whether the anterior end bent out (score +1) or in (score -1) or neither way (score 0) for each of the spermathecae (Fig. 2B). The two scores were then summed. Internal measurements were made on excised epigynes briefly dehydrated in absolute ethanol, immersed in clove oil and viewed from a dorsal (internal) aspect. For some of the borrowed material dissection of the female genitalia was not permitted. Which spermatheca was used for measurements was randomized. All measurement data (mm) are provided in the Appendix.

For these measurements to be repeatable, orientation is critical, as noted by previous workers, e.g. Barrientos & Ribera (1988) and Bolzern, Burckhardt & Hänggi (2013). Here, we use the orientations specified by Merrett (1980) for males and Oxford (2008) for females. Statistical analyses were performed using Minitab 17, SigmaPlot 13 and Microsoft Excel 2010 packages.

Molecular analyses

For the new DNA analyses, fresh specimens were collected between 2013 and 2016 by GSO, or by members of the British Arachnological Society and others, from locations across England and Wales and preserved in 98% ethanol (Table 1). In total, 20 *E. saeva*, 20 *E. duellica* and 10 *E. atrica s.s.* were taken from areas where the species are either allopatric (*E. saeva*, *E. duellica*) or constitute the most numerous species present (*E. atrica s.s.*). Two legs were removed from each specimen and anonymized by GSO with a random-number code before being sent to AB for DNA extraction and sequencing.

The ethanol was removed by incubating the cut tissue at 70°C for 30 min and the leg processed according to the protocol for the purification of total DNA from animal tissues (Spin-Column protocol) of the DNeasy Blood &

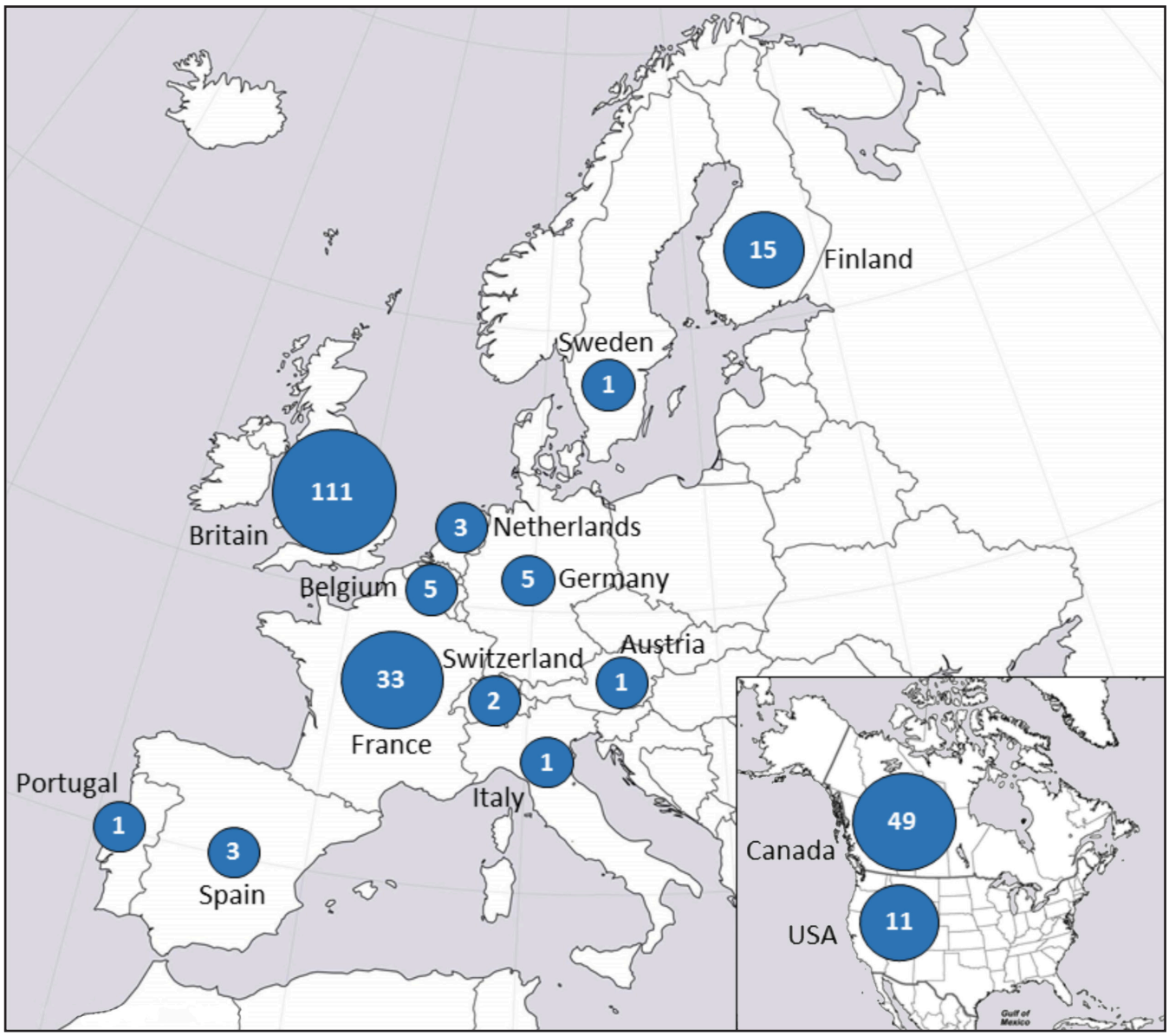


Fig. 1: Geographical distribution of specimens examined morphologically.

Tissue Kit (Qiagen). The DNA concentration of the resulting solution was measured using the fluorescent dye Picogreen and a Spectrofluorometer.

The amplification of two loci was undertaken by using the following primer pairs: LCO1490 (Folmer *et al.* 1994) and C1-N-2191 (Simon *et al.* 1994) for the mitochondrial cytochrome c oxidase subunit 1 gene (CO1, 678 bp), and TL-1-N-12718 (Hedin & Maddison 2001) and M510 (Murphy *et al.* 2006) for the mitochondrial nicotinamide adenine dinucleotide dehydrogenase subunit 1 (NADH1, 591 bp).

For the PCR, the Qiagen Hotstar polymerase reagents (Qiagen, Germany) were used. The following thermo-cycling conditions were applied: initial denaturation step at 95°C for 15 min, followed by 15 cycles touchdown at 94°C for 35 s, an annealing temperature from 60°C to 45°C for 90 s, and an extension temperature of 72°C for 90 s. After the touchdown cycling, 30 additional cycles were added at 94°C for 35 s, 50°C for 90 s, and 72°C for 90 s. Finally, the cycling was followed by an additional extension at 72°C for 5 min. The fragments were then sequenced applying the sequencing reaction method BigDye Terminator version 3.1. The capillary electrophoresis was undertaken by ecogenics.ch using an Applied Biosystems ABI3730xl DNA analyzer.

The new sequences were combined with those from Bolzern, Burckhardt & Hänggi (2013) and, for some analyses, with previous British sequences (Croucher 1998; Croucher, Oxford & Searle 2004) extracted from GenBank. Model testing and phylogenetic analyses were performed in MEGA 7.0.21 (Kumar, Stecher & Tamura 2016) with the evolutionary history inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model (Tamura 1992) using a discrete Gamma distribution (TN93+G). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value.

Analyses of the number of base substitutions per site (genetic distance) for the new and Bolzern, Burckhardt & Hänggi (2013) sequences were conducted using the Kimura

Code no.	Haplo-type	GenBank sequence no.		Sample ID no.	Species	Sex	Location	Date	OS map reference	Collector
		CO1	NADH1							
01	1	LT970942	LT969452	1672	S	♂	Kingsbridge, Devon	02/06/2013	SX733444	GSO
02	1	LT970943	LT969453	1861	D	♂	Bentley, Suffolk	05/09/2014	TM109368	Paul Lee
03	4	LT970944	LT969454	1974	D	♂	Aldeburgh, Suffolk	28/09/2015	TM448594	Ian Hughes
04	1	LT970945	LT969455	1857	S	♀	Barnstaple, Devon	31/08/2014	SS540327	Mike Towns
05	1	LT970946	LT969456	1674	S	♀	Dartmouth, Devon	03/06/2013	SX878514	GSO
06	4	LT970947	LT969457	1870	S	♀	Lower Bunbury, Cheshire	16/09/2014	SJ565578	GSO
07	1	LT970948	LT969458	1999	S	♀	Boscastle, Cornwall	20/06/2016	SX095908	GSO
08	1	LT970949	LT969459	2005	S	♀	Trebah Gardens, Cornwall	22/06/2016	SW768274	GSO
09	3	LT970950	LT969460	1847	S	♂	Plymouth, Devon	15/08/2014	SX462557	Peter Smithers
10	4	LT970951	LT969461	1964	D	♀	Rye, Kent	10/09/2015	TQ920202	GSO
11	1	LT970952	LT969462	1906	S	♀	Burnham-on-Sea, Somerset	-/09/2014	ST301493	Francis Farr-Cox
12	9	LT970953	LT969463	1753	A	♀	Holy Cross, Tyne & Wear	24/09/2013	NZ305671	GSO
13	1	LT970954	LT969464	1990	D	♀	Warwick, Warwickshire	23/05/2016	SP286649	GSO
14	1	LT970955	LT969465	2007	S	♀	Boscastle, Cornwall	23/06/2016	SX105916	GSO
15	1	LT970956	LT969466	1962	D	♀	Broxbourne, Hertfordshire	11/08/2015	TL360047	GSO
16	1	LT970957	LT969467	1852	S	♂	Honiton, Devon	27/08/2014	ST243046	Brian Johnson
17	1	LT970958	LT969468	1949	D	♂	Manton, Rutland	27/06/2015	SK876041	GSO
18	1	LT970959	LT969469	1839	S	♂	Bangor, Gwynedd	30/07/2014	SH544698	Richard Gallon
19	1	LT970960	LT969470	1995	D	♀	Charlecote, Warwickshire	25/05/2016	SP259564	GSO
20	9	LT970961	LT969471	1746	A	♀	Preston, Tyne & Wear	24/09/2013	NZ348693	GSO
21	1	LT970962	LT969472	1850	S	♀	Exeter, Devon	25/08/2014	SX943928	Keith Alexander
22	9	LT970963	LT969473	1720	A	♀	Jarrow, Tyne & Wear	23/09/2013	NZ335642	GSO
23	4	LT970964	LT969474	1930	D	♀	Hinxworth, Hertfordshire	01/04/2015	TL237403	GSO
24	1	LT970965	LT969475	1830	D	♂	Norwich, Norfolk	03/08/2014	TG218076	Pip Collyer
25	4	LT970966	LT969476	1900	D	♂	Basildon, Essex	17/09/2014	TQ738865	Peter Harvey
26	1	LT970967	LT969477	1971	D	♂	Shoreham, Kent	15/09/2015	TQ520609	GSO
27	1	LT970968	LT969478	1927	D	♀	Brent, London	14/10/2014	TQ199890	Edward Milner
28	1	LT970969	LT969479	1903	S	♂	Coryton, Cardiff	24/09/2014	ST141813	Simon Warmingham
29	1	LT970970	LT969480	1865	S	♀	Penycae, Wrexham	14/09/2014	SJ290447	GSO
30	4	LT970971	LT969481	1858	D	♀	Wicken, Cambridgeshire	05/09/2014	TL563705	GSO
31	9	LT970972	LT969482	1891	A	♀	Burnopfield, Co. Durham	24/09/2014	NZ166568	GSO
32	4	LT970973	LT969483	1987	D	♀	Holywell, Cambridgeshire	10/05/2016	TL341706	GSO
33	1	LT970974	LT969484	1989	D	♂	Oxford, Oxfordshire	13/05/2016	SP514069	GSO
34	1	LT970975	LT969485	1855	S	♂	Llanfarian, Ceredigion	27/08/2014	SN595770	John Warren
35	9	LT970976	LT969486	1779	A	♀	Seghill, Northumberland	25/09/2013	NZ295750	GSO
36	1	LT970977	LT969487	1959	S	♀	Porthgain, Pembrokeshire	16/07/2015	SM815324	Ian Hughes
37	9	LT970978	LT969488	1883	A	♀	Stanley, Co. Durham	24/09/2014	NZ195531	GSO
38	4	LT970979	LT969489	1898	D	♂	Doddington, Kent	20/09/2014	TQ951580	Tony Russell-Smith
39	1	LT970980	LT969490	1979	S	♂	Llandovery, Carmarthenshire	26/09/2015	SN769428	Keith Hyatt
40	9	LT970981	LT969491	1726	A	♀	Hebburn, Tyne & Wear	23/09/2013	NZ310642	GSO
41	6	LT970982	LT969492	1998	S	♀	Toller Porcorum, Dorset	19/06/2016	SY554990	GSO
42	4	LT970983	LT969493	1966	D	♀	Ivy Hatch, Kent	12/09/2015	TQ584534	GSO
43	1	LT970984	LT969494	1843	D	♀	South Lopham, Norfolk	13/08/2014	TM039794	Helen Smith
44	3	LT970985	LT969495	2004	S	♀	Tintagel, Cornwall	21/06/2016	SX056884	GSO
45	9	LT970986	LT969496	1888	A	♀	Marley Hill, Tyne & Wear	24/09/2014	NZ204581	GSO
46	2	LT970987	LT969497	1988	D	♀	Belton, Lincolnshire	10/05/2016	SK928394	GSO
47	9	LT970988	LT969498	1805	A	♀	Harlow Hill, Northumberland	26/09/2013	NZ078683	GSO
48	4	LT970989	LT969499	1970	D	♀	Sissinghurst, Kent	13/09/2015	TQ808382	GSO
49	1	LT970990	LT969500	1869	S	♀	Chester, Cheshire	15/09/2014	SJ406664	GSO
50	9	LT970991	LT969501	1769	A	♀	Blyth, Northumberland	25/09/2013	NZ318790	GSO
Croucher sequences										
A	6	AY138828	AY138817		S					
B	3	AY138829	AY138818		S					
C	4	AY138832	AY138820		D					
D	2	AY138830	AY138821		D					
E	1	AY138833	AY138819		D/S					
F	-	AY138831	-		D					

Table 1: Details of the new sequences (Code numbers 01 to 50) analysed in Figs. 9–11. Haplotype designations are based only on the 651 base pairs common with the sequences of Croucher, Oxford & Searle (2004) and Bolzern, Burckhardt & Hänggi (2013) (see text). Other columns provide information on species (A = *Eratigena atrica*, D = *E. duellica*, S = *E. saeva*), sex, location, date, Ordnance Survey map reference for each individual, and the name of the collector. The Croucher sequences (with GenBank identifiers) and the current haplotypes they match are shown at the bottom of the table.

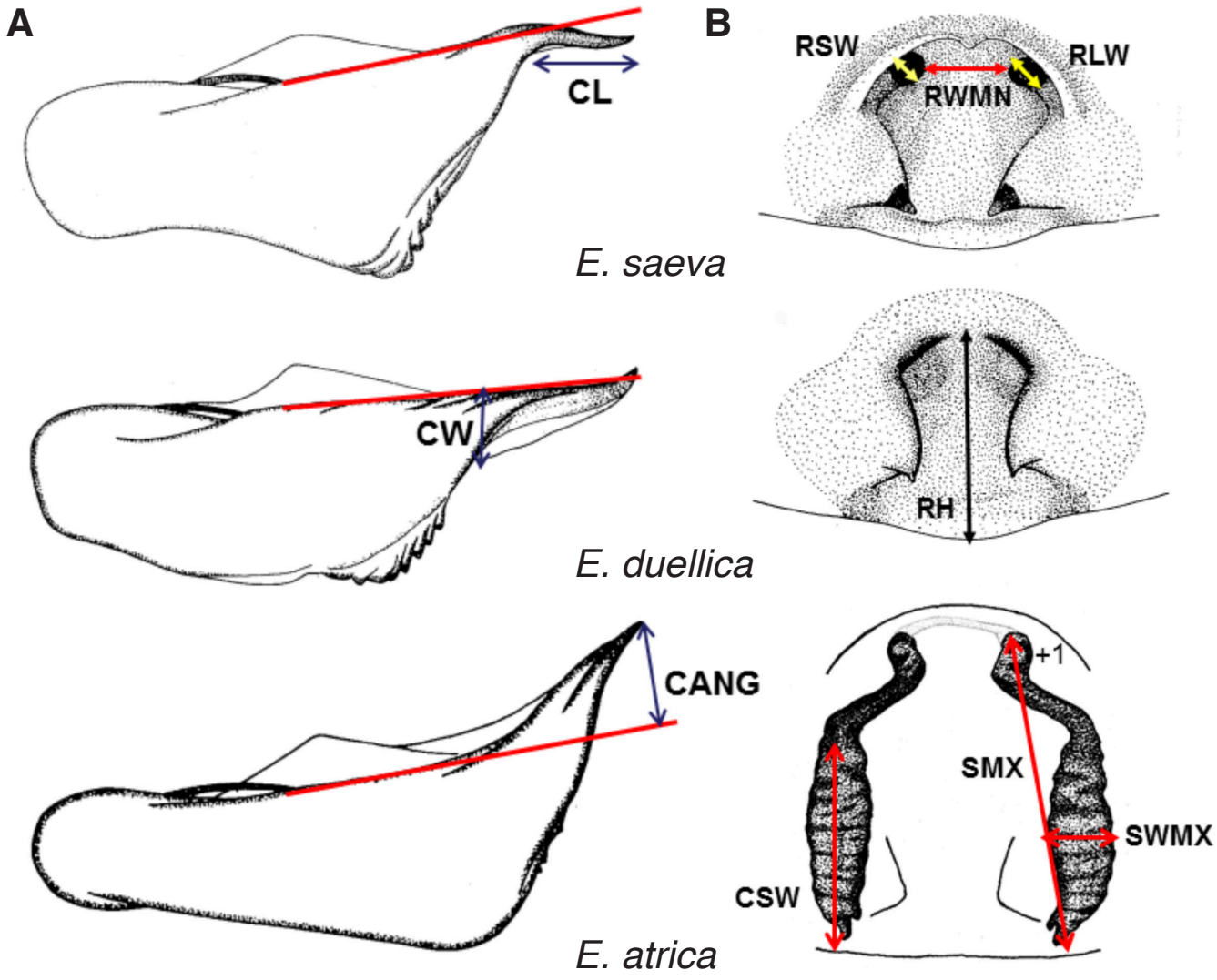


Fig. 2: New morphological characters introduced in this paper or which are specifically mentioned in the text. Other characters are described in Croucher *et al.* (2007). Line drawings modified from Merrett (1980), except for the vulva. **A** male, tegulum and conductor profiles showing the conductor angles (CANG) for the three species. Note that palps are illustrated with the ventral side uppermost, as in Merrett (1980). Red line continues the trajectory of the straight, ventral edge of the tegulum, conductor length (CL) and conductor width (CW) are also shown; **B** female, two new measurements made on external features: receptacle short width (RSW) and receptacle height (RH). Receptacle long width (RLW) and minimum width between receptacles (RWMN) are also shown. New measurements made on internal characters are spermatheca maximum height (SMX), spermatheca maximum width (SWMX), distance from the epigastric furrow to the major constriction in the spermatheca width (CSW) and whether the extreme anterior end of the spermatheca bends outwards (score +1, as here), inwards (-1) or does not bend at all (0).

2-parameter model (K2P, Kimura 1980) in MEGA 7.0.21. The rate variation among sites was modelled with a gamma distribution (shape parameter = 4). The analysis involved 65 nucleotide sequences. All three codon positions were included and any ambiguous positions were removed for each sequence pair. A total of 1227 positions were in the final dataset. Sequences for *Tegenaria domestica* and *Eratigena agrestis* from Bolzern, Burckhardt & Hänggi (2013) were included as outgroups.

Haplotype networks were constructed with concatenated datasets of CO1 and NADH1 in PopART (Leigh & Bryant 2015). Haplotypes from previous British work were assembled from separate GenBank CO1 and NADH1 (ND1) sequence files using information provided in Croucher (1998) and Croucher, Oxford & Searle (2004).

The new sequences reported here are deposited in GenBank with accession numbers provided in Table 1.

Results

Morphology

A total of 148 males and 113 females were used for morphometrics during the course of this study: Britain (68 males, 43 females), North America (36 males, 24 females), continental Europe (34 males, 36 females), and known hybrids (10 males, 10 females). Of these, only four females (continental Europe, 2; North America, 1; Britain, 1) did not fall cleanly into one of the putative species categories on first examination, and two more seemed to be not entirely typical (continental Europe). All males were readily identifiable. Thus, overall, 2.3% of specimens (5.3% of females) initially appeared problematic. In a few cases we disagreed with the original determiner but for all these specimens which species they represented was without doubt. In total, there were 10 species/source combinations for males: *E. saeva*: British (DNA) n = 7, British (additional) n =

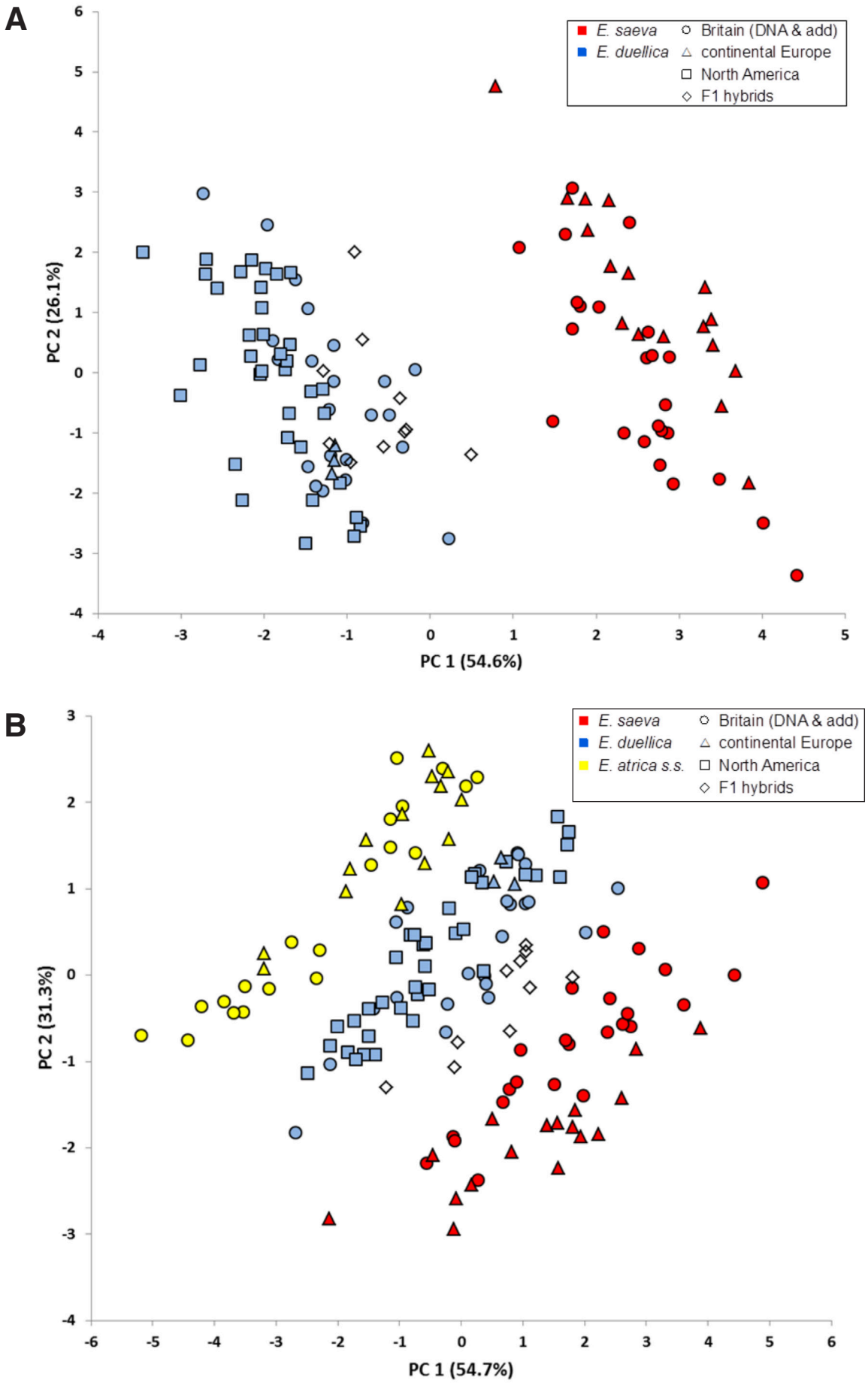


Fig. 3: Plots of Principal Component 1 (PC1) v. PC2 for male morphological characters. The proportion of the overall variance explained by each PC is shown in parentheses. **A** analysis of *E. saeva* and *E. duellica* using all morphological characters; **B** analysis of all three species but excluding the characters conductor width (CW) and conductor length (CL), which cannot be measured on *E. atrica s.s.*

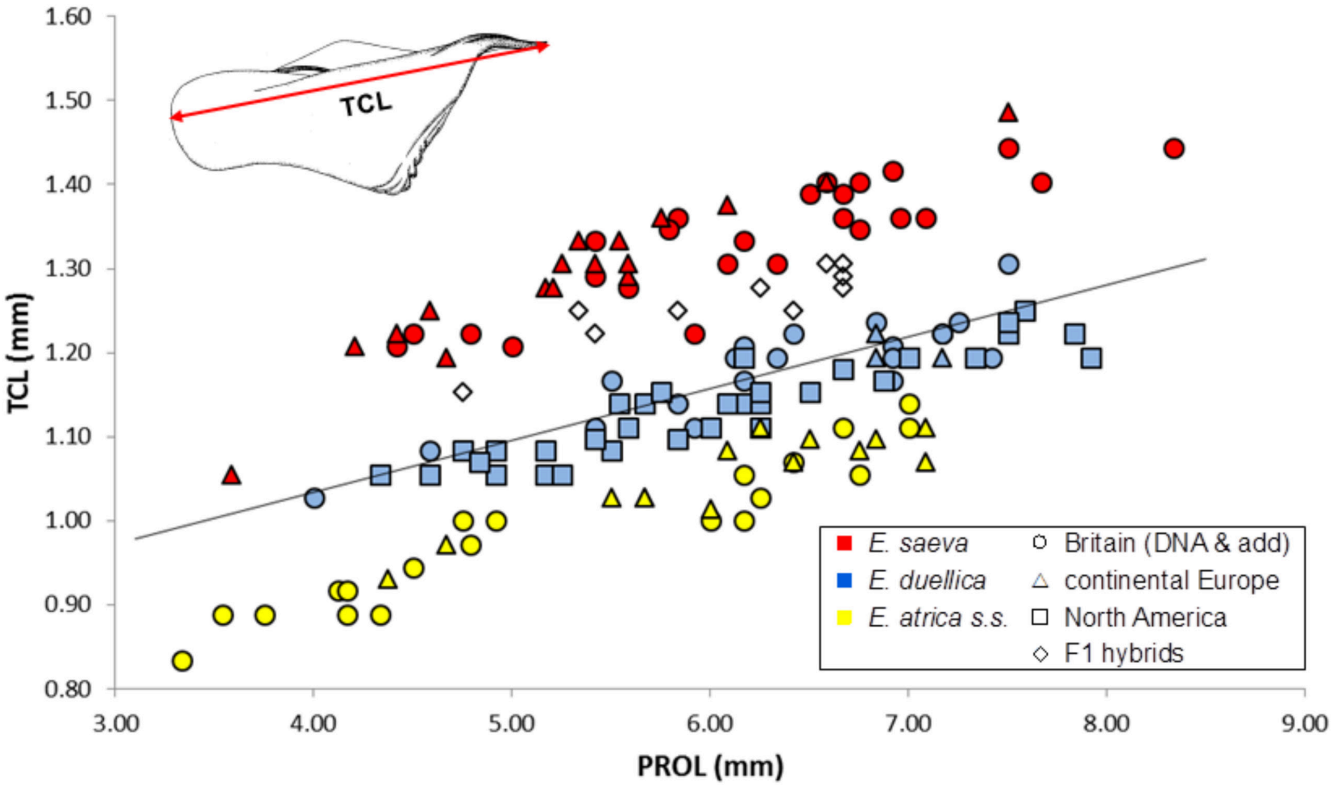


Fig. 4: Merrett plot of maximum tegulum + conductor length (TCL, inset) v. maximum prosoma length (PROL). The common regression line (from ANCOVA) is based on all data except for continental European *E. saeva*; see text.

18, continental Europe n = 17; *E. duellica*: British (DNA) n = 8, British (additional) n = 15, North America n = 36, continental Europe n = 3; *E. atrica s.s.*: British (additional) n = 20, continental Europe n = 14; known *E. saeva* × *E. duellica* F1 hybrids: n = 10. For females, nine species/source combinations comprised: *E. saeva*: British (DNA) n = 13, British (additional) n = 7, continental Europe n = 12; *E. duellica*: British (DNA) n = 12, North America n = 23, continental Europe n = 4; *E. atrica s.s.*: British (DNA) n = 10, continental Europe n = 16; known *E. saeva* × *E. duellica* F1 hybrids: n = 10.

Males

Principal component analysis (PCA), derived from a correlation matrix, was used as the method of ordination because it makes no *a priori* assumptions regarding the relationships between individuals. In alternative approaches, such as discriminant function analysis, specific groups are initially identified as training sets. A key aim of this study was to ascertain whether individuals from different populations cluster into three groups without such prior information. Two PCAs were performed on male characters. Conductor length (CL) and conductor width (CW) cannot be defined in *E. atrica s.s*. (Fig. 2A) and so one PCA used all characters but excluded *E. atrica s.s*., and the other included all species but excluded measurements of CL and CW. In the first analysis, a plot of the first two Principal Components (PCs), together explaining 81% of the overall variance, produces two clear clusters which mapped to *E. saeva* and *E. duellica* (Fig. 3A). Known *E. saeva* × *E. duellica* F1 hybrids fall between the two main clusters, although intermingled more with *E. duellica*. *Eratigena duellica* from North America and *E. saeva* from continental Europe are distributed even further apart on the plot than are specimens from Britain. If characters CL and CW are ignored, fairly tight clustering within species is again observed (Fig. 3B), with PC1 and PC2 together explaining 86% of the overall variance. In this analysis, the F1 hybrids fall more centrally between their parent species.

In a seminal paper, Merrett (1980) introduced a simple plot that separates males of the three putative species within the *E. atrica* group. A Merrett plot of maximum tegulum + conductor length (TCL) against maximum prosoma length (PROL) for all male data is shown in Fig. 4. Previous analyses have shown that the slopes of the plots for the three species are usually statistically identical but that the intercepts differ between them (Oxford & Smith 1987; Oxford & Plowman 1991; Croucher 1998; Croucher *et al.* 2007). An analysis of covariance (with PROL as covariate) showed that the slopes of the individual regression lines for the 10 different species/source combinations (defined above) differed significantly (interaction term, $F_{(9,128)} = 4.24$, $P < 0.001$); the continental Europe *E. saeva* has a significantly steeper slope in the Merrett plot than the other sources or species. Removing these data yields a non-significant interaction ($F_{(8,113)} = 0.98$, $P = 0.453$), i.e. the slopes are no longer significantly different, but the differences between elevations (adjusted means) are highly significant ($F_{(8,121)} = 186.43$, $P < 0.001$). Note that, because the points contributed by *E. saeva* from continental Europe are, if anything, further from those of other species, their removal will not lead to misleading conclusions regarding species identities. A pairwise multiple comparison procedure (Holm-Sidak method) reveals that elevations of the regression lines are

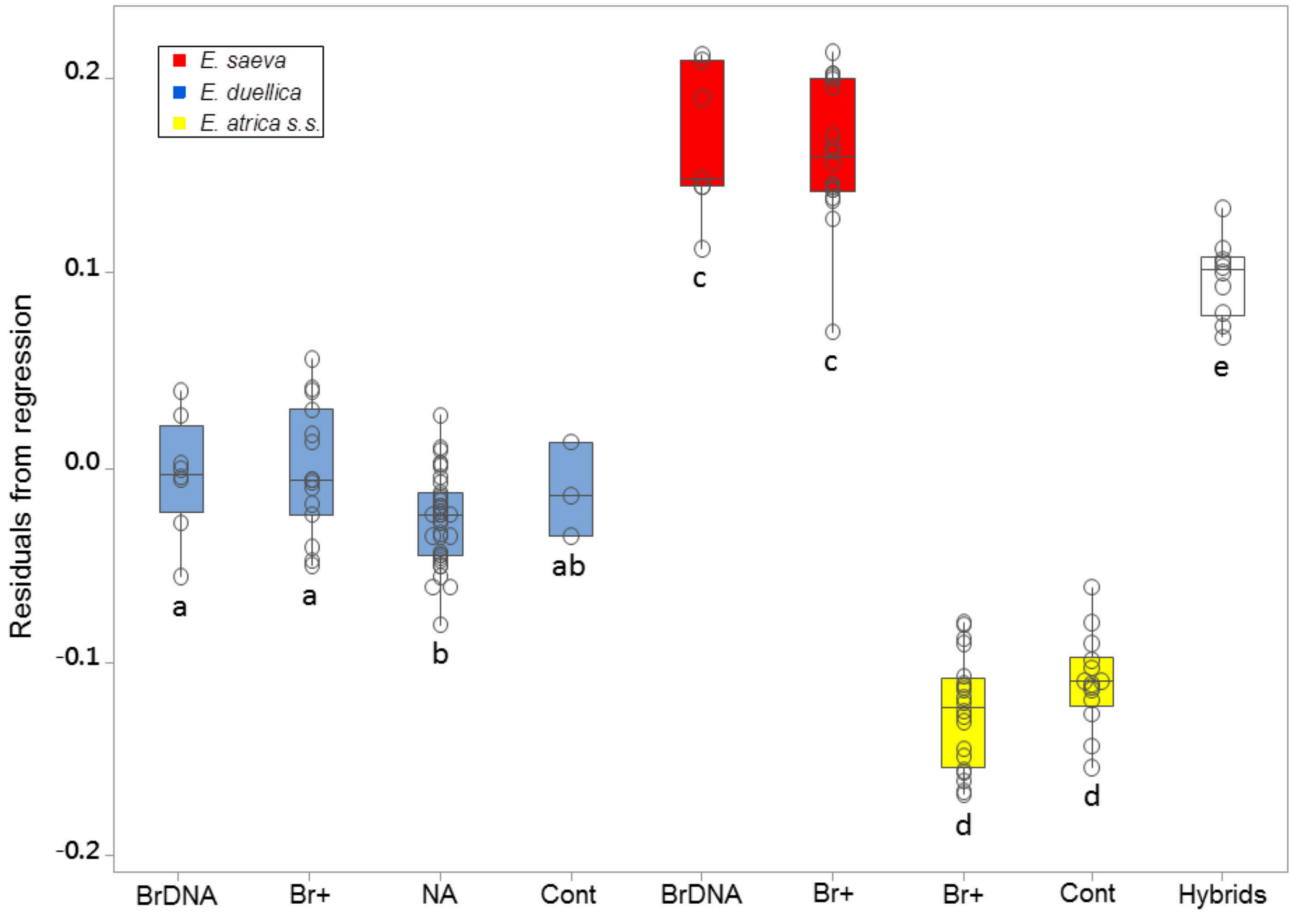


Fig. 5: Box and whisker plot of residuals from the Merrett plot in Fig. 4. Sources are as follows: BrDNA, British DNA samples; Br+, additional British material; NA, North America; Cont, continental Europe; Hybrids, F1 *E. saeva* × *E. duellica* hybrids. Note that there is no entry for continental European *E. saeva* (see text). Individual data points are shown as open circles, the interquartile ranges as coloured boxes and the medians by a horizontal line within each box. Letters below each box show the outcome of a post-hoc test (see text). Samples sharing a letter are not significantly different.

highly significantly different between species. Residuals from the common regression line fitted to the data (TCL = 0.788 + 0.061PROL) and their pairwise comparisons are shown in Fig. 5.

The new measurement, conductor angle (CANG), is shown to be of high diagnostic value. A normality test of the data across all 10 datasets failed (Shapiro-Wilks test, $P < 0.05$) but a Kruskal-Wallis one-way analysis of variance on ranks for this character was highly significant ($P < 0.001$). The plot in Fig. 6 shows that this one measure clearly differentiates the three species. The known hybrids fall close to the values for *E. duellica*. Post-hoc tests were not possible for the majority of pairwise comparisons.

Females

As for males, two PCAs were run for female data. The first was on cases where all characters, including internal ones, were measured (number of specimens, n = 95) and the second for external features alone (n = 110), lower than the total because key features could not be measured on three specimens, which were excluded from all analyses. In the PCA based on all characters, PC1 and PC2 together explain 64.8% of the overall variance (Fig. 7A) and provide a good separation between the putative species. The six specimens for which there was some doubt about species identity on first examination are shown as numbered black symbols on the plots. The origins of these specimens are as follows: #1, Goven, France; #2, Ille-et-Vilaine, France; #3, Andalucia, Spain; #4, Catalonia, Spain; #5 Vancouver Island, Canada; #6, Jersey, Channel Islands.

Two, initially classified by GSO as '*E. saeva*?' (#1, #2), from north-west France fall within the *E. saeva* points (Fig. 7A). A third specimen labelled '*E. atrica*?' by GSO (#3) lies close to the boundary between the *E. atrica s.s.* and *E. saeva* clusters. Two, one from continental Europe labelled '*Tegenaria* cf. *atrica*' by AB (#4) (Bolzern, Burckhardt & Hänggi, 2013) and #5 from North America classified by GSO as '*E. duellica*/*E. saeva*' fall between the *E. saeva* and *E. duellica* clusters, within the zone occupied by the known F1 hybrids between these species. For the sixth equivocal specimen, internal measurements could not be made.

The second plot (Fig. 7B), based only on external characters, shows a similar clustering of the putative species and placement of the equivocal, numbered specimens. Here PC1 and PC2 together explain 70.6% of the overall variance. Interestingly, in this plot most of the additional British *E. saeva*, all from the Channel Islands (crosses), tend to cluster with the known hybrids, despite being clearly assigned to *saeva* on first examination. Examination of the PCA coefficients associated with each character, and the individual measurements for the Channel Island specimens compared with others assigned to *E. saeva*, do not suggest a simple explanation for why these points fall where they do. The

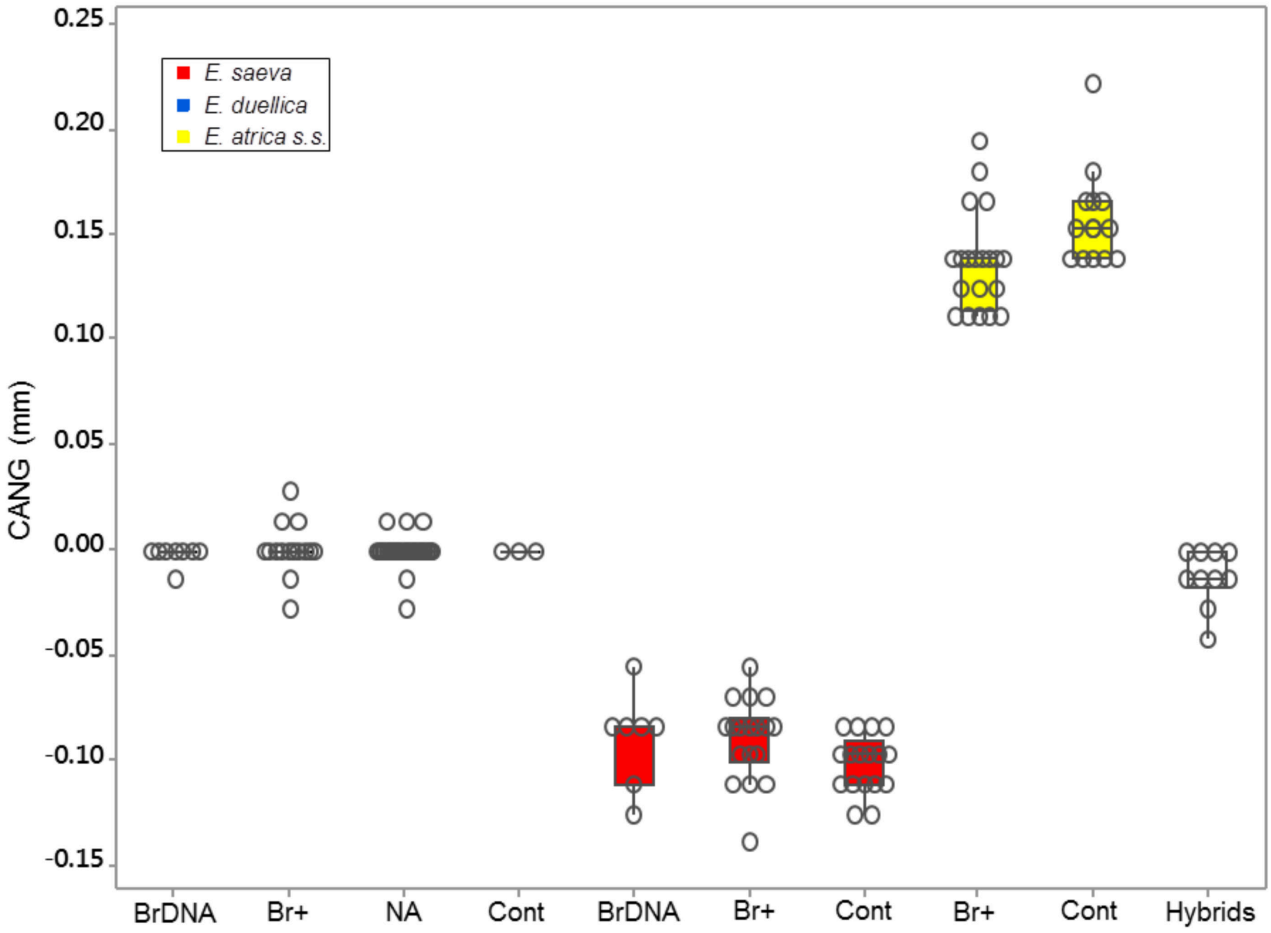


Fig. 6: Plot of conductor angle (CANG) (Fig. 2A) in the different samples (see text for the measurement of this character). The first four samples are of *E. duellica* but, because of the data distributions, no interquartile ranges (and thus colours) are shown. For sources, see legend to Fig. 5.

individual from this source initially identified as '*E. saeva*?' (#6), falls solidly within the *E. saeva* grouping.

Females of the three taxa can also be identified by considering ratios of simple external measurements. Fig. 8, for example, shows that on a RWMN/RH × RSW/RLW plot (characters shown in Fig. 2B), *E. duellica*, known F1 hybrids and *E. saeva*/*atrica s.s.* separate out along the RSW/RLW axis, whereas *E. saeva* and *E. atrica* form two clusters, with some degree of overlap, along the RWMN/RH axis. The RSW/RLW ratio measures how round the apertures to the spermathecae are, with a value of 1 representing a perfect circle. The lower limit of around 0.1 is a result of measuring the thickness of the anterior chitinized arch to the spermathecal openings in *E. duellica* and not that of the actual aperture itself. It is worth noting that, because the PCA procedure used is additive, these composite measures will not have contributed to the separation of species in Figs. 7–8, i.e. to some extent they are independent indicators of species identities. On this plot, equivocal specimens #1, #2 and #3 lie at the interface of the *E. saeva* and *E. atrica s.s.* clusters, and #4 and #6 are well within the *E. saeva* points. Once again, #5 falls with the F1 hybrids. The Channel Islands specimens here cluster firmly within *E. saeva*. Other plots such as RSW/RLW × SWMX (not shown) produce a similar separation of species.

Molecular analysis

Mitochondrial DNA from all 50 new specimens from Britain was successfully sequenced and comprised 1227 BPs. An analysis of the number of base substitutions per site between sequences (genetic distance) was performed for the new specimens and those of Bolzern, Burckhardt & Hänggi (2013) using *Tegenaria domestica* and *Eratigena agrestis* sequences from the Bolzern study as outgroups. The results confirm Bolzern, Burckhardt & Hänggi's (2013) observation of a small genetic distance between taxa within the *E. atrica* group. Thus, the mean K2P distance within the *E. atrica* group was 0.024 (min = 0.000, max = 0.059), compared with a mean from *E. agrestis* to the *E. atrica* group of 0.120 (min = 0.108, max = 0.166) and a mean from *Tegenaria* to *Eratigena* of 0.291 (min = 0.276, max = 0.375).

A maximum likelihood (ML) phylogeny of sequences including those of Bolzern, Burckhardt & Hänggi (2013) and Croucher (Croucher 1998; Croucher, Oxford & Searle 2004) is shown in Fig. 9. For this analysis, the dataset was trimmed manually so that only complete sequences with an overlap of 100% were used: a total of 651 base pairs. In a phylogeny based on the full sequences (not shown), the essence of the relationships between the haplotypes is exactly the same, but with a few more minor branches. In Fig. 9, each of Croucher's haplotypes is represented just once in the phylogeny (his haplotype F is not included as it was based solely on a CO1 sequence). It is the genetic

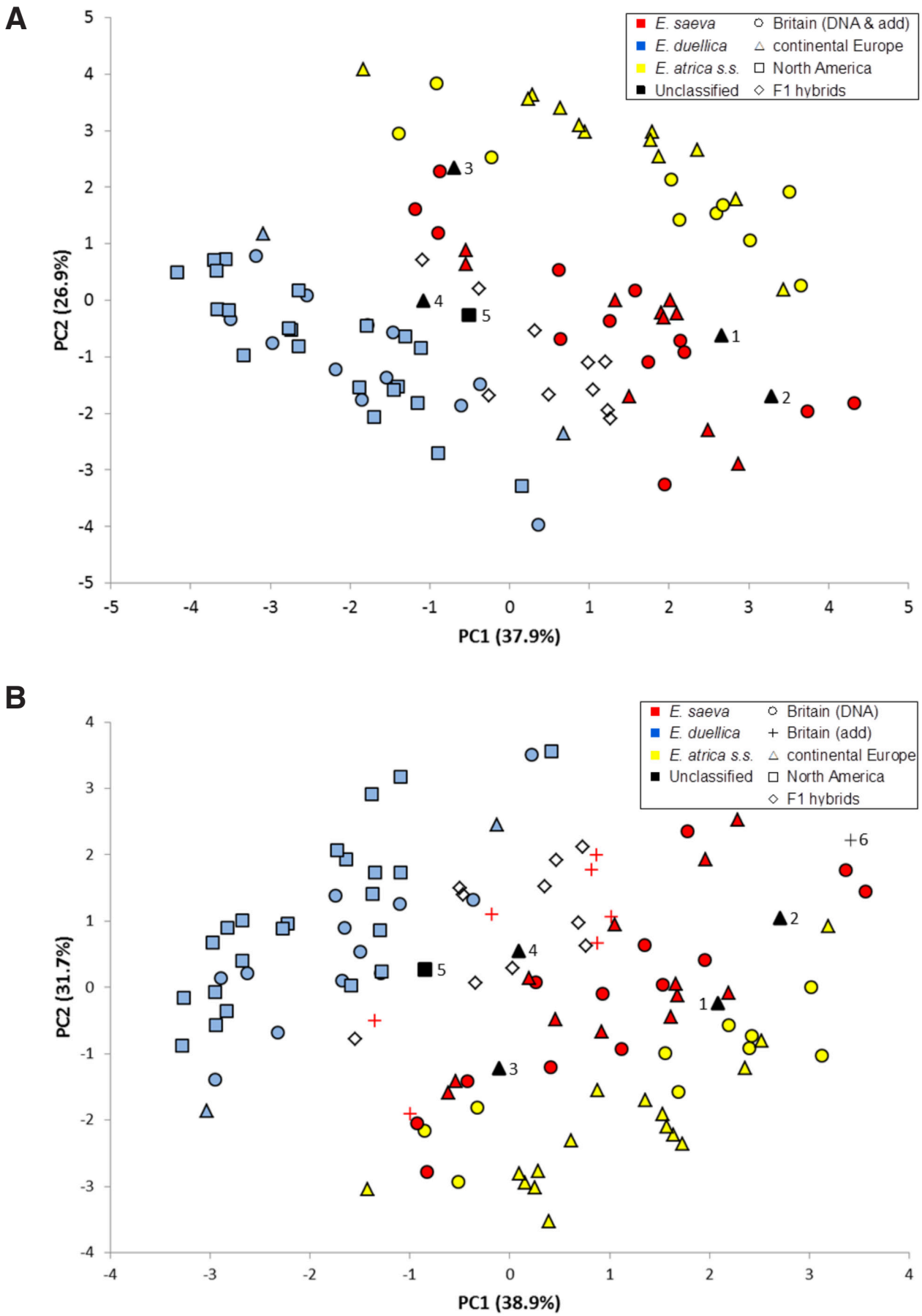


Fig. 7: Plots of Principal Component 1 (PC1) v. PC2 for female morphological characters. The proportion of the overall variance explained by the PCs are shown in parentheses. Numbered black symbols indicate the six individuals that did not, on first inspection, fall into one or other of the classical species. Their sources are as follows: #1, Goven, France; #2, Ille-et-Vilaine, France; #3, Andalucia, Spain; #4, Catalonia, Spain; #5 Vancouver Island, Canada; #6, Jersey, Channel Islands. **A** analysis of females using both external and internal morphological characters; **B** analysis of females using only external morphological characters.

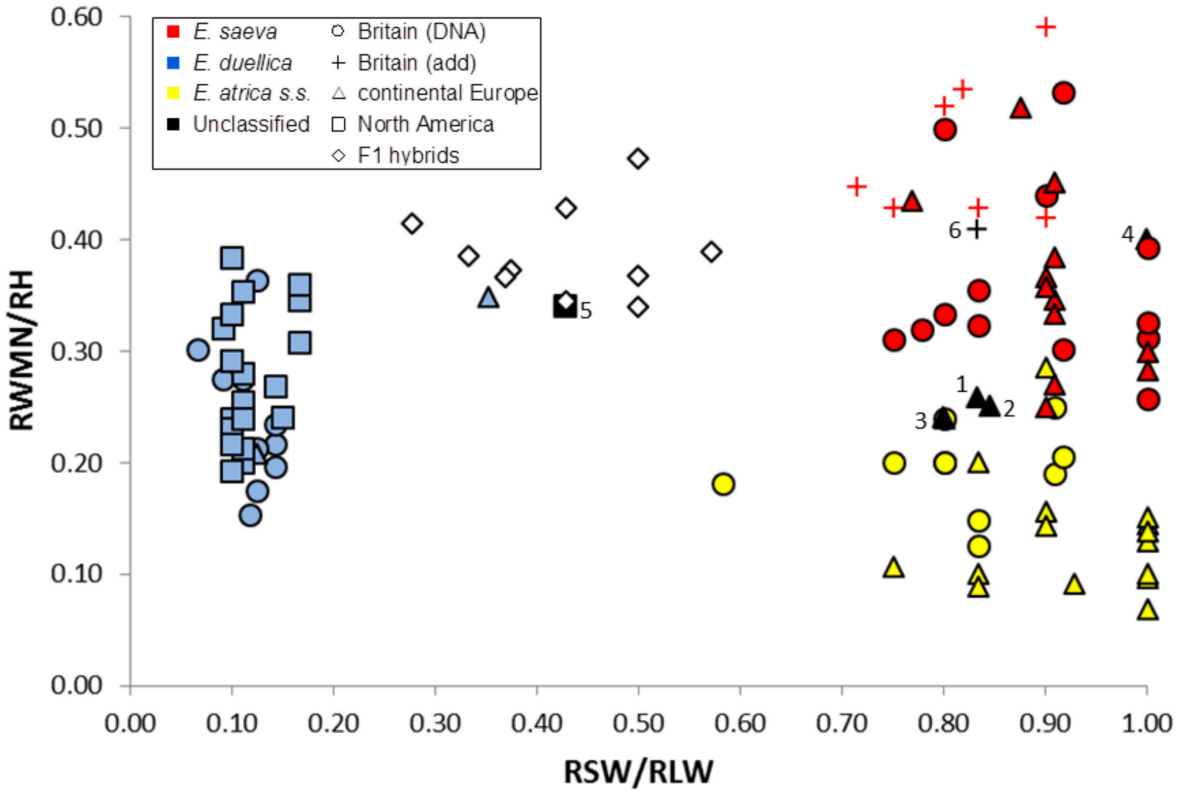


Fig. 8: Plot of ratios of the minimum width between receptacles (RWMN)/top of receptacle to epigastric furrow (RH) v. receptacle short width (RSW)/receptacle long width (RLW) (Fig. 2B) for all females. For numbered symbols, see legend to Fig. 7.

structure and relationships within the *E. atrica* group that is of interest here and so, for clarity, no outgroups are shown. Bolzern, Burckhardt & Hänggi (2013) and Bolzern & Hänggi (2016) provided the broader context of where this group lies with respect to other *Eratigena* and *Tegenaria* species. Unfortunately, some of the critical specimens sequenced by Bolzern, Burckhardt & Hänggi (2013) could not be re-located in the Naturhistorisches Museum Basel and so their identifications were not confirmed in the present study. These specimens are shown in Figs. 9–10 as symbols with darker borders.

The ML phylogeny shows a complex pattern with respect to the distributions of species identified morphologically (Fig. 9) and reveals 10 haplotypes, which are displayed as a network in Fig. 10 (where all of Croucher's specimens are counted in). *Eratigena atrica s.s.* falls out as a reasonably coherent group comprising haplotypes 8 (n = 2) and 9 (n = 13). Haplotype 10 (n = 1), which clusters nearby, was from one of the small group of females that could not readily be identified (#4, Figs. 7–8). Although haplotypes 8 and 9 differ in just one base position, haplotype 9 differs from haplotype 10 at 28 base positions (Fig. 10). The individual comprising haplotype 7, identified by Bolzern, Burckhardt & Hänggi (2013) as *E. saeva* (but not confirmed here), also clusters to some extent with *E. atrica* but is separated by a number of mutations (Fig. 10). The well-sampled haplotypes 1 and 4 comprise individuals of both *E. saeva* and *E. duellica*. Haplotype 1 was found commonly in both *E. saeva* (n = 17) and *E. duellica* (n = 11), whereas haplotype 4 was predominantly found in *E. duellica* (n = 11) but also in one individual of *E. saeva* (Figs. 9–10). The other haplotypes (2, 3, 5, and 6) derived from monospecific samples of from one to five individuals (Figs. 9–10). Of course, it is not known whether larger samples of these haplotypes would include additional species.

The geographical distribution in Britain of the six haplotypes identified in the new material examined here is shown in Fig. 11, together with the sampling locations of addition specimens used for morphometrics. Ordnance Survey 10 km squares in which both *E. saeva* and *E. duellica* have been sampled are shown in grey; to the east of this zone lies the broadly allopatric area of *E. duellica* and to the west the broadly allopatric area of *E. saeva* (Croucher *et al.* 2007; Oxford 2011). The only known self-sustaining population of *E. atrica* in Britain is from the area around Newcastle upon Tyne (upper light green points) (Oxford & Smith 2014). Croucher's haplotypes A to E (Croucher 1998; Croucher, Oxford & Searle 2004), all from individuals collected along the south coast of England, were matched with the current haplotypes, and are plotted with the same colour code in Fig. 11. Haplotype F, from a single individual collected in central southern England was not found in the present study. This haplotype was based on just a CO1 sequence and is therefore not shown in Figs. 9–10.

Haplotype 1 is clearly very widely distributed across England and Wales, irrespective of species. The relatively local geographical clustering of specimens of haplotype 3 and of haplotype 6, both in *E. saeva*, is pronounced, despite the fact that Croucher's specimens were collected 20 years earlier. They underline the relatively poor dispersal ability of *Eratigena* species, despite the great potential for human transportation. Haplotype 4 is predominantly associated with *E. duellica* but also appeared in a single *E. saeva* just west of the zone of overlap between the species. Finally, all the specimens of *E. atrica* from the Newcastle area were of a single haplotype.

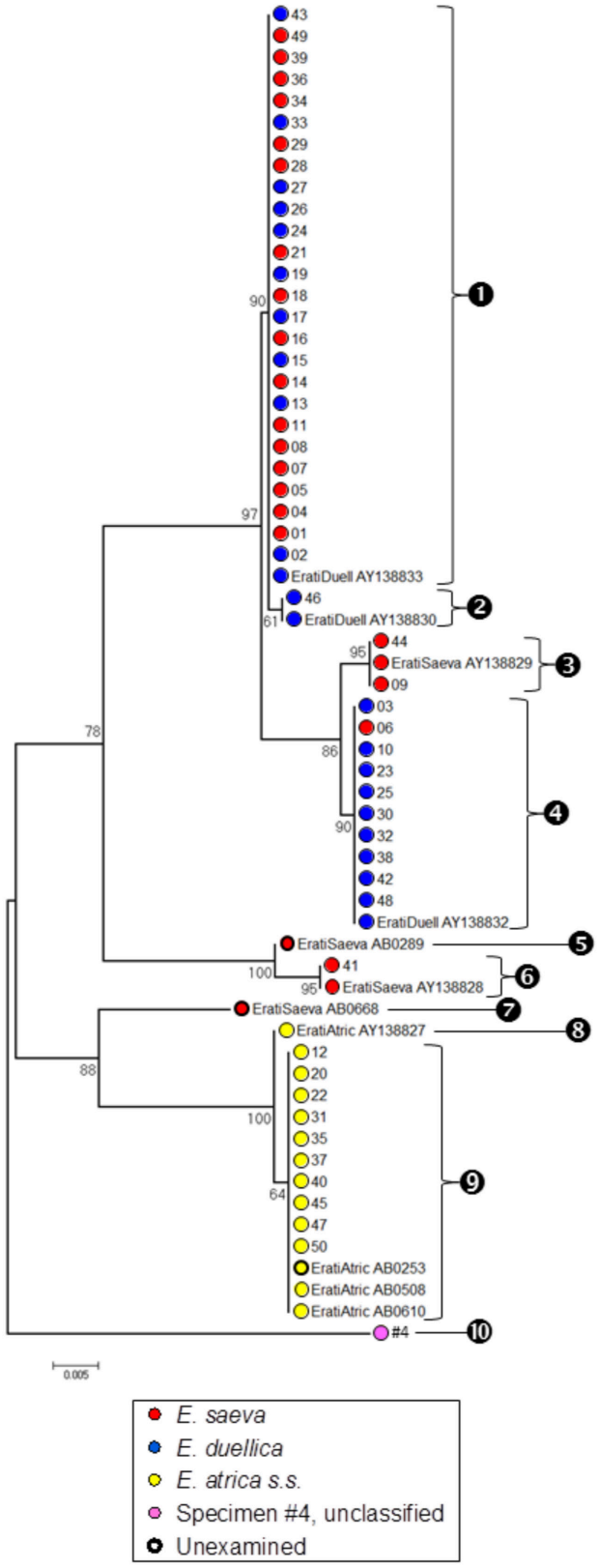


Fig. 9: Phylogeny based on the 651 base pairs common to 62 sequences from the present study and those from Croucher, Oxford & Searle (2004). The tree with the highest log likelihood (-1231.8080) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bolzern, Burckhardt & Hänggi (2013) specimens that were not re-examined in the present study are indicated: the identification is as in the original paper. Simple numbers denote new DNA sequences from the current study. Sample codes starting with AY are from Croucher, Oxford & Searle (2004) and those starting with AB from Bolzern, Burckhardt & Hänggi (2013). Haplotypes are numbered 1 to 10 on the right.

Discussion

Bolzern, Burckhardt & Hänggi (2013) concluded that low genetic divergence between previously recognized taxa within the *Eratigena atrica* group, and the difficulties identifying constituent species morphologically, indicated that there was, in fact, just one, albeit variable, species *E. atrica*. This inference was rapidly accepted by many arachnologists and museum curators, and is formally endorsed in the World Spider Catalog (2018). This one-species proposal, however, is difficult to reconcile with the results of British research stretching back almost four decades (Merrett 1980; Oxford & Plowman 1991; Croucher 1998, Croucher *et al.* 2007; Oxford 2009, 2011; Oxford & Smith 2014), as acknowledged by Bolzern, Burckhardt & Hänggi (2013). The present study set out to test whether the situation regarding these species in Britain was different in morphological and/or molecular aspects to that on the European mainland, the source of almost all of Bolzern, Burckhardt & Hänggi's (2013) samples and thus the basis for their one-species conclusion. As well as examining the morphology of British material, specimens from the European mainland and North America were also included, as were laboratory-produced *E. saeva* × *E. duellica* F1 hybrids.

The hypothesis that the traditional species may have hybridized into a single variable taxon in continental Europe, but not over most of southern and central Britain, should manifest itself as a blurring of clusters (species) in morphological space. Examination of a small number of morphological characters previously shown to be of taxonomic value, together with those introduced here for the first time, demonstrate clearly that the vast majority of specimens of both sexes, irrespective of source, fall into one of three groups on PCA plots (Figs. 3, 7). The distributions of points tend to be more sharply defined for males (Fig. 3) than for females (Fig. 7) but, nonetheless, for both the non-overlapping groupings correspond to the traditional taxa *E. atrica s.s.*, *E. saeva* and *E. duellica*. For males at least, data points from continental specimens, especially for *E. saeva* and *E. duellica*, tend to be even further apart on PCA plots than those from Britain and North America. This is the reverse of what would be expected if continental specimens were, essentially, a hybrid swarm. Known first-generation hybrids between *E. saeva* and *E. duellica* fall between their parental clusters and demonstrate that these, at least, should be discernible in samples from the wild. These conclusions are strongly reinforced by analysing Merrett plots and measurements of conductor angles.

For six females, the initial designations of species was uncertain. The positions of these individuals vary on different plots. Two specimens examined from Spain (#3 and #4) lie on the PCA plots near the *atrica*/*saeva* and *saeva*/*duellica* interfaces, respectively, (Figs. 7–8) but on the RWMN/RH × RSW/RLW plot #4 falls well within the *E. saeva* cluster. The two '*E. saeva*?' from northern France (#1 and #2) cluster within the *E. saeva* points but tend towards the *E. atrica* end of the cluster. The uncertain specimen from Jersey (#6) clearly falls within *E. saeva*. Most intriguingly, one female from Canada clustered on all plots with known *E. saeva*/*E. duellica* hybrids. Whereas *Eratigena duellica* is widespread in the Pacific Northwest, and *E. atrica* has more

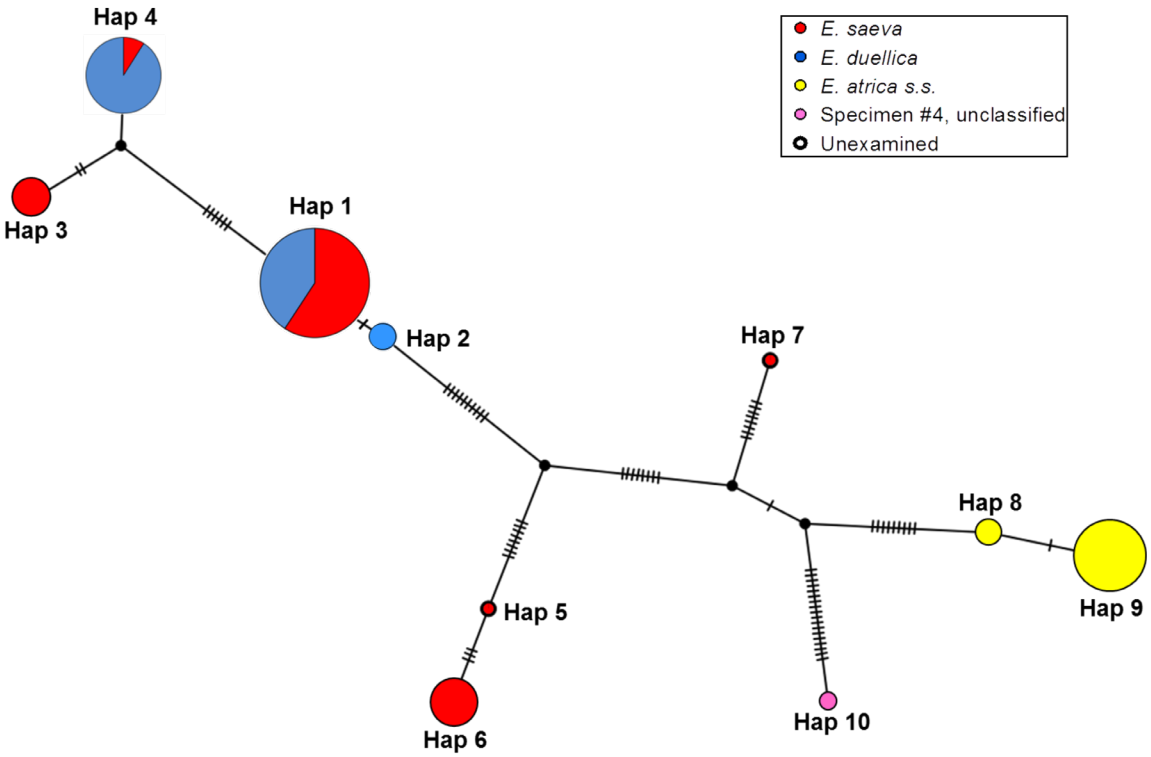


Fig. 10: Haplotype network for those identified in Fig. 9. Size of circles approximates to sample size. The diagram includes all of the Croucher specimens shown in Fig. 11 and additional ones mentioned in the text. Black dots at nodes indicate hypothetical sequences that were not identified in current samples. Cross bars on the lines linking haplotypes denote the number of mutations separating one sequence from another.

recently been reported from four Canadian provinces (Pickavance & Dondale 2006; D. Buckle, pers. comm.), *E. saeva* has not to our knowledge been confirmed in North America. Mention of this species by Roth (1968) is almost certainly based on a misidentification. The presence of what appears to be an F1 hybrid suggests the importation of *E. saeva* into Vancouver Island, Canada, on at least one occasion.

The morphological examination of specimens from across the core range of *Eratigena atrica s.l.* suggests that they form three clusters in morphospace that correspond with the traditional three species *E. saeva*, *E. duellica*, and *E. atrica s.s.* Material from continental Europe is no different in this respect than that from Britain or North America; the widespread hybridization of species in Europe suggested by Bolzern, Burckhardt & Hänggi (2013) is not supported by the new data. The classification of two out of three specimens from Spain was to some extent problematic. Barrientos & Ribera (1988), using material from the northern half of Spain, concluded that the three species could not be separated reliably and formed a complex which they suggested might be regarded as a single species, foreshadowing the conclusions of Bolzern, Burckhardt & Hänggi (2013). They paid much attention to the shape of the tibial apophysis of the pedipalp (a character not used here), and pointed out that the outline changed markedly with the angle of view. Similar problems with viewing angle are mentioned by Bolzern, Burckhardt & Hänggi (2013). It seems likely, therefore, that at least some of the apparent blurring between species in the *E. atrica* group reported by these authors resulted from inconsistent positioning of specimens. Here, we have used strict positioning rules for both male and female features (Merrett 1980; Oxford 2008) and have been able to classify readily all males and almost 95% of females. However, there remains the possibility that in parts of Spain there has been extensive hybridization and introgression between species, a process that is known to be occurring to some extent in parts of northern England and Scotland (Oxford & Smith 1987; Oxford & Plowman 1991; Croucher *et al.* 2007; Oxford 2011) and in the zone of parapatry further south (Fig. 11).

Although there is some overlap in characters used in the initial identification of specimens and those used to examine relationships more quantitatively, for example the roundness of the spermathecal openings in females and the shape and angle of the conductor tip in males, this would not necessarily lead to three clusters on PCA and other plots. Additionally, Merrett plots for males involve lengths not used in identification, but they too indicate three groups.

Molecular analyses of new British material, together with sequences from Bolzern, Burckhardt & Hänggi (2013), reveal a complex situation. The low K2P genetic differentiation of 0.024 (mean, min = 0.000, max = 0.059) between the constituent *Eratigena atrica* group species is comparable to that observed *within* species of other spiders (e.g. Barrett & Hebert 2005; Blagoev *et al.* 2013; Čandek & Kuntner 2015; Astrin *et al.* 2016). However, published interspecific distance values for spiders span a range which includes zero and haplotypes can be shared by good species. For example, Astrin *et al.* (2016) report four morphologically distinct pairs out of 598 species examined (1.3%) that share haplotypes. Indeed, Lasut, Nentwig & Kropf (2015) showed that not only CO1 sequences, but also those of nuclear markers ITS2, 28S, and H3, failed to differentiate the theridiid species *Enoplognatha ovata* and *E. latimana*. This seems

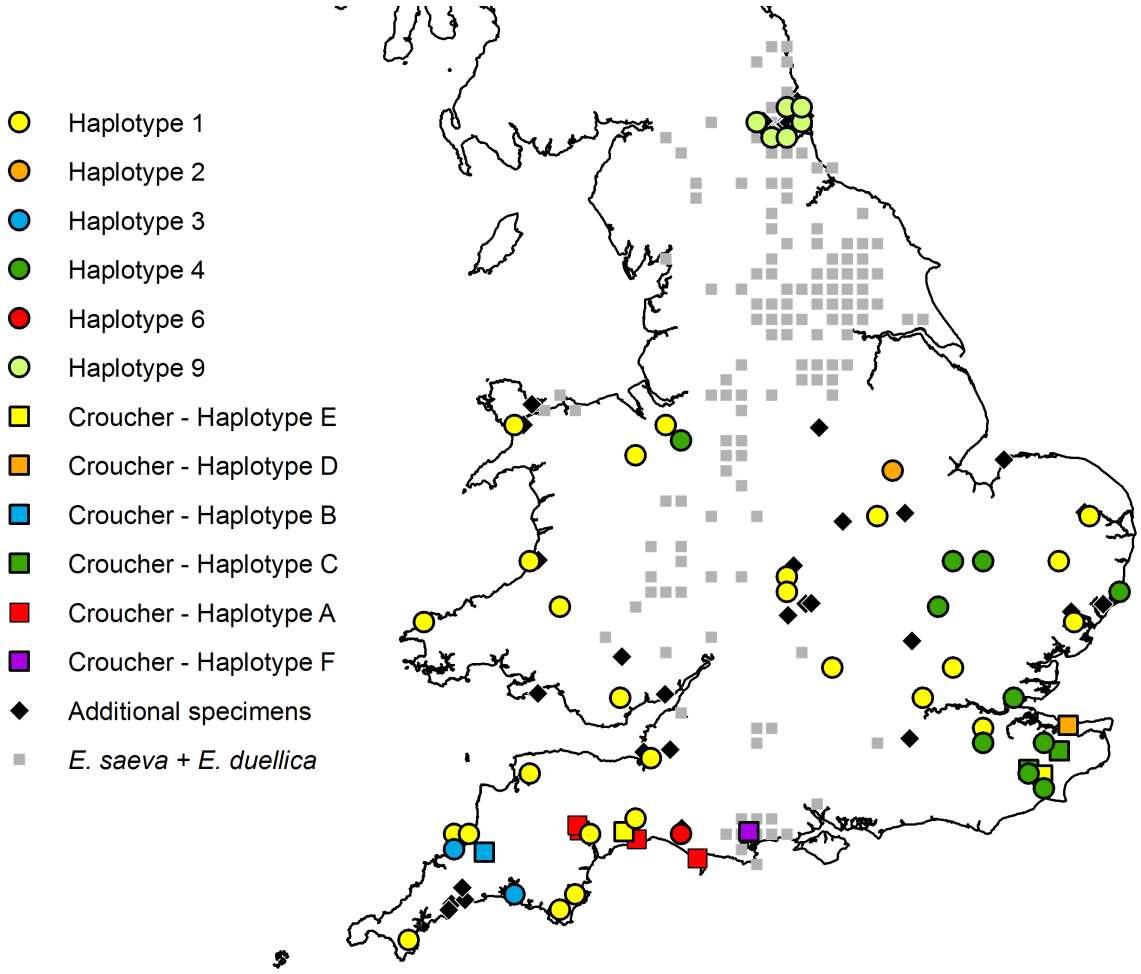


Fig. 11: Map showing the distributions of haplotypes in England and Wales. Croucher haplotypes derive from Croucher, Oxford & Searle (2004). The pale grey squares show 10 km squares of the National Grid where both *E. saeva* and *E. duellica* have been reported. Areas to the west of this band contain predominantly *E. saeva*, and to the east predominantly *E. duellica*. Equivalent haplotypes between the present study and that of Croucher, Oxford & Searle (2004) are shown in the same colour. Croucher's haplotype F was based on a CO1 sequence only and is therefore not shown in Figs. 9 and 11. Note that the colour scheme for haplotypes used here is not the same as for species identity in other figures. Map © Crown Copyright/database right 2017. An Ordnance Survey/EDINA supplied service.

also to be the case with the *Eratigena atrica* group, in which morphology indicates three taxa but the genetic distances are lower than that normally reported between species.

The overall genetic distance between the *E. atrica* group species will depend on the mutational distances between haplotypes and the degree to which haplotypes are shared. One striking feature of the haplotype network (Fig. 10) is the extremely high number of mutations between many adjacent sequences. Although two specimens (representing haplotypes 5 and 7) were not identified in the present study, they are not critical to an interpretation of the patterns of genetic variation within the *E. atrica* group. On the basis of present data, only two haplotypes in Britain are found in more than one species. Two aspects of the haplotype network demand explanation: the high mutational distances between haplotypes mentioned above, and the British distributions of haplotypes within and between species.

The most likely explanation for the deep mutational divisions between many haplotypes is differentiation in ancient, allopatric populations. Croucher, Oxford & Searle (2004) estimated crude times for the divergence of members of the *E. atrica* group, based on a relatively small number of mtDNA sequences and assuming a molecular clock. They suggested that *E. atrica* had split from the *E. saeva*/*E. duellica* lineage approximately 3 million years ago, and that *E. saeva* and *E. duellica* differentiated approximately 1.5 million years ago. On the phylogeny (Fig. 9) and the haplotype network (Fig. 10), *E. atrica* specimens form a relatively coherent group, which is separated from *E. saeva* and *E. duellica* (although one unexamined supposed *E. saeva* specimen, haplotype 7, falls relatively close to *E. atrica*). These divergence times include a period when species ranges were likely subject to expansion and contraction in concert with Quaternary glacial-interglacial cycles. Small, isolated groups of these nascent species may have formed numerous times in southern refugia during glacial advances and within which mtDNA sequence differentiation could have occurred. Ballooning in *Tegenaria*/*Eratigena* species has not been reported (Bell *et al.* 2005), which would reduce further their natural ability to disperse.

Haplotype 1, in Britain at least, is common in both *E. saeva* and *E. duellica*. Although this might be a result of differentiation within one of the species, and subsequent transfer to the other through hybridization and introgression, the fact that up to four of the other haplotypes appear to be species specific may suggest an alternative explanation. This ubiquitous haplotype might represent an ancient sequence that has not yet been lost in one of the species by lineage

sorting; these are, after all, relatively young taxa. Haplotype 4 was recovered from 11 *E. duellica* and a single *E. saeva*, and was also the haplotype found in one North American *E. duellica* fully sequenced by Croucher (Croucher 1998; Croucher, Oxford & Searle 2004). The sharing of haplotype 4 could also reflect incomplete lineage sorting although, given the synanthropic nature of these species, recent hybridization is another possibility. It is inevitable that individuals will occasionally be transported from one allopatric area to another and there, as a result of rare hybridization and subsequent backcrossing (Kennett & Dalingwater 1986; Croucher & Oxford 2014), nuclear markers (= morphology) will be hybridized away leaving, if the introduced individual was female, a mitochondrial record of the event (Croucher, Oxford & Searle 2004; Oxford 2011). In this way haplotypes characteristic of one species can sometimes appear in the soma of another. Other examples of good morphological species of arachnids that share haplotypes are provided by the lycosids *Pardosa lugubris* and *P. saltans*, the thomisids *Xysticus audax* and *X. cristatus* (Astrin *et al.* 2016), salticids in the *Habronattus* species complex (Maddison & Hedin 2003; Hedin & Lowder 2009) and *Promitobates* species of Brazilian harvestmen (Bragagnolo *et al.* 2015). The presence of divergent haplotypes 3 and 6 in two fairly localized patches in south-west England (Fig. 11) are likely to represent independent, human-assisted colonization events and local spread. Until the distributions of haplotypes in continental Europe are established, the possible sources of these and other haplotypes remain unknown. The single haplotype in specimens of *E. atrica* from Newcastle upon Tyne is not unexpected. Oxford & Smith (2014) speculated that this population was established many decades, if not centuries, ago by an introduction from either continental Europe or the Republic of Ireland, when there was likely to have been a powerful founder effect. The haplotype is the same as that found in three specimens from continental Europe. Both of Croucher's *E. atrica s.s.* specimens (Croucher 1998; Croucher, Oxford & Searle 2004), one from the south of France and the other from Castleknock, Co. Dublin, Republic of Ireland, constitute haplotype 8 (Figs. 9–10), which differs from haplotype 9 by one substitution.

The evidence presented here poses a conflict between morphology, which strongly indicates three species in the *Eratigena atrica* group, and mtDNA markers which may suggest one (*E. atrica s.l.*) or possibly two (*E. saeva* + *E. duellica*, *E. atrica s.s.*) species. Such discrepancies are not unusual. Both sexual and natural selection have been proposed as the drivers of genital, behavioural and ecological divergences between nascent species (e.g. Arnqvist 1998; Kraus 2000; Hosken & Stockley 2004; Eberhard 2010a,b; Safran *et al.* 2013; Lackey & Boughman 2016; Barnard *et al.* 2017). If these changes are rapid compared with the rate of lineage sorting of mitochondrial sequences between taxa, mostly as a result of genetic drift (but see Galtier *et al.* 2014), discrepancies between morphological (nuclear) and molecular signals will result (e.g. McKay *et al.* 2013; Willis, Farias & Ortí 2014). In such cases, morphology should override mtDNA data in delimiting taxa since it is morphology (often in combination with behaviour), rather than neutral molecular sequences, that generates pre-zygotic isolating mechanisms (e.g. Barnard *et al.* 2017). This is the situation in the *Eratigena atrica* group, at least between *E. saeva* and *E. duellica*. Oxford and Croucher (2014) demonstrated that there was a mechanical barrier to hybridization between these two species; during interspecific pairings the male palp failed to engage for long enough for sperm to be transferred. Only two out of 65 interspecific crosses (3%) produced live offspring compared with 48 out of 55 intraspecific crosses (87%). The differential might be even higher in the wild where there is the possibility of multiple matings and interspecific sperm competition. Oxford & Croucher (2014) also showed that once F1 hybrids had been generated they crossed *inter se* and backcrossed with both parent species as readily as intraspecific matings and produced fully viable offspring. Thus, the major factors maintaining the integrity of these two species are their largely allopatric distributions in central and southern England and Wales, and the morphological mismatch between male palps and female epigynes when they do meet. Once the initial mechanical barrier to hybridization is breached, the opportunity for introgression between the species is enhanced, and appears to be responsible for the greater morphological similarity between good *E. saeva* and good *E. duellica* in the recently colonized north of England, where species are more sympatric, than those in the south (Oxford & Smith 1987; Croucher 1998; Croucher *et al.* 2007). No laboratory crosses have been attempted between *E. atrica s.s.* and the other two species, but extensive collecting in the Newcastle upon Tyne area of northern England, where all three species occur, has failed to reveal specimens that are not readily assignable (Oxford & Smith 2014).

In conclusion, we have demonstrated that *Eratigena atrica s.l.* is not a single, variable species, and that the original concept of an *E. atrica* group comprising *E. saeva*, *E. duellica* and *E. atrica s.s.* is correct. In other words, we reject the synonymy of *E. duellica*, *E. atrica s.s.*, and *E. saeva* as *E. atrica*, proposed by Bolzern, Burckhardt & Hänggi (2013).

Acknowledgements

We gratefully acknowledge the financial support provided by the Systematics Association and the Linnean Society's Systematics Research Fund (GSO) and the Basler Stiftung für biologische Forschung (AB) towards sequencing costs, and a British Arachnological Society grant (GSO) for fieldwork expenses. Museum curators and private individuals who allowed us to examine specimens are acknowledged under Material and methods. The following kindly provided material from Britain for DNA extraction: Keith Alexander, Pip Collyer, Francis Farr-Cox, Richard Gallon, Peter Harvey, Ian Hughes, Keith Hyatt, Brian Johnson, Paul Lee, Edward Milner, Tony Russell-Smith, Helen Smith, Peter Smithers, Mike Towns, Simon Warmingham, and John Warren. Don Buckle provided information on *Eratigena* species in North America. We thank Peter Merrett for permission to adapt illustrations from his 1980 paper, and for drawing the vulva in Fig. 2B. Sara Goodacre and Kanchon Dasmahapatra provided invaluable initial advice and Mark Williamson kindly commented on an early draft of this paper. Finally,

we thank two anonymous reviewers, whose perceptive comments helped to improve the final version.

Author contributions: GSO conceived the study, undertook all morphometric measurements and analyses, constructed the final phylogenetic tree and haplotype network and drafted the manuscript; AB extracted DNA, organised the sequencing, produced an initial phylogeny and calculated genetic distances between and within species.

References

ARNQVIST, G. 1998: Comparative evidence for the evolution of genitalia by sexual selection. *Nature* **393**: 784–786.

ASTRIN, J. J., HÖFER, H., SPELDA, J., HOLSTEIN, J., BAYER, S., HENDRICH, L., HUBER, B. A., KIELHORN, K-H., KRAMMER, H-J., LEMKE, M., MONJE, J. C., MORINIÀRE, J., RULIK, B., PETERSEN, M., JANSSEN, H. & MUSTER, C. 2016: Towards a DNA barcode reference database for spiders and harvestmen of Germany. *PLoS ONE* **11**: e0162624.

BARNARD, A. A., FINCKE, O. M., McPEEK, M. A. & MASLY, J. P. 2017: Mechanical and tactile incompatibilities cause reproductive isolation between two young damselfly species. *Evolution* **71**: 2410–2427.

BARRETT, R. D. H. & HEBERT, P. D. N. 2005: Identifying spiders through DNA barcodes. *Canadian Journal of Zoology* **83**: 481–491.

BARRIENTOS, J. A. & RIBERA, C. 1988: Algunas reflexiones sobre las especies del grupo "*atrica*", en la Península Ibérica (Araneae, Agelenidae, *Tegenaria*). *Revue Arachnologique* **7**: 141–162.

BELL, J. R., BOHAN, D. A., SHAW, E. M. & WEYMAN, G. S. 2005: Ballooning dispersal using silk: world fauna, phylogenies, genetics and models. *Bulletin of Entomological Research* **95**: 69–114.

BLAGOEV, G. A., NIKOLOVA, N. I., SOBEL, C. N., HEBERT, P. D. N & ADAMOWICZ, A. J. 2013: Spiders (Araneae) of Churchill, Manitoba: DNA barcodes and morphology reveal high species diversity and new Canadian records. *BMC Ecology* **13**: 44.

BOLZERN, A. & HÄNGGI, A. 2016: Revision of the Nearctic *Eratigena* and *Tegenaria* species (Araneae: Agelenidae). *Journal of Arachnology* **44**: 105–141.

BOLZERN, A., BURCKHARDT, D. & HÄNGGI, A. 2013: Phylogeny and taxonomy of European funnel-web spiders of the *Tegenaria-Malthonica* complex (Araneae: Agelenidae) based upon morphological and molecular data. *Zoological Journal of the Linnean Society* **168**: 723–848.

BRAGAGNOLO, C., PINTO-DA-ROCHA, R., ANTUNES, M. & CLOUSE, R. M. 2015: Phylogenetic and phylogeography of a long-legged harvestman (Arachnida: Opiliones) in the Brazilian Atlantic Rain Forest reveals poor dispersal, low diversity and extensive mitochondrial introgression. *Invertebrate Systematics* **29**: 386–404.

BRIGNOLI, P. M. 1978: Quelques notes sur les Agelenidae, Hahniidae, Oxyopidae et Pisauridae de France et d'Espagne (Araneae). *Revue Suisse de Zoologie* **85**: 265–294.

ČANDEK, K. & KUNTNER, M. 2015: DNA barcoding gap: reliable species identification over morphological and geographical scales. *Molecular Ecology Resources* **15**: 268–277.

CARSTENS, B. C. & KNOWLES, L. L. 2007: Estimating species phylogeny from gene-tree probabilities despite incomplete lineage sorting: an example from *Melanoplus* grasshoppers. *Systematic Biology* **56**: 400–411.

CRAWFORD, R. & LOCKET, G. H. 1976: The occurrence of *Tegenaria gigantea* Chamberlin and Ivie (1935) in North America and Western Europe. *Bulletin of the British Arachnological Society* **3**: 199.

CROUCHER, P. J. P. 1998: *Evolutionary interactions of two colonising species of large house spider* (*Araneae*: Tegenaria *spp*.). D.Phil. thesis, University of York, UK.

CROUCHER, P. J. P., JONES, R. M., SEARLE, J. B. & OXFORD, G. S. 2007: Contrasting patterns of hybridization in large house spiders (*Tegenaria atrica* group, Agelenidae). *Evolution* **61**: 1622–1640.

CROUCHER, P. J. P., OXFORD, G. S. & SEARLE, J. B. 2004: Mitochondrial differentiation, introgression and phylogeny of species in the *Tegenaria atrica* group (Araneae, Agelenidae). *Biological Journal of the Linnean Society* **81**: 79–89.

EBERHARD, W. G. 2010a: Evolution of genitalia: theories, evidence, and new directions. *Genetica* **138**: 5–18.

EBERHARD, W. G. 2010b: Rapid divergent evolution of genitalia: theory and data update. *In* J. L. Leonard & A. Córdoba-Aguilar (eds.), *The evolution of primary sexual characters in animals*. New York: Oxford University Press: 40–78.

FOLMER, O., BLACK, M., HOEH, W., LUTZ, R. & VRIJENHOEK, R. 1994: DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**: 294–299.

GALTIER, N., NABHOLZ, B., GLÉMIN, S. & HURST, G. D. D. 2009: Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology* **18**: 4541–4550.

HEDIN, M. C. & MADDISON, W. P. 2001: A combined molecular approach to phylogeny of the jumping spider subfamily Dendryphantinae (Araneae: Salticidae). *Molecular Phylogenetics and Evolution* **18**: 386–403.

HEDIN, M. & LOWDER, M. C. 2009: Phylogeography of the *Habronattus amicus* species complex (Araneae: Salticidae) of western North America, with evidence for localized asymmetrical mitochondrial introgression. *Zootaxa* **2307**: 39–60.

HOSKEN, D. J. & STOCKLEY, P. 2004: Sexual selection and genital evolution. *Trends in Ecology and Evolution* **19**: 87–93.

KENNETT, J. A. B. & DALINGWATER, J. E. 1986: A progress report on using aspects of reproduction as criteria for distinguishing two closely related British *Tegenaria* species. *In* W. G. Eberhard, Y. D. Lubin & B. C. Robinson (eds.), *Proceedings of the 9th international congress of arachnology, Panama 1983*. Washington, DC: Smithsonian Institution Press: 139–142.

KIMURA, M. 1980: A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**: 111–120.

KRAUS, O. 2000: Why no subspecies in spiders? *In* S. Toft & N. Scharff, (eds.), *European arachnology 2000*. Århus: Århus University Press: 303–314.

KUMAR, S., STECHER, G. & TAMURA, K. 2016: MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**: 1870–1874.

LACKEY, A. C. R. & BOUGHMAN, J. W. 2017: Evolution of reproductive isolation in stickleback fish. *Evolution* **71**: 357–372.

LEIGH, J. W. & BRYANT, D. 2015: PopART: full-feature software for haplotype network construction. *Methods in Ecology and Evolution* **6**: 1110–1116.

LASUT, L., NENTWIG, W. & KROPF, C. 2015: Two species or just one? DNA barcoding fails in *Enoplognatha* spp. (abstract). *Proceedings of the 29th European congress of arachnology.* Brno: 106.

LOCKET, G. H. 1975: The identity of Blackwall's *Tegenaria saeva* (Araneae, Agelenidae). *Bulletin of the British Arachnological Society* **3**: 85–90.

MADDISON, W. P. & HEDIN, M. C. 2003: Phylogeny of *Habronattus* jumping spiders (Araneae: Salticidae), with consideration of genitalic and courtship evolution. *Systematic Entomology* **28**: 1–21.

McKAY, B. D., MAYS, H. L., WU, Y., LI, H., YAO, C-T., NISHIUM, I. & ZOU, F. 2013: An empirical comparison of character-based and coalescent-based approaches to species delimitation in a young avian complex. *Molecular Ecology* **22**: 4943–4957.

MERRETT, P. 1980: Notes on the variation, identification and distribution of British species of the *Tegenaria atrica* group (Araneae, Agelenidae). *Bulletin of the British Arachnological Society* **5**: 1–8.

MERRETT, P., RUSSELL-SMITH, A. & HARVEY, P. 2014: A revised check list of British spiders. *Arachnology* **16**: 134–144.

MURPHY, N. P., FRAMENAU, V. W., DONNELLAN, S. C., HARVEY, M. S., PARK, Y.-C. & AUSTIN, A. D. 2006: Phylogenetic reconstruction of the wolf spiders (Araneae: Lycosidae) using sequences from the 12S rRNA, 28S rRNA, and NADH1 genes: implications for classification, biogeography, and the evolution of web building behavior. *Molecular Phylogenetics and Evolution* **38**: 583–602.

OXFORD, G. S. 2008: Identification of *Tegenaria gigantea* and *T. saeva*. *Spider Recording Scheme News* **62**: 21–23, *In Newsletter of the British Arachnological Society* **113**.

OXFORD, G. S. 2009: Historical distributions in Britain of two species of large house spiders, *Tegenaria saeva* and *T. gigantea* (Araneae, Agelenidae), and their evolutionary implications. *Bulletin of the British Arachnological Society* **14**: 297–302.

OXFORD, G. S. 2011: Large house spiders in the British Isles: past, present and future. *British Wildlife* **23**: 34–41.

OXFORD, G. S. & CROUCHER, P. J. P. 2014: Many a slip: dissecting the causes of reproductive isolation in two species of *Tegenaria* (Agelenidae). *Biological Journal of the Linnean Society* **113**: 355–367.

OXFORD, G. S. & PLOWMAN, A. 1991: Do Large House spiders *Tegenaria gigantea* and *T. saeva* (Araneae, Agelenidae) hybridise in the wild? – A multivariate approach. *Bulletin of the British Arachnological Society* **8**: 293–296.

OXFORD, G. S. & SMITH, C. D. 2014: Distributions of species in the *Tegenaria atrica* group (Araneae: Agelenidae) in north-east England: chance, contingency and priority. *Arachnology* **16**: 145–153.

OXFORD, G. S. & SMITH, C. J. 1987: The distribution of *Tegenaria gigantea* Chamberlin & Ivie, 1935 and *T. saeva* Blackwall, 1844 (Araneae, Agelenidae) in Yorkshire. *Bulletin of the British Arachnological Society* **7**: 123–127.

PICKAVANCE, J. R. & DONDALE, C. D. 2006: An annotated checklist of the spiders of Newfoundland. *Canadian Field-Naturalist* **119**: 254–275.

PRAGER, E. M., SAGE, R. D., GYLLENSTEN, U., THOMAS, W. K., HUBNER, R., JONES, C. S., NOBLE, L., SEARLE, J. B. & WILSON, A. C. 1993: Mitochondria1 DNA sequence diversity and the colonization of Scandinavia by house mice from East Holstein. *Biological Journal of the Linnean Society* **50**: 85–122.

ROTH, V. D. 1968: The spider genus *Tegenaria* in the western hemisphere. *American Museum Novitates* **2323**: 1–33.

SAFRAN, R. J., SCORDATO, E. S. C., SYMES, L. B., RODRIGUEZ, R. L. & MENDELSON, T. C. 2013: Contributions of natural and sexual selection to the evolution of premating reproductive isolation: a research agenda. *Trends in Ecology and Evolution* **28**: 643–650.

SIMON, C., FRATI, F., BECKENBACH, A., CRESPI, B., LIU, H. & FLOOK, P. 1994: Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* **87**: 651–701.

STERN, D. L. 2013: The genetic causes of convergent evolution. *Nature Reviews, Genetics* **14**: 751–764.

TAMURA, K. 1992: Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. *Molecular Biology and Evolution* **9**: 678–687.

WILLIS, S. C., FARIAS, I. P. & ORTÍ, G. 2014: Testing mitochondrial capture and deep coalescence in Amazonian cichlid fishes (Cichlidae: *Cichla*). *Evolution* **68**: 256–268.

WORLD SPIDER CATALOG 2018: *World spider catalog, version 19.0*. Bern: Natural History Museum, online at https://wsc.nmbe.ch

Appendix

Source of specimens and morphological measurements (mm)

Grouping = species/source combinations (as defined in the text); **Code no**. = as shown in Table 1; **Collection/ Sample ID no**. = collection codes (defined below) + identifiers for samples within collections; **PROW** = prosoma width; **PROL** = prosoma length. Females: **APR** = tip of apophysis to posterior edge of receptacle opening; **RWMX** = maximum distance between receptacle openings; **RWMN** = minimum distance between receptacle openings; **RLW** = receptacle opening long width; **RSW** = receptacle opening short width; **RH** = top of receptacle opening to epigastric furrow; **BEND** = for each spermatheca score +1 if anterior end bends outwards, -1 if it bends inwards, 0 of there is no bend, two scores summed; **SMX** = total length of spermatheca; **CSW** = distance from epigastric furrow to major constriction in spermatheca width; **SWMX** = maximum spermatheca width; * = continuous ridge between openings; ** = distorted; n/a = internal features not examined. Males: **TCL** = tegulum + conductor length; **TW** = tegulum width; **TWMX** = maximum tegulum width; **CW** = conductor width; **CL** = conductor length; **CANG** = conductor angle. Collections: **DMNS** = Denver Museum of Nature and Science, Denver; **FMNH** -= Finnish Museum of Natural History, Helsinki; **GSO** = Geoff Oxford, York; **JP** = Julien Pétillon, Rennes; **MM** = Manchester Museum; **NHM** = Natural History Museum, London; **NMB** = Naturhistorisches Museum Basel; **RB** = Robert Bosmans, Gent; **RBCM** = Royal British Columbia Museum, Victoria; **RW** = Rick West, Vancouver Island (now GSO).

FEMALES

Grouping Origin	Code no.	Collection/Sample ID no.	PROW	PROL	APR	RWMX	RWMN	RLW	RSW	RH	BEND	SMX	CSW	SWMX
Eratigena atrica														
British (DNA)														
Britain	12	GSO 1753	4.8750	6.7917	0.8333	0.4167	0.1944	0.1111	0.0833	0.9722	2	0.9722	0.4861	0.1944
Britain	20	GSO 1746	5.0000	7.0000	0.7500	0.4444	0.1111	0.1667	0.1389	0.8889	2	0.8889	0.5278	0.1806
Britain	22	GSO 1720	4.4583	6.6667	0.7500	0.3889	0.1667	0.1389	0.1111	0.8333	2	0.8611	0.5278	0.1944
Britain	31	GSO 1891	3.7083	5.2500	0.5278	0.5000	0.1806	0.1528	0.1389	0.7222	2	0.6667	0.4167	0.1667
Britain	35	GSO 1779	4.7917	6.8333	0.7639	0.4444	0.1667	0.1528	0.1389	0.8750	2	0.8889	0.5833	0.2222
Britain	37	GSO 1883	4.7917	6.9167	0.7639	0.4444	0.1667	0.1667	0.0972	0.9167	2	0.8889	0.5556	0.1667
Britain	40	GSO 1726	3.8333	5.2500	0.5833	0.3889	0.1111	0.1667	0.1389	0.7500	2	0.7222	0.4167	0.1667
Britain	45	GSO 1888	4.2500	5.9583	0.5833	0.4167	0.1667	0.1389	0.1111	0.6944	2	0.7500	0.5139	0.1667
Britain	47	GSO 1805	4.5833	6.4167	0.7361	0.5000	0.1667	0.1389	0.1111	0.8333	2	0.8889	0.5556	0.1944
Britain	50	GSO 1769	4.9583	7.1667	0.7500	0.5139	0.1944	0.1667	0.1528	0.9444	2	0.9167	0.5833	0.2222
Continental Europe														
Germany		NHM Numberg #1	4.3333	6.0000	0.6389	0.4444	0.1667	0.1667	0.1389	0.8333	n/a	n/a	n/a	n/a
France		MM 10520(a)	4.0000	5.9583	0.6667	0.3333	0.0833	0.1111	0.0833	0.7778	2	0.8056	0.5556	0.1806
France		MM 10520(b)	3.5000	4.9167	0.5278	0.4167	0.1667	0.1389	0.1111	0.6944	2	0.6944	0.4167	0.1389
Sweden		NMB 26414 [610]	5.5417	7.9167	0.7361	0.4722	0.2500	0.1389	0.1250	0.8750	2	0.9167	0.5000	0.1944
France		NMB 26412 [552]	4.6667	6.5833	0.6250	0.3333	0.1111	0.1389	0.1389	0.8611	2	0.8611	0.5278	0.1944
Belgium		NMB 26368 [198]	4.3750	6.4167	0.6389	0.4306	0.1250	0.1389	0.1389	0.8611	2	0.7778	0.4444	0.1528
Switzerland		NMB 26387a [508]	4.8333	7.0000	0.6944	0.4444	0.1389	0.1667	0.1667	0.9167	2	0.8889	0.5556	0.1667
Switzerland		NMB 26387b [508]	4.1667	6.2500	0.7222	0.3889	0.1389	0.1389	0.1250	0.8889	2	0.9167	0.5833	0.1806
Austria		NMB 26374 [265]	3.7500	5.5833	0.6111	0.3889	0.0833	0.1667	0.1389	0.8333	2	0.8056	0.5000	0.1667
Finland		FMNH 37198	4.5000	6.3750	0.5556	0.3333	0.0556	0.1389	0.1389	0.8056	2	0.8611	0.4722	0.1944

FEMALES (cont.)

Grouping / Origin	Code no.	Collection/Sample ID no.	PROW	PROL	APR	RWMX	RWMN	RLW	RSW	RH	BEND	SMX	CSW	SWMX
***Eratigena atrica* (cont.)**														
Continental Europe (cont.)														
Finland		FMNH 28670	4.0000	5.9167	0.5833	0.3611	0.1111	0.1111	0.1111	0.8056	2	0.8056	0.4444	0.1806
Finland		FMNH 44708	4.3333	6.5000	0.7222	0.3611	0.0833	0.1389	0.1389	0.8611	2	0.8333	0.5000	0.1944
Finland		FMNH 72723	4.2500	6.0833	0.6667	0.4167	0.0833	0.1944	0.1806	0.9167	2	0.8889	0.4722	0.1944
Belgium		RB BOS#2	3.9167	5.6250	0.6250	0.3889	0.1111	0.1389	0.1250	0.7778	n/a	n/a	n/a	n/a
Belgium		RB BOS#7b	3.7500	5.3750	0.6389	0.3472	0.0833	0.1389	0.1389	0.8333	n/a	n/a	n/a	n/a
Netherlands		RB BOX#11	4.8750	6.9167	0.6944	0.3889	0.0833	0.1667	0.1389	0.9444	n/a	n/a	n/a	n/a
Eratigena saeva														
British (DNA)														
Britain	04	GSO 1857	4.5833	6.4167	0.5833	0.5833	0.2778	0.1389	0.1389	0.8889	2	0.7778	0.6111	0.2639
Britain	05	GSO 1674	3.9167	5.6667	0.6111	0.5417	0.3056	0.1389	0.1389	0.7778	2	0.6944	0.5556	0.2500
Britain	06	GSO 1870	4.4583	6.3333	0.6528	0.5833	0.2500	0.1667	0.1250	0.8056	2	0.7222	0.5833	0.2500
Britain	07	GSO 1999	4.2500	5.9167	0.6944	0.5278	0.2778	0.1389	0.1111	0.8333	2	0.7500	0.5833	0.2639
Britain	08	GSO 2005	3.7500	5.4167	0.5556	0.4722	0.2222	0.1250	0.0972	0.6944	2	0.6111	0.4722	0.2500
Britain	11	GSO 1906	4.0417	5.5417	0.5556	0.4722	0.3056	0.1389	0.1250	0.6944	2	0.6111	0.4306	0.2500
Britain	14	GSO 2007	5.5000	7.5833	0.6944	0.5972	0.2500	0.1667	0.1667	0.9722	2	0.8333	0.6944	0.2500
Britain	21	GSO 1850	4.3333	6.1667	0.5972	0.5833	0.3611	0.1389	0.1111	0.7222	2	0.6944	0.5833	0.2500
Britain	29	GSO 1865	4.4167	6.5000	0.6250	0.6667	0.3056	0.1667	0.1389	0.8611	2	0.7500	0.5833	0.2500
Britain	36	GSO 1959	4.8750	6.7500	0.6389	0.5694	0.2639	0.1667	0.1528	0.8750	2	0.7500	0.5556	0.2778
Britain	41	GSO 1998	5.3333	7.5417	0.7500	0.6389	0.3056	0.1667	0.1389	0.9444	2	0.8194	0.5833	0.2639
Britain	44	GSO 2004	4.8333	6.8333	0.6111	0.7778	0.4444	0.1667	0.1528	0.8333	2	0.6667	0.5833	0.2917
Britain	49	GSO 1869	3.4167	4.7917	0.6250	0.5000	0.2222	0.1389	0.1389	0.6806	2	0.6389	0.5278	0.2222
British (add.)														
Jersey		NHM Jersey #2a	4.6667	6.5417	0.4722	0.6389	0.3611	0.1389	0.1111	0.6944	n/a	n/a	n/a	n/a
Jersey		NHM Jersey #2b	4.9167	6.8333	0.5833	0.5833	0.3333	0.1667	0.1250	0.7778	n/a	n/a	n/a	n/a
Guernsey		NHM Guernsey #1a	4.9167	7.0000	0.5000	0.6667	0.4167	0.1528	0.1250	0.7778	n/a	n/a	n/a	n/a
Guernsey		NHM Guernsey #1b	3.8333	5.3333	0.5000	0.6389	0.3611	0.1389	0.1250	0.6111	n/a	n/a	n/a	n/a
Guernsey		NHM Guernsey #1c	4.5000	6.4583	0.6111	0.6389	0.3333	0.1667	0.1389	0.7778	n/a	n/a	n/a	n/a
Jersey		NHM Jersey #1a	3.5833	5.1667	0.5278	0.5000	0.2917	0.1389	0.1250	0.6944	n/a	n/a	n/a	n/a
Jersey		NHM Jersey #1b	4.5833	6.5833	0.5833	0.7500	0.3611	0.1944	0.1389	0.8056	n/a	n/a	n/a	n/a
Continental Europe														
France		JP JP-1	4.7083	6.8333	0.6111	0.6111	0.3889	0.1111	0.0972	0.7500	2	0.7222	0.5833	0.2778
France		JP JP-4	4.6250	6.6667	0.6528	0.5556	0.2500	0.1528	0.1528	0.8333	2	0.7778	0.5833	0.2500
France		JP JP-6	4.0833	6.0833	0.6250	0.5556	0.3056	0.1389	0.1250	0.8333	2	0.7222	0.5972	0.2500
France		JP JP-7	3.6250	5.4167	0.5556	0.5556	0.2500	0.1528	0.1389	0.7222	2	0.6667	0.5000	0.2639
France		JP JP-9	5.4167	7.5833	0.5694	0.6667	0.3889	0.1528	0.1389	0.8611	2	0.8056	0.5833	0.2778
France		JP JP-13	4.7500	6.8333	0.6250	0.4722	0.2361	0.1250	0.1250	0.8333	2	0.7222	0.5833	0.2778
France		JP JP-19	4.5833	6.7500	0.6667	0.5556	0.2778	0.1528	0.1389	0.8333	2	0.7639	0.5556	0.2500
France		JP JP-20	4.7500	7.0000	0.6944	0.5139	0.2361	0.1528	0.1389	0.8750	2	0.7361	0.4861	0.2639
Finland		FMNH 89150	5.1250	7.1667	0.6111	0.6389	0.3750	0.1806	0.1389	0.8611	2	0.8056	0.5556	0.2778
Finland		FMNH 89149	4.4167	6.1667	0.5556	0.5278	0.2778	0.1389	0.1250	0.7778	n/a	n/a	n/a	n/a
Spain		RB BOS#5	5.0833	7.5000	0.4722	0.4444	0.1667	0.1389	0.1250	0.6667	2	0.6111	0.4167	0.2222
France		RB BOS#12b	3.7500	5.3333	0.5556	0.5556	0.2778	0.1528	0.1389	0.7222	n/a	n/a	n/a	n/a
Eratigena duellica														
British (DNA)														
Britain	10	GSO 1964	3.9167	5.5000	0.4722	0.5556	0.1667	0.1944	0.0278	0.7083	-2	0.6944	0.5556	0.2222
Britain	13	GSO 1990	4.1667	5.7500	0.5000	0.5833	0.2222	0.2222	0.0278	0.6111	-2	0.6389	0.5556	0.2222
Britain	15	GSO 1962	4.0833	5.7500	0.4167	0.5833	0.1389	0.2500	0.0278	0.6944	-2	0.6389	0.4861	0.2222
Britain	19	GSO 1995	4.5000	6.4167	0.5278	0.6111	0.2222	0.2500	0.0278	0.8056	-2	0.8056	0.6667	0.2222
Britain	23	GSO 1930	5.8333	7.9167	0.5972	0.5833	0.2222	0.3056	0.0278	0.8056	-2	0.7778	0.6111	0.2778
Britain	27	GSO 1927	4.5000	6.3333	0.4722	0.5833	0.2222	0.2083	0.0139	0.7361	-2	0.6944	0.4722	0.2500
Britain	30	GSO 1858	5.2917	7.4167	0.5000	0.4722	0.1389	0.2222	0.0278	0.7917	-2	0.8056	0.5556	0.2500
Britain	32	GSO 1987	4.5833	6.6667	0.5833	0.5556	0.1389	0.2222	0.0278	0.6528	0	0.6944	0.5556	0.2222
Britain	42	GSO 1966	5.0000	6.9167	0.5000	0.5556	0.1389	0.2500	0.0278	0.6528	-2	0.7222	0.5556	0.2500
Britain	43	GSO 1843	4.3333	6.6667	0.5139	0.5000	0.1111	0.2361	0.0278	0.7222	-2	0.7222	0.5833	0.2222
Britain	46	GSO 1988	4.6250	6.5000	0.5278	0.5278	0.1389	0.1944	0.0278	0.7083	-2	0.6944	0.5278	0.3056
Britain	48	GSO 1970	3.8750	5.5000	0.4306	0.4444	0.1389	0.1944	0.0278	0.6389	-2	0.6667	0.5556	0.2222
Continental Europe														
Portugal		MM 10679	3.5000	4.9167	0.5139	0.4722	0.1389	0.2222	0.0278	0.6667	-2	0.7222	0.5417	0.2222
Finland		FMNH 89024	3.7917	5.5000	0.3889	0.5139	*	0.2500	0.0278	0.6806	0	0.6944	0.5556	0.2500
Finland		FMNH 89021	5.0833	6.9167	0.3889	0.6389	0.3056	0.2361	0.0833	0.8750	2	0.8056	0.5556	0.2500
Finland		FMNH 89023	3.8333	5.5000	0.4167	0.5000	*	*	0.0278	0.6389	0	n/a	n/a	n/a
North America														
Canada		RW BC#1	4.5417	6.5000	0.4583	0.5000	0.2222	0.1667	0.0278	0.7222	-2	0.7500	0.5833	0.2222
Canada		RW BC#1	4.5417	6.5000	0.4583	0.5000	0.2222	0.1667	0.0278	0.7222	-2	0.7500	0.5833	0.2222
Canada		RW BC#2	4.2500	6.0833	0.5139	0.5556	0.1944	0.1944	0.0278	0.7222	-2	0.7222	0.5556	0.2222
Canada		RW BC#3	5.1250	7.0833	0.5278	0.5556	0.1806	0.2500	0.0278	0.7083	-2	0.7500	0.5833	0.2222
USA		DMNS DMNS ZA7513	4.9167	7.0833	0.4722	0.5694	0.2361	0.2500	0.0278	0.6667	0	0.7778	0.5000	0.2222
USA		DMNS DMNS ZA11125	5.0833	7.2500	0.4444	0.5556	0.1667	0.2778	0.0278	0.6944	-2	0.8056	0.5000	0.2500

FEMALES (cont.)

Grouping / Origin	Code no.	Collection/Sample ID no.	PROW	PROL	APR	RWMX	RWMN	RLW	RSW	RH	BEND	SMX	CSW	SWMX
***Eratigena duellica* (cont.)**														
North America (cont.)														
USA		DMNS DMNS ZA1990	5.4167	7.5833	0.4444	0.5833	0.2778	0.2778	0.0278	0.7222	0	0.8056	0.4583	0.2500
Canada		RBCM ENT 009-004554	4.7500	6.8750	0.4722	0.5833	0.2500	0.2500	0.0417	0.7222	-2	0.7222	0.4722	0.2222
Canada		RBCM ENT 011-011703(a)	4.4167	6.0833	0.4167	0.5556	0.1944	0.2778	0.0278	0.6667	0	0.7500	0.4444	0.2222
Canada		RBCM ENT 011-011703(b)	3.6667	5.0833	0.4167	0.5000	0.1944	0.2500	0.0278	0.6944	-2	0.6944	0.5139	0.1944
Canada		RBCM ENT 006-005800	5.0833	7.4167	0.4167	0.5833	0.2500	0.3056	0.0278	0.7778	**	0.8056	0.5278	0.2222
Canada		RBCM ENT 010-001929	3.8750	5.5833	0.4444	0.5139	0.1944	0.2500	0.0278	0.6944	-2	0.8056	0.5278	0.2083
Canada		RBCM ENT 006-005784	4.7917	6.9167	0.4722	0.4722	0.1528	0.2500	0.0278	0.7639	-2	0.8333	0.5556	0.2222
Canada		RBCM ENT 006-005785	4.1667	6.0833	0.4722	0.6111	0.2222	0.2778	0.0278	0.6667	-2	0.6667	0.4444	0.2222
Canada		RBCM ENT 991-056851	5.8333	8.2500	0.5556	0.6111	0.1944	0.2778	0.0417	0.8056	-2	0.8056	0.5139	0.2500
Canada		RBCM ENT 000-000411	4.5000	6.9167	0.4444	0.5556	0.1944	0.2778	0.0417	0.8056	-2	0.7917	0.5000	0.2500
Canada		RBCM ENT 991-056849	4.0833	5.5000	0.4722	0.5000	0.1389	0.2500	0.0278	0.6528	-2	0.6944	0.3889	0.2222
Canada		RBCM ENT 991-056853	4.3333	5.8333	0.4167	0.5556	0.1944	0.2778	0.0278	0.6667	-2	0.6944	0.4167	0.1944
Canada		RBCM ENT 010-002130	4.5000	6.4167	0.4444	0.5417	0.1667	0.2778	0.0278	0.7222	-2	0.7222	0.4722	0.2222
Canada		RBCM ENT 006-005798	3.8750	5.6667	0.4722	0.5556	0.1389	0.2778	0.0278	0.6389	-2	0.6389	0.4167	0.1944
Canada		RBCM ENT 991-056860	4.0000	5.6667	0.4167	0.5833	0.2500	0.2500	0.0417	0.6944	-2	0.6389	0.4444	0.1944
Canada		RBCM ENT 991-056859	4.3333	6.5000	0.4444	0.5833	0.1667	0.2500	0.0278	0.6944	-2	0.7222	0.4722	0.1944
Canada		RBCM ENT 011-0011691	3.4167	4.7500	0.4167	0.5000	*	0.3056	0.0278	0.6111	-2	0.6667	0.4444	0.1944
Canada		RBCM ENT 011-0011696	3.9167	5.8333	0.4167	0.5278	0.1389	0.2778	0.0278	0.7222	-2	0.6944	0.4167	0.1944
***E. saeva* × *E. duellica* hybrids**														
Laboratory		GSO 12-1-A	4.7500	6.7917	0.6389	0.5833	0.2778	0.1944	0.0833	0.8056	2	0.7778	0.5556	0.2500
Laboratory		GSO 12-2-K	4.6250	6.6667	0.5000	0.5833	0.3056	0.2500	0.0833	0.7917	0	0.7778	0.5278	0.2500
Laboratory		GSO 12-4-I	4.1667	5.4167	0.4722	0.5139	0.2361	0.1944	0.0972	0.6944	2	0.7222	0.5278	0.2222
Laboratory		GSO 12-2-B	4.7500	6.7917	0.5833	0.6111	0.3056	0.2222	0.0833	0.8194	2	0.8056	0.5556	0.2500
Laboratory		GSO 12-1-K	4.0000	5.9583	0.5972	0.5833	0.3333	0.1944	0.0833	0.7778	2	0.7222	0.4444	0.2222
Laboratory		GSO 12-4-G	5.0000	6.9167	0.6389	0.6389	0.3056	0.2639	0.0972	0.8333	2	0.7778	0.5556	0.2500
Laboratory		GSO 12-5-C	4.3333	6.3333	0.5972	0.5833	0.2917	0.1944	0.1111	0.7500	2	0.7222	0.5278	0.2500
Laboratory		GSO 12-1-Q	4.7917	7.0000	0.5833	0.6389	0.3611	0.2222	0.1111	0.7639	2	0.8056	0.5833	0.2361
Laboratory		GSO 12-1-O	4.7083	6.7500	0.5833	0.5556	0.3056	0.2500	0.0694	0.7361	2	0.7778	0.6111	0.2500
Laboratory		GSO 12-1-J	4.7917	6.7500	0.6250	0.5417	0.2917	0.1667	0.0833	0.7917	0	0.7778	0.5833	0.2500
Species uncertain														
British (add.)														
Jersey		NHM Jersey #1c	5.2500	7.3333	0.8056	0.7222	0.3750	0.1667	0.1389	0.9167	n/a	n/a	n/a	n/a
Continental Europe														
Spain		NMB 26385 [570]	4.9167	7.2500	0.4167	0.5000	0.2778	0.1111	0.1111	0.6944	0	0.6111	0.3889	0.2222
Spain		NMB 20693 [?]	4.6667	6.2500	0.5556	0.4167	0.1667	0.1389	0.1111	0.6944	2	0.6667	0.4306	0.1667
France		JP JP-2	4.7500	6.8750	0.7222	0.5000	0.2222	0.1667	0.1389	0.8611	2	0.7778	0.6111	0.2917
France		JP JP-15	5.2083	7.6667	0.6944	0.5556	0.2222	0.1806	0.1528	0.8889	2	0.8194	0.5833	0.3056
North America														
Canada		RW BC#4	4.4167	6.3333	0.5417	0.5694	0.2361	0.1944	0.0833	0.6944	2	0.6944	0.5556	0.2222

MALES

Grouping / Origin	Code no.	Collection/Sample ID no.	PROW	PROL	TCL	TW	TWMX	CW	CL	CANG
Eratigena atrica										
British (add.)										
Britain		GSO 1396	3.5833	4.9167	1.0000	0.4861	0.2778	n/a	n/a	0.1111
Britain		GSO 1399	5.0417	6.6667	1.1111	0.5833	0.3056	n/a	n/a	0.1389
Britain		GSO 1400	2.9583	4.1250	0.9167	0.4306	0.2778	n/a	n/a	0.1250
Britain		GSO 1402	4.4167	6.4167	1.0694	0.5417	0.2917	n/a	n/a	0.1806
Britain		GSO 1405	2.5417	3.5417	0.8889	0.4167	0.2500	n/a	n/a	0.1389
Britain		GSO 1420	4.7500	6.7500	1.0556	0.5000	0.2778	n/a	n/a	0.1944
Britain		GSO 1421	4.2917	6.1667	1.0000	0.5278	0.3056	n/a	n/a	0.1389
Britain		GSO 1535	4.1667	6.0000	1.0000	0.5000	0.2917	n/a	n/a	0.1250
Britain		GSO 1546	2.9583	4.1667	0.9167	0.4028	0.2639	n/a	n/a	0.1111
Britain		GSO 1574	2.5833	4.1667	0.8889	0.5000	0.2500	n/a	n/a	0.1389
Britain		GSO 1575	4.5000	6.2500	1.0278	0.5278	0.2778	n/a	n/a	0.1389
Britain		GSO 1582	2.4583	3.3333	0.8333	0.3889	0.2083	n/a	n/a	0.1250
Britain		GSO 1615	3.3750	4.7917	0.9722	0.5000	0.2639	n/a	n/a	0.1389
Britain		GSO 1618	3.3750	4.7500	1.0000	0.4861	0.2917	n/a	n/a	0.1111
Britain		GSO 1689	3.2500	4.5000	0.9444	0.4167	0.2778	n/a	n/a	0.1111
Britain		GSO 1724	4.4167	6.1667	1.0556	0.5278	0.3194	n/a	n/a	0.1389
Britain		GSO 1737	3.1667	4.3333	0.8889	0.4306	0.2639	n/a	n/a	0.1111
Britain		GSO 1770	5.0833	7.0000	1.1389	0.5278	0.3333	n/a	n/a	0.1667
Britain		GSO 1777	2.7500	3.7500	0.8889	0.4583	0.2361	n/a	n/a	0.1389
Britain		GSO 1892	5.0000	7.0000	1.1111	0.4861	0.3056	n/a	n/a	0.1667

MALES (cont.)

Grouping Origin	Code no.	Collection/Sample ID no.	PROW	PROL	TCL	TW	TWMX	CW	CL	CANG
***Eratigena atrica* (cont.)**										
Continental Europe										
Germany		NHM 1916.16.1	4.4167	6.4167	1.0694	0.5278	0.2917	n/a	n/a	0.1667
Germany		NHM Nümberg #2a	4.4167	6.0000	1.0139	0.4583	0.2917	n/a	n/a	0.1528
Germany		NHM Nümberg #2b	3.9583	5.5000	1.0278	0.4722	0.2917	n/a	n/a	0.1528
Germany		NHM Nümberg #2c	3.2083	4.3750	0.9306	0.4722	0.2778	n/a	n/a	0.1528
Italy		NMB 26410 [566]	5.1667	7.0833	1.1111	0.4444	0.2778	n/a	n/a	0.1528
France		JP JP-14b	4.1250	5.6667	1.0278	0.5278	0.3472	n/a	n/a	0.1389
Finland		FMNH 9128	5.0000	7.0833	1.0694	0.5139	0.3056	n/a	n/a	0.1389
Finland		FMNH 72725	4.5833	6.5000	1.0972	0.5417	0.3333	n/a	n/a	0.1389
Finland		FMNH 109908	4.8333	6.7500	1.0833	0.5833	0.3194	n/a	n/a	0.1389
Netherlands		RB BOS#1	4.0833	5.6667	1.0278	0.4722	0.2917	n/a	n/a	0.1667
Belgium		RB BOS#7a	4.7500	6.2500	1.1111	0.6111	0.3056	n/a	n/a	0.2222
Netherlands		RB BOS#8	4.1667	6.0833	1.0833	0.5833	0.3333	n/a	n/a	0.1667
Belgium		RB BOS#10	3.2500	4.6667	0.9722	0.4444	0.2500	n/a	n/a	0.1389
France		RB BOS#3	4.7500	6.8333	1.0972	0.5278	0.3194	n/a	n/a	0.1806
Eratigena saeva										
British (DNA)										
Britain	01	GSO 1672	4.6667	6.6667	1.3889	0.5278	0.4167	0.0556	0.1944	-0.0833
Britain	09	GSO 1847	5.2500	6.9583	1.3611	0.5556	0.3194	0.0278	0.1667	-0.1111
Britain	16	GSO 1852	6.0000	8.3333	1.4444	0.6667	0.4167	0.0417	0.1944	-0.1250
Britain	18	GSO 1839	3.5000	5.0000	1.2083	0.4167	0.3750	0.0556	0.1806	-0.0833
Britain	28	GSO 1903	3.1667	4.4167	1.2083	0.4722	0.3472	0.0417	0.1250	-0.0833
Britain	34	GSO 1855	3.9167	5.4167	1.3333	0.4583	0.3750	0.0417	0.1667	-0.0556
Britain	38	GSO 1979	4.6667	6.5833	1.4028	0.5556	0.4167	0.0556	0.2083	-0.0833
British (add.)										
Britain		GSO 834	5.5000	7.6667	1.4028	0.5833	0.4861	0.0556	0.1944	-0.1111
Britain		GSO 832	3.2500	4.5000	1.2222	0.5278	0.4028	0.0278	0.1667	-0.0833
Britain		GSO 830	3.4167	4.7917	1.2222	0.4722	0.3611	0.0278	0.1806	-0.0833
Britain		GSO 820	4.5000	6.3333	1.3056	0.4722	0.3889	0.0278	0.1667	-0.1111
Britain		GSO 2002	4.4167	6.0833	1.3056	0.5278	0.3889	0.0278	0.1667	-0.0833
Britain		GSO 1997	4.6667	6.5000	1.3889	0.5139	0.3889	0.0417	0.1944	-0.1111
Britain		GSO 1907	5.2500	7.5000	1.4444	0.4722	0.4167	0.0417	0.1944	-0.0972
Britain		GSO 1904	4.0417	5.7917	1.3472	0.5000	0.4028	0.0278	0.1667	-0.0694
Britain		GSO 1856	3.9583	5.4167	1.2917	0.4722	0.3333	0.0417	0.2222	-0.1389
Britain		GSO 1851	4.8333	6.6667	1.3611	0.4444	0.3611	0.0556	0.2222	-0.0833
Britain		GSO 1848	4.9583	6.9167	1.4167	0.6111	0.4583	0.0556	0.2083	-0.0694
Britain		GSO 1838	4.8333	6.7500	1.3472	0.5139	0.4028	0.0417	0.2222	-0.0833
Britain		GSO 1188	4.1667	5.8333	1.3611	0.5278	0.4167	0.0417	0.1944	-0.0972
Britain		GSO 1058	4.8333	6.7500	1.4028	0.5000	0.4167	0.0417	0.2222	-0.0833
Britain		GSO 1054	4.1667	5.9167	1.2222	0.4861	0.3889	0.0417	0.1806	-0.0694
Britain		GSO 1050	5.0833	7.0833	1.3611	0.5833	0.4028	0.0417	0.1944	-0.0556
Britain		GSO 1016	4.0000	5.5833	1.2778	0.4861	0.3750	0.0417	0.1667	-0.0833
Jersey		NHM Jersey #1d	4.2917	6.1667	1.3333	0.5139	0.4306	0.0417	0.1944	-0.0972
Continental Europe										
France		JP JP-3	4.0000	5.5833	1.2917	0.5417	0.4444	0.0417	0.0417	-0.1250
France		JP JP-5	3.9167	5.7500	1.3611	0.5556	0.4444	0.0417	0.0417	-0.1111
France		JP JP-8	3.1250	4.2083	1.2083	0.4444	0.4028	0.0417	0.0417	-0.1250
France		JP JP-10	3.9167	5.5417	1.3333	0.5417	0.4306	0.0556	0.0556	-0.0972
France		JP JP-11	3.6667	5.2500	1.3056	0.5278	0.4444	0.0417	0.0417	-0.1111
France		JP JP-12	3.9167	5.3333	1.3333	0.5000	0.4167	0.0556	0.0556	-0.0833
France		JP JP-14a	3.2500	4.6667	1.1944	0.4444	0.3611	0.0278	0.0278	-0.0833
France		JP JP-17	2.5417	3.4167	1.0556	0.4167	0.3333	0.0278	0.0278	-0.0833
France		JP JP-18	4.7500	7.3333	1.4722	0.5278	0.4444	0.0417	0.0417	-0.1111
France		RB BOS#4	3.0833	4.4167	1.2222	0.4861	0.3889	0.0278	0.0278	-0.0972
France		RB BOS#6a	3.4167	4.5833	1.2500	0.4444	0.3889	0.0417	0.0417	-0.1111
France		RB BOS#6b	3.8750	5.4167	1.3056	0.5556	0.4167	0.0417	0.0417	-0.0972
France		RB BOS#9a	4.2917	6.0833	1.3750	0.5556	0.4444	0.0417	0.0417	-0.1111
France		RB BOS#9b	4.5833	6.5833	1.4028	0.5278	0.4444	0.0278	0.0278	-0.0833
France		RB BOS#9c	4.0833	5.5833	1.3056	0.5556	0.4306	0.0278	0.0278	-0.0972
France		RB BOS#12c	3.5000	5.1667	1.2778	0.5000	0.4028	0.0278	0.0278	-0.0972
France		RB BOS#12b	3.6667	5.2083	1.2778	0.4861	0.3611	0.0278	0.0278	-0.0972
Eratigena duellica										
British (DNA)										
Britain	02	GSO 1861	4.5833	6.1667	1.1667	0.3611	0.3611	0.1250	0.2222	-0.0139
Britain	03	GSO 1974	5.1667	7.1667	1.2222	0.4722	0.3056	0.1111	0.3056	0.0000
Britain	17	GSO 1949	4.4583	6.1667	1.1389	0.4167	0.2500	0.1111	0.2222	0.0000
Britain	24	GSO 1830	4.7500	6.4167	1.2222	0.4444	0.3472	0.0972	0.2361	0.0000
Britain	25	GSO 1900	5.2917	7.2500	1.2361	0.4028	0.3333	0.1111	0.2500	0.0000
Britain	26	GSO 1971	3.7917	5.2500	1.0556	0.4167	0.3056	0.1111	0.2083	0.0000

MALES (cont.)

Grouping Origin	Code no.	Collection/Sample ID no.	PROW	PROL	TCL	TW	TWMX	CW	CL	CANG
***Eratigena duellica* (cont.)**										
British (DNA) (cont.)										
Britain	33	GSO 1989	5.0000	6.8333	1.2361	0.4167	0.2778	0.0833	0.1944	0.0000
Britain	38	GSO 1898	5.1250	6.9167	1.2083	0.3611	0.3611	0.1111	0.2917	0.0000
British (add.)										
Britain		GSO 949	5.2500	6.9167	1.1667	0.3889	0.3889	0.1389	0.2222	0.0000
Britain		GSO 947	5.0833	7.1667	1.2222	0.4583	0.4444	0.1667	0.3056	0.0000
Britain		GSO 908	4.9583	6.9167	1.1944	0.4167	0.3472	0.1111	0.2778	0.0000
Britain		GSO 805	4.9167	7.0000	1.1944	0.4722	0.3611	0.1111	0.2222	0.0000
Britain		GSO 2012	4.5417	5.9167	1.1111	0.3611	0.2778	0.0833	0.2222	0.0139
Britain		GSO 1896	5.2500	7.4167	1.1944	0.4028	0.3333	0.1111	0.2778	0.0000
Britain		GSO 1829	3.9583	5.4167	1.1111	0.4167	0.3056	0.0972	0.2361	0.0000
Britain		GSO 1374	4.4167	6.1667	1.2083	0.4167	0.3611	0.1389	0.2778	-0.0139
Britain		GSO 1369	4.1667	6.1250	1.1944	0.4444	0.3889	0.1111	0.2361	0.0139
Britain		GSO 1368	3.2500	4.5833	1.0833	0.3750	0.3056	0.1111	0.1944	0.0278
Britain		GSO 1235	4.2500	6.3333	1.1944	0.4444	0.3611	0.0972	0.1944	0.0000
Britain		GSO 1072	2.8750	4.0000	1.0278	0.3333	0.3194	0.1250	0.2639	0.0000
Britain		GSO 1062	5.5833	7.5000	1.3056	0.4722	0.4028	0.1389	0.2500	-0.0278
Britain		GSO 1056	3.8750	5.5000	1.1667	0.4444	0.3611	0.1389	0.2500	0.0000
Britain		GSO 1055	4.0833	5.8333	1.1389	0.4167	0.3611	0.1389	0.2500	0.0000
Continental Europe										
Finland		FMNH 89018	5.0833	7.1667	1.1944	0.4444	0.3056	0.1111	0.2500	0.0000
Finland		FMNH 89022	5.0833	6.8333	1.1944	0.4722	0.3333	0.1111	0.3056	0.0000
Finland		FMNH 89019	5.0000	6.8333	1.2222	0.4306	0.3056	0.1111	0.2500	0.0000
North America										
USA		DMNS DMNS ZA11826	5.1667	7.5000	1.2222	0.3889	0.3611	0.1389	0.2778	0.0000
USA		DMNS DMNS ZA7513(a)	5.2500	7.3333	1.1944	0.3611	0.3333	0.1528	0.3056	0.0000
USA		DMNS DMNS ZA7513(b)	3.4583	4.3333	1.0556	0.3056	0.2917	0.1389	0.3056	0.0000
USA		DMNS DMNS ZA11824	5.0833	7.0000	1.1944	0.3472	0.3056	0.1389	0.2917	0.0000
USA		DMNS DMNS ZA11822(a)	5.7500	7.9167	1.1944	0.3889	0.3611	0.1389	0.2778	0.0000
USA		DMNS DMNS ZA11822(b)	4.0000	5.5000	1.0833	0.3750	0.3611	0.1389	0.2639	0.0000
USA		DMNS DMNS ZA11823	4.5833	6.1667	1.1389	0.4444	0.3194	0.1111	0.2500	0.0000
USA		DMNS DMNS ZA16837	4.7500	6.2500	1.1389	0.4167	0.3333	0.1250	0.2778	0.0000
Canada		RBCM ENT 011-011700	5.1667	6.6667	1.1806	0.4167	0.2917	0.1111	0.2917	-0.0278
Canada		RBCM ENT 012-013733	4.2500	5.8333	1.0972	0.3611	0.3056	0.1250	0.3056	0.0139
Canada		RBCM ENT 991-56854	5.0000	6.8750	1.1667	0.3889	0.3056	0.1111	0.2639	0.0000
Canada		RBCM ENT 006-005796	3.4167	4.9167	1.0556	0.3472	0.2917	0.1111	0.2778	0.0000
Canada		RBCM ENT 012-013743	4.5000	6.2500	1.1528	0.3056	0.2917	0.1389	0.3056	0.0000
Canada		RBCM ENT 991-056858	4.2917	6.0833	1.1389	0.4028	0.3056	0.1250	0.2639	0.0139
Canada		RBCM ENT 006-005786	3.5833	4.5833	1.0556	0.4028	0.3056	0.1111	0.2639	0.0000
Canada		RBCM ENT 011-0011701	3.9583	5.5417	1.1389	0.3611	0.3194	0.1250	0.2778	0.0000
Canada		RBCM ENT 992-022363	5.5833	7.5833	1.2500	0.4306	0.3611	0.1389	0.2500	0.0000
Canada		RBCM ENT 006-005793	3.6250	4.9167	1.0833	0.3889	0.3333	0.1111	0.2361	0.0000
Canada		RBCM ENT 006-005798	4.2500	6.2500	1.1111	0.4167	0.3056	0.0972	0.2778	0.0000
Canada		RBCM ENT 011-0011707	3.5833	4.9167	1.0556	0.3750	0.3333	0.1111	0.2500	0.0000
Canada		RBCM ENT 006-005801	5.5417	7.8333	1.2222	0.4444	0.3611	0.1250	0.2778	0.0000
Canada		RBCM ENT 011-0011704	4.0000	5.6667	1.1389	0.4167	0.3194	0.1111	0.2778	0.0000
Canada		RBCM ENT 006-005792	5.0833	7.0000	1.1944	0.5000	0.3333	0.1389	0.2778	0.0000
Canada		RBCM ENT 991-056848	4.4583	6.1667	1.1944	0.4306	0.3611	0.1250	0.2917	0.0000
Canada		RBCM ENT 010-001928	5.0000	7.5000	1.2361	0.5000	0.3611	0.1389	0.3056	0.0000
Canada		RBCM ENT 011-0011705	4.1667	5.5833	1.1111	0.4444	0.3333	0.1111	0.3056	0.0000
Canada		RBCM ENT 001-0011693	3.5833	4.7500	1.0833	0.3611	0.3194	0.1111	0.2778	0.0000
Canada		RBCM ENT 011-0011698(a)	3.9167	5.4167	1.0972	0.3750	0.3056	0.1111	0.2500	0.0000
Canada		RBCM ENT 011-0011698(b)	3.7500	5.1667	1.0556	0.3472	0.3056	0.1111	0.2778	0.0000
Canada		RBCM ENT 011-0011698(c)	3.8333	4.8333	1.0694	0.3333	0.2778	0.1111	0.2778	-0.0139
Canada		RBCM ENT 991-063887	4.1667	5.7500	1.1528	0.3611	0.3194	0.1389	0.2639	0.0000
Canada		RBCM ENT 011-0011696	4.1667	6.0000	1.1111	0.4167	0.3194	0.1250	0.2917	0.0000
Canada		RBCM ENT 001-010801(a)	3.8333	5.2500	1.0556	0.3889	0.3056	0.1250	0.2222	0.0000
Canada		RBCM ENT 001-010801(b)	4.5833	6.5000	1.1528	0.4167	0.3056	0.1111	0.2222	0.0139
Canada		RBCM ENT 991-056857	3.6667	5.1667	1.0833	0.3611	0.3194	0.1111	0.2222	0.0000
Canada		RBCM ENT 011-0011684	4.5000	6.2500	1.1111	0.3333	0.3056	0.0833	0.2361	0.0000
***E. saeva* × *E. duellica* hybrids**										
Laboratory		GSO 12-4-D	3.4583	4.7500	1.1528	0.3889	0.3333	0.0833	0.2222	-0.0139
Laboratory		GSO 12-2-A	4.6667	6.6667	1.2778	0.4444	0.3611	0.1250	0.3056	0.0000
Laboratory		GSO 12-6-A	3.8333	5.4167	1.2222	0.4722	0.3472	0.1389	0.3194	-0.0139
Laboratory		GSO 12-2-I	4.5833	6.2500	1.2778	0.4167	0.3611	0.1111	0.3333	0.0000
Laboratory		GSO 12-5-D	4.2500	5.8333	1.2500	0.4722	0.3750	0.1389	0.2778	-0.0417
Laboratory		GSO 12-6-B	3.7500	5.3333	1.2500	0.4167	0.3611	0.1111	0.2778	-0.0139
Laboratory		GSO 12-4-A	4.5833	6.6667	1.3056	0.5000	0.4167	0.1111	0.2500	0.0000
Laboratory		GSO 12-1-I	4.4167	6.4167	1.2500	0.4722	0.3889	0.1111	0.2917	-0.0139
Laboratory		GSO 12-5-A	4.5833	6.6667	1.2917	0.4444	0.3611	0.1111	0.2778	0.0000
Laboratory		GSO 12-3-G	4.5000	6.5833	1.3056	0.4583	0.3333	0.1111	0.2639	-0.0278