# Taxonomic review and phylogenetic analysis of central European *Eresus* species (Araneae: Eresidae)

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Ladybird spiders (*Eresus* spp.) have attracted scientific interest since the 18th century, but taxonomical knowledge of the genus is unsatisfactory. Early classification based on colour and size variation divided European *Eresus* into numerous species. These were later lumped into one predominant morphospecies, *Eresus niger/E. cinnaberinus*, which could be found from Portugal to Central Asia. Here, we perform a major revision of *Eresus* from northern and central Europe using morphological, phenological, habitat, distribution and molecular data. Three species, *E. kollari*, *E. sandaliatus* and *E. moravicus* sp. n. were distinguished. The name *E. niger* (originally *Aranea nigra*) cannot be used as the name *A. nigra* was used for a previous spider species. The name *E. cinnaberinus* is considered a *nomen dubium*. The three species differ in size, colour pattern, shape of prosoma and copulatory organs, phenology, and have slightly different habitat requirements. No morphologically intermediate forms were recorded. In contrast to distinct morphology and phenology, the genus is genetically complex. Genetically, the mitochondrial haplotypes of *E. sandaliatus* and *E. moravicus* sp. n. are monophyletic, whereas those of *E. kollari* are paraphyletic. Eastern central European *E. kollari* is likely a hybrid lineage between *E. sandaliatus* and the monophyletic western central European *E. kollari*. Because eastern and western European *E. kollari* are morphologically and phenologically indistinguishable, we did not formally split them. However, detailed population-based research in the future may partition *E. kollari* into additional species.

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## Introduction

Ladybird spiders, *Eresus* spp., are certainly among the most beautiful spiders present in central Europe. In recent years, they have become flagship species for the conservation of relic rocky steppes in several European countries, yet their taxonomy remains unresolved. *Eresus* spiders are characterized by considerable intraspecific variability in colour and size. The most constant specific character is the shape of the copulatory organs. As the differences are tiny, their use in species delimitation is only practical when numerous individuals are compared. In central and northern Europe, *Eresus* spp. are restricted to highly fragmented xerothermic steppe habitats. The spiders possess an elusive lifestyle confined to inconspicuous burrows. Only the adult males can be found on the soil surface for a few weeks each year when they are seeking females (Nørgaard 1941). Males are considerably smaller

than females; the opisthosoma is usually red with two or three pairs of black spots. This aposematic colouration resembling ladybirds protects them against predators during their diurnal search for females (Cloudsley-Thompson 1995). The much larger females live hidden in burrows their entire life and are usually uniformly black. The distinct sexual dimorphism in both colour and size makes it difficult to match sexes of the same species unless both come from the same locality.

Species discrimination in *Eresus* is further complicated by remarkably limited gene flow (Johannesen *et al.* 1998). This may act at two levels. First, the behaviour of *Eresus* spp. may lead to significant genetic differences between neighbouring populations. This is predominately caused by juveniles settling near the maternal nest (ballooning has never been observed) and by restricted male mating-dispersal. Males searching for females have been reported to move, at most,

76 m (Baumann 1997). The second level of divergence can be viewed in a historical context, in which xerothermic steppes, the habitat of *Eresus* species, expanded and receded in the Palearctic region during the Pleistocene climatic oscillations (Iversen 1964). Historical habitat fragmentation may break down the genetic cohesion of taxa resulting in cryptic speciation via genetic drift (Ramirez 1995; Bond *et al.* 2001; Ramirez & Chi 2004). Alternatively, range expansion during interglacial phases may bring previously isolated lineages into secondary contact, which, depending on the interaction between gene flow and selection, may either merge lineages or reinforce differences. These historical processes may have contributed to the diversity (and taxonomical confusion) of *Eresus*. Therefore, phena demonstrating various degrees of differentiation might occur in the genus *Eresus*. This is the case for European *Eresus*, where highly divergent genetic lineages are more or less allopatrically distributed (Johannesen & Veith 2001).

Taxonomical research of the genus *Eresus* dates back to 1778, when Martini and Goeze described a male from Bavaria, Germany, and named it *Aranea sandaliata*. In the following decade, the spectacular *Eresus* males were described four times under different names without reference to the already described species (Petagna 1787; Olivier 1789; Villers 1789; Rossi 1790). Hahn (1821), Brullé (1832) and Koch (1836) were the first to distinguish more than one *Eresus* species. However, they used colouration as the only character for discrimination and elevated some infraspecific varieties to the specific level (e.g. Koch 1836, 1846). Due to the remarkable sexual dimorphism, males and females of the same species were given different names (Brullé 1832; Koch 1846). Koch (1850) even established a new genus, *Erythrophorus*, for *Eresus* males. Unfortunately, insufficient descriptions and lost type material during this early phase of taxonomical study has brought about considerable confusion in the nomenclature of the genus. Simon first noticed that *Eresus* forms differ not only in colouration, but also in other characters, for example, the shape of the prosoma and copulatory organs. He distinguished at least 13 species and three subspecies (e.g. Simon 1873, 1895, 1910), but later downgraded some (e.g. *Eresus tricolor*) to forms of *E. cinnaberinus* and completely neglected others (e.g. *E. annulatus*) (Simon 1914). Even during this period, some authors were describing new species without considering contemporary knowledge (e.g. Lucas 1864; Franganillo 1918). Kulczyński (1903) and Herman (1879) recorded colour forms without treating them taxonomically. Later, however, the majority of arachnologists ignored this variability and used the names *E. cinnaberinus/niger* for all specimens from Spain to China, sometimes synonymizing even obviously different species (e.g. *E. tristis* synonymized with *E. cinnaberinus* by Nenilin & Pestova 1986). Modern taxonomic research on *Eresus* was initiated by the description of *E. crassitibialis* from the Canary Islands (Wunderlich 1987).

Interestingly, the identity of this species was questioned, even by its own author (Wunderlich 1992). So far, 48 descriptions of new *Eresus* taxa have been published (Platnick 2007), but only three of them, namely those of *Eresus lucasi* Simon, 1873, *Eresus semicanus* Simon, 1908 and *Eresus daliensis* Yang & Hu, 2002 (new synonym of *Stegodyphus tibialis* (O. P.-Cambridge, 1869)), contain descriptions of both sexes.

Due to the complications mentioned above, a generic revision of *Eresus* is impossible today. Sufficient materials for delimitation of taxa are available from only a few regions, for example, from central and northern Europe. Ratschker & Bellmann (1995) and Ratschker (1995) made an important contribution to the taxonomy of *Eresus* from this region. These authors recognized three species, *E. cinnaberinus*, *E. sandaliatus* and *Eresus* sp., which were delimited primarily by male leg colouration and conductor and spermatheca variation. However, the absence of a taxonomic revision, the poor inclusion of several species in *E. cinnaberinus*, and the very schematic drawings made contemporary arachnologists doubt the validity of this delimitation.

In the present paper, we perform a taxonomical revision based on extensive material from central Europe. We compare morphology, phenology, habitats, distribution and molecular phylogenetics of the taxa. Our study revealed that the genus *Eresus* in central Europe is composed of at least three species, *Eresus kollari* Rossi 1846 and *E. moravicus* sp. n. (prior varieties of *E. cinnaberinus*) and *E. sandaliatus* (Martini & Goeze, 1778). The species are difficult to distinguish based on a single morphological character, but not when using multicharacter analysis. Specific discreteness is corroborated by ecological features such as phenology and distributions.

## Materials and methods
### Morphology
We studied 495 males, 220 females and 130 juveniles of *E. kollari*; 50 males, 19 females and 10 juveniles of *E. moravicus* sp. n. and 42 males, 60 females and 11 juveniles of *E. sandaliatus*. We used the following morphological characters for species delimitation: colour pattern, body size, shape of the prosoma, and shape of male and female copulatory organs (Table 1). Morphological characters used to distinguish some other *Eresus* species, as well as some species of the genus *Stegodyphus*, such as position of eyes and their relative size, relative length of legs and the shape of male forelegs (Simon 1873; Kraus & Kraus 1988) were uniform among the studied species and thus were not considered.

### Microscopy
To investigate the shape of the palpal organs, palps from each species were dried at room temperature, mounted on a stub, coated with gold and observed by scanning electron microscopy.

**Table 1** Morphological characters used for species delimitation in central European *Eresus*.

| Character | *E. kollari* morphotype | *E. moravicus* sp. n. | *E. sandaliatus* |
|---|---|---|---|
| **Both sexes** | | | |
| Cephalic part of prosoma in lateral view | Prominent (Fig. 4D) | Very prominent (Fig. 4E) | Slightly prominent (Fig. 4F) |
| Width of cephalic part of prosoma | Slightly wider than thoracic part | Remarkably wider than thoracic part | Almost as wide as thoracic part |
| Area among posterior median eyes and posterior lateral eyes | Almost flat (Fig. 4D) | Gibbous (Fig. 4E) | Almost flat (Fig. 4F) |
| **Males** | | | |
| Prosoma length (mm) | 2.6–4.2 (mean 3.6) | 3.5–5.6 (mean 4.6) | 2.9–4.1 (mean 3.6) |
| Black hairs on chelicerae | Relatively long | Relatively short | Relatively short |
| Hind legs | Proximally red, distally black (Fig. 4A) | Red (Fig. 4B) | Black (Fig. 4C) |
| Usual number of black spots on opisthosoma | 4 | 4 | 6 |
| White hairs on opisthosoma | Usually present | Usually present | Usually absent |
| Conductor in lateral view | Wrinkled, much longer than wide (Fig. 5A) | Wrinkled, slightly longer than wide (Fig. 5B) | Almost smooth, as long as wide (Fig. 5C) |
| Terminal tooth on conductor | Small, almost straight (Fig. 5A) | Strong, incurvated towards groove (Fig. 5B) | Strong, almost straight (Fig. 5C) |
| Groove on conductor | Small, V-shaped (Fig. 5A) | Large, round (Fig. 5B) | Large, U-shaped (Fig. 5C) |
| **Females** | | | |
| Prosoma length (mm) | 3.6–6.1 (mean 4.7) | 5.9–9.9 (mean 7.5) | 4.2–7.2 (mean 5.4) |
| Orange hairs on prosoma | Absent | Present | Absent |
| Anterior longitudinal bar in epigyne | Wide (Fig. 4G) | Inconsiderable (Fig. 4H) | Inconsiderable (Fig. 4I) |
| Epigynal fissures anteriorly incurvated | Remarkably sidewards (Fig. 4G) | Slightly centrally (Fig. 4H) | Sidewards (Fig. 4I) |
| Spermathecae reach to the side | As far as copulatory ducts (Fig. 4J) | Further than copulatory ducts (Fig. 4K) | Further than copulatory ducts (Fig. 4L) |
| Lobation of spermathecae | Distinct (Fig. 4J) | Very distinct (Fig. 4K) | Indistinct (Fig. 4L) |
| Anterior section of copulatory ducts | Elliptical, heavily sclerotised (Fig. 4J) | Elliptical, heavily sclerotised (Fig. 4K) | Circular, finely sclerotised (Fig. 4L) |

To investigate the shape of vulvae, they were dissected, cleared with glycerol and observed under a dissecting microscope.

### Biology of investigated species

Habitat preferences were based on surveys carried out in the Czech and Slovak Republics, and compared with data available from the revised material. We studied 46 localities of *E. kollari*, four localities of *E. moravicus* sp. n. and nine localities of *E. sandaliatus* between 1995 and 2005. The vegetation of the study localities was classified following Chytrý (2007).

We failed to find any interspecific differences in burrows and capturing webs. The burrows and capturing webs of all three species appeared to be identical to those described for *E. sandaliatus* by Nørgaard (1941).

The phenology of *Eresus* was quantified from our own observations at the study sites and by comparing sampling dates of the collected material.

### Molecular phylogenies

Molecular phylogenies were based on mitochondrial sequence analysis and allozyme allele frequency analysis. Mitochondrial analyses were based on 548 bp of the mitochondrial genome spanning the ribosomal RNA (16S)–tRNA(Leu) and NADH dehydrogenase subunit I (ND1) genes, amplified with the primers LR-N-12945 and N1-J-12261 (Hedin 1997). PCR products were sequenced using an ABI-377 automatic sequencer. Sequences were aligned with the Sequence Navigator program (ABI) (default settings). All mutations and/or gaps were confirmed by eye. Allozyme analysis was based on 24 loci analysed by Johannesen & Veith (2001). Mitochondrial DNA (mtDNA) amplification and allozyme running conditions were identical to those previously described by Johannesen & Veith (2001).

For allozyme analyses, we compared Czech populations of *E. kollari* (Český kras/Koda (Number of individuals ($N$) = 20), Prague/Podbaba ($N$ = 7) and Divoká Šárka ($N$ = 9)) and *E. sandaliautus* (Křivoklátsko/Velká Pleš ($N$ = 10)) to a geographical subset of previously published populations of these species (Johannesen & Veith 2001) (see Appendix A). The regions/species were previously found to be discrete; hence, we limited the number of populations per region or species to a maximum of four. For Danish *E. sandaliatus*, we included two populations with different mtDNA sublineages (Addit and Allinge). Allozyme phylogenetic trees were based on maximum-likelihood (ML) analysis implemented with the subroutine CONTML in PHYLIP (options: global rearrangements allowed, random addition of taxa, jumble = 100,

**Table 2** Species and sample locations from the Czech Republic and Slovakia. *Eresus kollari* and *E. sandaliatus* are syntopic (syn) at the Koda site.

| | | | Haplotype | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Species | Locality | N | E-a-1 | E-a-3 | E-a-4 | E-a-5 | E-a-6 | E-b-1 | E-d-2 | E-d-3 |
| *E. kollari* | Divoká Šárka | 5 | 4 | | | | | | | 1 |
| *E. kollari* | Koda (syn) | 8 | | 3 | 5 | | | | | |
| *E. kollari* | Podbaba* | 3 | 3 | | | | | | | |
| *E. kollari* | Milovická stráň | 1 | | | | 1 | | | | |
| *E. sandaliatus* | Velká Pleš | 6 | 5 | | | | | | 1 | |
| *E. sandaliatus* | Koda (syn) | 4 | | | | | 4 | | | |
| *E. moravicus* | Květnice | 2 | | | | | | 2 | | |
| *E. moravicus* | Bojnice* | 3 | | | | | | 3 | | |

*Data from Johannesen & Veith (2001).

random population as grouping taxon) (Felsenstein 1993). Significant branching patterns were estimated by 95% confidence intervals on branch lengths. Furthermore, we included *Eresus* from Italy for hierarchical outgroup comparison to understand divergence and specificity between the lineages examined in this study. These Italian spiders are genetically distinct for allozyme and mtDNA (Johannesen & Veith 2001) and probably represent a separate species; they will be examined in taxonomical detail later. The outgroup species *Eresus walckenaeri* (Johannesen & Veith 2001; Johannesen *et al.* 2005) was used as a root for ML analysis and for rooting the bootstrap consensus tree.

mtDNA was analysed from all three central European species from specimens analysed for allozyme polymorphisms, excluding *E. sandaliatus* from Sweden ($N = 3$), and Czech *E. kollari* (Pálava/Milovická stráň, $N = 1$), *E. sandaliatus* (Český kras/Koda, $N = 4$) and *E. moravicus* sp. n. (Tišnov/Květnice, $N = 2$), which were only available as alcohol samples (Table 2). The Koda populations of *E. kollari* and *E. sandaliautus* are syntopic. Phylogenetic analyses were performed with maximum-parsimony (MP), distance analysis with sequences joined by neighbor-joining (NJ) and ML using PAUP v4.08b for Macintosh (Swofford 1999). For MP, all characters were weighted equally and indels were treated as fifth bases. Haplotype relationships were analysed using a heuristic search with random addition of sequences. For NJ analysis, we applied the LogDet distance model and the distance model found by the Bayesian Information Criterion (BIC) in MODELTEST 3.8 (Posada & Crandall 1998). Model scores were estimated among ingroup haplotypes. BIC parameter estimates were used for ML analysis. MP bootstrapping was performed using the heuristic search option, while ML was, due to computational limitations, performed with the 'fast-stepwise' procedure. The resulting consensus tree did not differ from the ML heuristic search. Consensus trees for all tree algorithms were based on the 50% majority rule and 2000 bootstrap searches. As for allozyme analyses (see above), Italian *Eresus* sp. were included for hierarchical outgroup

comparison and *E. walckenaeri* was used for outgroup routing of the mtDNA phylogeny.

Gene tree analysis (TCS 1.3, Clement *et al.* 2000) was employed to resolve geographical samples and lineages involved in a possible introgression event including *E. sandaliatus* and eastern German and Czech *E. kollari* (Clade E-a, see Results). Because gene tree analysis allows haplotypes to be evolutionary intermediates, this analysis may resolve the introgression events in shallow phylogenies better than the phylogenetic tree analyses employed above.

### Abbreviations

*Synonymics.* is, insufficient; D, description; C, figure of whole body; B, bulbus; E, epigyne; V, vulva.

*Morphology.* Fe, femur; Pt, patella; Ti, tibia; Mt, metatarsus; PME, posterior median eyes; PLE, posterior lateral eyes.

*Material.* a, area; h, hill; l, lake; m, mountains; r, natural reserve; J, juvenile.

*Collections.* BMNH, British Museum of Natural History, London; BT, B. Thaler, Innsbruck; CRI, Výzkumný ústav rostlinné výroby (Crop Research Institute), Prague; CS, C. Szinetár, Szombathely, Hungary; HNHM, Magyar Természettudományi Múzeum, Budapest; HUJ, The Hebrew University, Jerusalem; JL, J. C. Ledoux, Solignac sur Loire, France; JP, J. Prouza, Hradec Králové, Czech Republic; JS, J. Svatoň, Martin, Slovakia; MBCG, Museo Civico di Scienze Naturali 'E. Caffi', Bergamo, Italy; MHNG, Muséum d'Histoire naturelle, Genève; MNHN, Muséum national d'Histoire naturelle, Paris; MR, M. Řezáč, Praha; NHRS, Naturhistoriska Riksmuseet, Stockholm; NMBE, Naturhistorisches Museum der Burgergemeinde Bern; NMPC, Národní muzeum, Praha; NMW, Naturhistorisches Museum, Wien; PG, P. Gajdoš, Nitra, Slovakia; PU, The Zoology Department of the Perm University, Russia; RMNH, Nationaal Natuurhistorisch Museum, Leiden, the Netherlands; SK, S. Korenko, Banská

**Fig. 1** A–C. Drawings of *Eresus kollari* copulatory organs showing the morphological characters. —A. conductor. —B. epigyne. —C. vulva. Abbreviations: b, bar; cd, copulatory duct; e, embolus; f, fissure; fd, fertilisation duct; g, groove; l, lamella; s, spermatheca; t, terminal tooth.
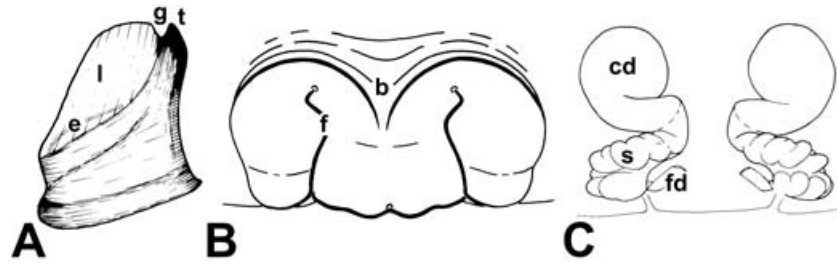
Bystrica, Slovakia; SMF, Naturmuseum Senckenberg, Frankfurt am Main; SZMN, Siberian Zoological Museum, Novosibirsk; UL, Univerza v Ljubljani, Slovenia; VMH, Vihorlatské múzeum, Humenné, Slovakia; ZMHB, Museum für Naturkunde, Humbold Universität, Berlin; ZMUM, Zoological Museum of the Moscow University.

## Results

### Genus Eresus *Walckenaer, 1805*

*Colour pattern.* Colouration in *Eresus* is based on the colour of the cuticle and the colour of the plumose setae. The cuticle is lighter (rusty or orange) in places with red setae, than in places with only black setae. The studied *Eresus* species were especially different in the pattern of red hairs on the legs of males, and the orange hairs on chelicerae and the cephalic part of the prosoma of females.

*Body shape and size.* Body size and relative height and width of the cephalic part of the prosoma were found to be important characters. The shape of the prosoma slightly correlates with the body size (larger specimens have a relatively more pronounced cephalic part); the cephalic part is more pronounced in males.

*Copulatory organs.* Due to a high degree of shape uniformity, copulatory organs are not as useful for discrimination between *Eresus* species as they are for other spider groups. In males, only the shape of the conductor is species specific. It is composed of a sclerotized terminal tooth that protects the tip of the embolus. The tooth is separated from the less sclerotized part of the conductor (lamella) by a groove in the central European species (Fig. 1A).

The epigyne is composed of a simple pit with two longitudinal fissures (Figs 1B and 2). The frontal part of the fissures, where the openings of the copulatory ducts are located, is curved either laterally or medially. The frontal part of the vulva is composed of a loop of massive copulatory ducts (Fig. 1C). The degree of sclerotization is partly species specific. The distal part of the vulva is mainly composed of spermathecae. These are lobed, and eversed to the sides. Fertilization ducts are tiny, directed promediodorsally and situated at the base of spermathecae. There is considerable variability in the shape of the vulvae within species (Fig. 3), but the degree of spermatheca lobation and general proportions of the vulvae are species specific. Unfertilized females experience post-adult moulting (Gerhardt 1928). This causes changes in the shape of both the epigyne and the vulva (Kraus & Kraus 1988). Epigynes of post-adult females are usually larger, with a sharp longitudinal bar and with wide fissures (e.g. Nenilin & Pestova 1986: fig. 3; Fig. 2Q). Vulvae are characterized by narrower loops of the copulatory ducts. The pre-epigynum, the structure present in *Eresus* subadult females, can be confused with the epigyne (e.g. Locket & Millidge 1951; Roberts 1985; Feng 1990). Pre-epigynes are smaller, less sclerotized and only slightly wider than the area bordered by fissures (Fig. 2R); both copulatory ducts and spermathecae are small and simple (Fig. 3R).

*Eresus kollari* Rossi 1846 (figs 1A–C, 2A–J, 2R, 3A–J, 3R, 4ADGJ, 5AD, 6).
*Eresus kollari* Rossi 1846: D♀ (17–18).
**Misidentifications**
*Eresus cinnaberinus* Walckenaer 1806: D♂ (43–44), C♂ (fig. 10), Paris: Boulogne; Hahn 1821: C♂ (pl. 5, fig. A), Spain, Portugal, France, Italy, Hungary; Walckenaer 1830: D♂♀ (38–39), C♂ (fig. 7), France?; Walckenaer 1837: C♂ (pl. 11, fig. 7D, after Walckenaer 1806); Simon 1873: D♂ (341, 343–344), France; Thorell 1873: D♂ (421), Austria; Herman 1879 (variety α): D♂ (292), Slovakia, Romania; Chyzer & Kulczyński 1891: D♂ (152–153), D♀ (152–153, only 'typical form'), E (pl. 6, fig. 22a), Hungary: Tokaj, Szerencs, Almádi, Zeng; Becker 1896: D♂♀ (2–3), C♂ (pl. 1, fig. 1a), is.C♀ (pl. 1, fig. 1), B (pl. 1, fig. 1d), is.E (pl. 1, fig. 1b), Belgium?; Leist 1994: C♂♀ (p. 306), Germany: Heidelberg; Ratschker & Bellmann 1994: D♂♀ (217), V (fig. 2), Germany?; Ratschker & Bellmann 1995: D♂♀ (807–809), V (fig. 4, after Ratschker & Bellmann 1994); Roberts 1998: D♂♀ (80), B (80), V (80); Walter 1999: C♂ (fig. 1), Switzerland; Bellmann 2001: D♂♀ (38), C♂ (19, 39), C♀ (39, except for the middle two females), V (38, after Ratschker & Bellmann 1994), Germany?; Johannesen & Veith 2001: specimens West (western Germany) and East (eastern Germany, Czech Republic); Song *et al.* 2001: V (fig. 35A, after Ratschker & Bellmann 1994).
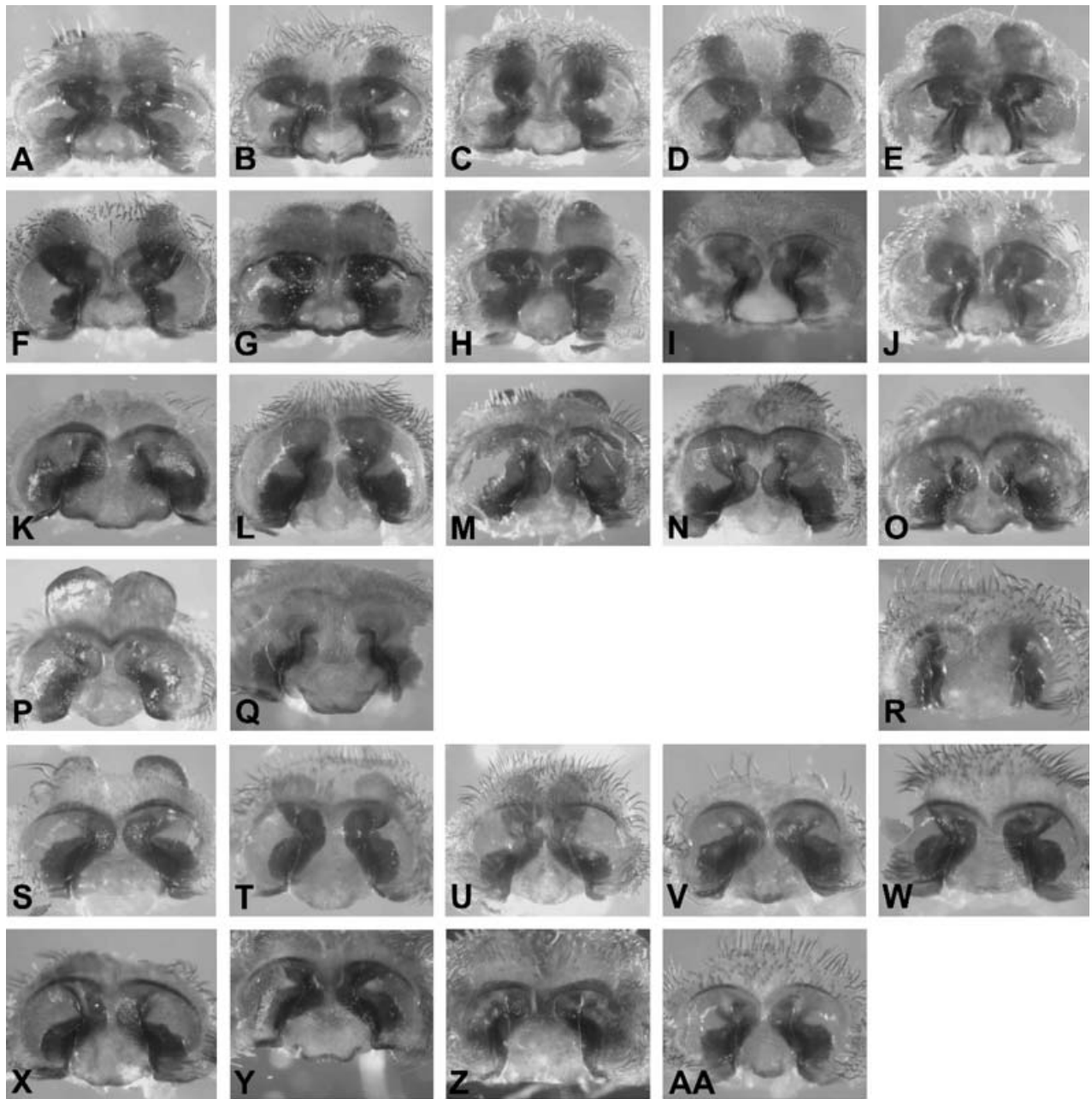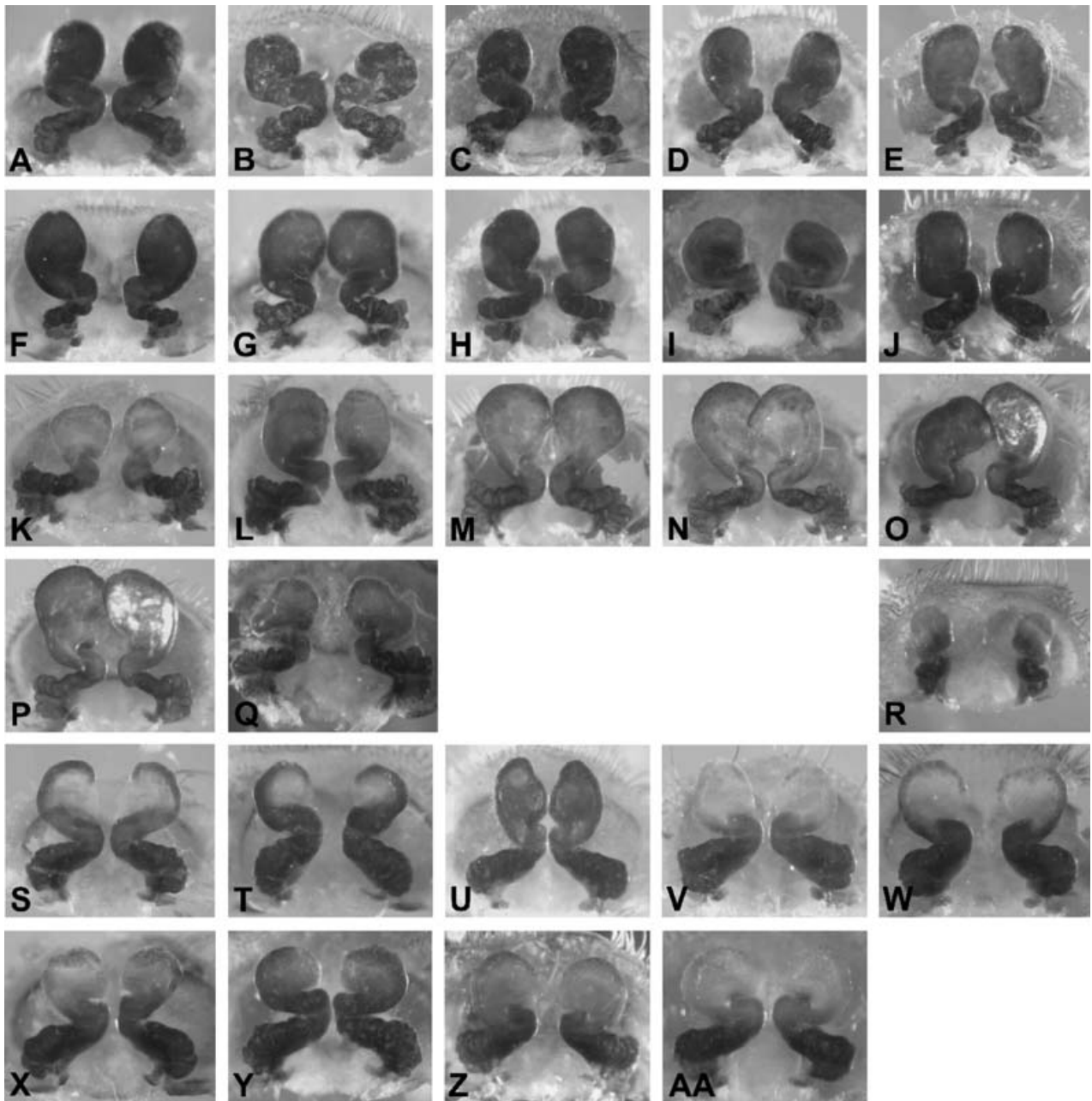
**Fig. 2** A–AA. Variation of epigynes, ventral view. —A to J, R. *Eresus kollari*. —A. Greece: Dirrachi. —B. Czech Rep.: Dolany. —C. Czech Rep.: Havraníky. —D. Czech Rep.: Srbsko. —E. Czech Rep.: Lovosice. —F. Czech Rep.: Milovice. —G. Czech Rep.: Chraberce. —H. Czech Rep.: Ctiněves. —I. Austria: Baden (syntype). —J. Czech Rep.: Vrané nad Vltavou. —R. Czech Rep.: Praha-Liboc (pre-epigynum). —K to Q. *Eresus moravicus* sp. n. —K. Czech Rep.: Tišnov. —L. Slovakia: Plášť'ovce. —M to P. Slovakia: Banská Bystrica. —Q. Slovakia: Nitra. —S to AA. *Eresus sandaliatus.* —S. Czech Rep.: Roztoky u Křivoklátu. —T. Czech Rep.: Beroun. —U. Denmark: Enebærbakken. —V. Czech Rep.: Žloukovice. —W. Denmark: Mossö. —X. Czech Rep.: Nezabudice. —Y. Czech Rep.: Nová Rabyně. —Z. Czech Rep.: Karlova Ves. —AA. Czech Rep.: Úhošť'any.

*Eresus quatuorguttatus* Hahn 1832: D♂ (45–47), C♂ (fig. 35), Hungary.

*Eresus 4-guttatus* Koch 1838: D♂ (104–105), C♂ (fig. 316), Italy.

*Erythrophorus quadriguttatus* Koch 1874: D♂ (10–11), Germany.

*Eresus niger* Simon 1892: B (fig. 204); Bösenberg 1903: D♂♀ (411), C♂ (pl. 39, fig. 606D), is.C♀ (pl. 39, fig. 606A), B

**Fig. 3** A–AA. Variation of vulvae, dorsal view. —A to J, R. *Eresus kollari.* —A. Greece: Dirrachi. —B. Czech Rep.: Dolany. —C. Czech Rep.: Havraníky. —D. Czech Rep.: Srbsko. —E. Czech Rep.: Lovosice. —F. Czech Rep.: Milovice. —G. Czech Rep.: Chraberce. —H. Czech Rep.: Ctiněves. —I. Austria: Baden (syntype). —J. Czech Rep.: Vrané nad Vltavou. —R. Czech Rep.: Praha-Liboc (pre-vulva). —K to Q. *Eresus moravicus* sp. n. —K. Czech Rep.: Tišnov. —L. Slovakia: Plášť'ovce. —M to P. Slovakia: Banská Bystrica. —Q. Slovakia: Nitra. —S to AA. *Eresus sandaliatus.* —S. Czech Rep.: Roztoky u Křivoklátu. —T. Czech Rep.: Beroun. —U. Denmark: Enebærbakken. —V. Czech Rep.: Žloukovice. —W. Denmark: Mossö. —X. Czech Rep.: Nezabudice. —Y. Czech Rep.: Nová Rabyně. —Z. Czech Rep.: Karlova Ves. —AA. Czech Rep.: Úhošť'any.

(pl. 39, fig. 606C), is.E (pl. 39, fig. 606B, subadult), Germany; Kulczyński 1903: D♂ (637), B (pl. 1, figs 1–2), Hungary?; Lessert 1910: D♂ (33), D♀ (32–33, partly), Switzerland; Simon 1914 (typical form): D♀ (69), France; Roewer 1928: is.D♂♀ (127), B (443), probably after Chyzer & Kulczyński 1891; Reimoser 1931: D♂♀ (59, more species), is.C♂ (pl. 2, fig. 5), B (pl. 2, fig. 6), E (pl. 2, fig. 7), Germany?; Locket & Millidge 1951: D♂ (50), B (fig. 19A), is. E (fig. 19B, subadult);

Wiehle 1953: D♂♀ (71–72), is.C♂ (fig. 156), B (fig. 159), E (fig. 160), V (fig. 161), Germany; Loksa 1969: D♂♀ (19–20), is.C♂ (fig. 12), B (fig. 13A), E (fig. 13D), Hungary; Miller 1971: D♂♀ (54–55), E (pl. 2, fig. 9), Czech Republic; Heimer & Nentwig 1991: D♂♀ (52), B (fig. 112.1, after Locket & Millidge 1951), E (fig. 112.3, after Miller 1971), V (fig. 112.4, after Wiehle 1953); Haupt & Haupt 1993: D♂♀ (55), C♂ (p. 55); Ratschker 1992: E (fig. 58, 60), V (fig. 59, 61), Switzerland: Zeneggen, Germany: Lower Saxony: Unterlüß.

The following references formerly attributed to *E. cinnaberinus* in a broad sense (Platnick 2007) could possibly refer to *E. kollari*, however, they do not allow for undoubted identification.

*Eresus cinnaberinus* Walckenaer 1805: is.D♂ (21), is.B (pl. 3, fig. 25), France?; Walckenaer 1837: D♂ (395–396, several species including *E. kollari* and *E. sandaliatus*); Koch 1838: is.D♂ (106–108), is.C♂ (fig. 318), S France; Ratschker & Bellmann 1994: is.B (fig. 1, after Ratschker 1992: fig. 131); Ratschker & Bellmann 1995: is.B (fig. 3, after Ratschker & Bellmann 1994); Bellmann 2001: C♀ (39, third from the top); Song *et al.* 2001: D (79–80), is.C♂ (fig. 35B), C♂ (fig. B), B (fig. 35C, after Song 1987; fig. 35D, after Ratschker & Bellmann 1994).

*Eresus niger* Simon 1895: D♂ (331), Russia: Kandagatai, Altai; Planet 1905: is.D♂ (51–53), is.C♂ (51), France; Reimoser 1931: D♂♀ (59, more species), is.C♂ (pl. 2, fig. 5), B (pl. 2, fig. 6), E (pl. 2, fig. 7), Germany?; Nakatsudi 1942: D♂ (8), is.C♂ (fig. 1A), is.B (fig. 1B), is.E (fig. 1C), China; Namkung 1964: is.D♂ (33), is.B (fig. 5B–C), is.E (fig. 5A), Korea; Tyschenko 1971: is.D♂♀ (58), former USSR; Hu 1984: D♂ (51–52), is.B (pl. 47, figs 3–4), China; Feng 1990: C♂ (fig. 6), is.B (pl. 6, figs 3–4), is.E (pl. 6, fig. 2, subadult), China; Ratschker 1992: B (figs 131–132), NE Spain: Porte Vendres.

### Nomina dubia

*Aranea nigra* Petagna 1787: is.D♂ (34), preoccupied by Fabricius (1775); replaced by Walckenaer (1805); Petagna 1792: is.D♂ (437).

*Aranea cinnaberina* Olivier 1789: is.D♂ (221).

*Aranea moniligera* Villers 1789: is.D♂ (128), is.C♂ (pl. 11, fig. 8).

*Aranea quatuorguttata* Rossi 1790: is.D♂ (135), is.C♂ (pl. 1, figs 8–9).

*Eresus ater* Walckenaer 1805: D♂ (21, after Petagna 1787).

*Chersis dubius* Walckenaer 1837: D♂ (392, after Petagna 1787).

*Eresus illustris* Koch 1838: D♂ (105–106), C♂ (pl. 88, fig. 317).

*Type specimens of the species mentioned in synonymics. Eresus cinnaberinus:* Italy: Florence (♂ holotype, 18th century, M. Towson, repository unknown, not examined). *Eresus illustris:*

unknown location, the author assumed they came from Hungary (4♂ syntypes, 19th century, repository unknown, not examined). *Eresus kollari:* Austria: Baden near Vienna (1♀ syntype August 1841, K. Kollar, NMW, 1896.VIII.475, Koelbel's estate, examined; 1♀ syntype July 1842, F.W. Rossi, repository unknown, not examined). *Eresus moniligerus:* France: Lyon (♂ holotype, 18th century, repository unknown, not examined). *Eresus niger, E. ater, E. dubius:* Italy: Regio di Calabria: Aspromonte (♂ holotype, 18th century, repository unknown, not examined). *Eresus quatuorguttatus:* Italy: Florence (♂ holotype, 18th century, repository unknown, not examined).

*Localities and material examined.* ALBANIA: Tomori (2♂ 14.VIII. 1935, A.H.G. Alston & N.Y. Landwith, BMNH).

AUSTRIA: (1♀, MNHN). *Niederösterreich:* Vienna (4♂, T. Thorell, NHRS; 5♂, NMW); Mödling (2♂ 21.VIII. 1960, G.E.J. Nixon, BMNH); Mödling, Kalenderberg (1♂ 17.IX.–15.X. 1977, NMW); Brühl near Mödling, Rogenhofer (1♀2 J 1862, NMW); Vienna, Leopoldsberg, Wiener Wald (2♂ 28.VIII.–24.IX. 1977, NMW); Hainburg (1♂, NMW); Ober-Weiden, Marchfeld (2♂, H. Franz, NMW); Baden (1♂ 1890, F. von Feiller, NMW). *Burgenland:* Parndorfer Platte (1♂ 1988, K. Thaler, NMW); Weiden, Zitzmannsdorfer Wiesen (1♀, F. Kasy, NMW);

BULGARIA: (1♂ 1964, R. Braun, SMF).

CROATIA: Dalmatia, Zara vecchia, Vrana (1♀ 1891, R. Sturany, NMW).

CZECH REPUBLIC: Numerous materials from this country will be listed elsewhere.

FRANCE: Paris (1♂, T. Thorell, NHRS, *E. cinnaberinus* — det. E. Simon); (1♀, HUJ); Ardèche, Sainte Eulalie (1♂ 12.IX. 2001, J. Johannesen, MR); Cévennes (1♂ VIII. 1965, J.C. Ledoux, JL); Essonne, Puiselet le Marais (8♂ 28.VIII. 2005, J.C. Ledoux, JL); Haute Loire, Solignac sur Loire (2♀ 20.–21.VIII. 2004, J.C. Ledoux, JL); Chansi (1♂ 11.X. 1914, CNMP); Seine et Marne, Fontainebleau (2♂ 22.IX. 1910, Benoist, MNHN, *E. niger* — det. E. Simon); Cascade de la Beaume (2♂ 5.IX.19?35, MNHN). *Hautes Alpes:* Le Prozel near Briançon (2♂ IX. 1952, Y. Bertrand, NMPC); Aspres lès Corps (2♂ VIII.58, J. Jarrige, MR); Serre, Lomb (Serralongue in Pyrénées Orientales?) (30♂2♀4 J, E. Simon, MNHN).

GERMANY: (1♂ 24.IX. 1931, H. Wiehle, SMF); Stromberg (1♂, summer 1901, ZMHB); Pfynwald (2♂ 19.VII. 1979, 1♂ 22.IX. 2000, Kiels, NMBE); Nahe, Rotenfels (1J, J. Johannesen, MR). *Baden-Wuerttemberg:* Kaiserstuhl m. near Freiburg im Breisgau (1♂ 22.X. 1966, O. Kraus; 1♂ X. 1968, Buch; 1♂ 1984, J. Wunderlich; SMF); Waldhof near Mannheim (1♂ 30.IX. 1925, G. Ochs, SMF); Ochsenburg near Karlsruhe (1♂ VII. 1965, H. Hiebsch, SMF). *Bavaria:* Nürnberg (1♂1♀ 1882, L. Koch, NMW). *Berlin:* Berlin, Pfaueninsel (1♂ 1968, J. Wunderlich, SMF). *Brandenburg:*

Summt near Berlin (3♂ 18.IX.1937, M. Ehlers, ZMHB); Beelitz near Postdam (12♂ 7.IX.1913; K. Heijn, ZMHB); Schönow near Bernau (1♂ 30.IX.1926, Walther; 1♀ 23.X.1937, M. Ehlers; ZMHB); Rathenow near Brandenburg, Brunnerstädt (1♂ 18.IX.1921, ZMHB); Gasta near Berlin (1♂ 26.V.1991, Echelt, ZMHB). *Hesse:* Frankfurt am Main (2♂ 2.I.1905, 2♂, L. von Heyden, SMF); Kelsterbach am Main near Frankfurt am Main (2♂ 24.IX.1949, H. Hesse, SMF); Florsheim am Main (1♂5♀ 20.V.1951, H. Hesse, SMF); Florsheim am Main, Falkenberg (1♂, W. Hororst, SMF); Jugenheim (1♂ 1910, 2♂ 1913, K. Dietz (Dieke?) (SMF). *Rhineland-Palatinate:* ruin Hammerstein am Rhein near Neuwied (1♀ 25.II.1905, P. Bertkau, SMF); Mombach near Mainz (1♂, L. von Heyden, SMF); Lorch am Rhein (1♂ 7.IX.1951, R. Langer, SMF); Ochtendung near Koblenz (3♂, J. Johannesen, MR). *Saxony-Anhalt:* Dessau (1♂ IX. 1926, Hoppe; 1♀ 21.IX.1930, H. Wiehle; SMF); Hettstedt near Magdeburg (1♂ 2.IX.1942, 1♀ 1951, H. Wiehle, SMF). *Thuringia:* Kyffhäuser near Leipzig, near Frankenhauser Quelle (1♀1J 11.VII.1938, ZMHB); Kyffhäuser near Leipzig, near Bad Frankenhausen (2♂ 10.XI.1964, M. Moritz, ZMHB); Kyffhäuser near Leipzig, Kesabenberg (1♂ 21.IX.1964, M. Moritz, ZMHB).

GREECE: Dirrachi (1♀ V.2003, A. Kiefer, MR).

HUNGARY: Nyíregyháza (1♂ 2.X.1898, L. Hollós, HNHM); Vertes, Czákberény (1♂ 20.IX.1991, V.V. Dubatolov, SZMN), Gödöllö (1♀1880, Steindachner, NMW); Kiskunsý sands (1♂ 23.X.2001, K. Kunbaracs, CS); Sas hegy h., Budapest (1♂ 16.IX.1994, CS); (2♂1 J, Steindachner, NMW).

ITALY: Triest (3♂, Krauss, NMW); (1♂, ZMHB). *Abruzzo:* Grupo del Velino, Piano di Pezza (1♂ IX.1924, Alzona, MBCG); Sopra Castel del Monte, national park Gran Sasso (2J 23.VII.2004, P. Pantini, MBCG).

KAZAKHSTAN: Kustanai a., Naurzum r. (1♂ 15.IX.1989, T.M. Brugina, ZMUM); Uralsk a., Dzhanybek (1♀ 12.VI.1982, K.G. Mikhailov, ZMUM).

KYRGYZSTAN: western Tian-Shan m., Sary-Chelek r., Iri-Kel l. (1♂ 5.VIII.1983, 1♂ 18.IX. 1983, A.B. Ravkin, ZMUM); western Tian-Shan m., Fergansky m., Yarodar (1♂ 26.IX.1983, K.Y. Eskov, ZMUM); Kurskoe, north shore of Issyk-Kul l. (1♂1J 10.VIII. 1980, S.L. Zonstein, ZMUM); Issyk-Kul a., Kungei Alatau m., Chon-Uryukty boundary (1♀ 23.VI.1983, S.V. Ovchiunikov, ZMUM); Baubashata m., Maek boundary (1♂ 22.X.1980, S.L. Zonstein, ZMUM).

MACEDONIA: Doiran-Sunfer (1♂1♀ 1935, C.F. Roewer, SMF).

MONTENEGRO: (2♀, BMNH).

ROMANIA: Tâmpa near Braşov (1♂ 9.X.1904, E.J. Lehmann, ZMHB);

RUSSIA: Altai m., Volchikha a. (1♂ 9.VII.1999, D.V. Logunov, SZMN); Tassor, south Ural (1♂ 11.–12.IX.1999, D.V. Logunov, PU); Ilmenskii r., south Ural (1♂ 25.VII.1999, D.V. Logunov, PU); Novosibirsk a., 27 km ESE of Zdvinsk, Chany l., Kargat river mouth (5♂ 10.IX.1989, V.P. Pekin, SZMN); Novosibirsk a., 13 km W of Karasuk, l. Troitskoe (1♂ 7.IX.1989, V.P. Pekin, SZMN); Bashkortostan, Bashart (3♂, I.V. Stelaer, SZMN); Southern Russia, Valuiki (1♂, Velitschkovski, NMW).

SLOVAKIA: *Hronská pahorkatina:* Štúrovo (4♂ 1965, J. Buchar, NMPC). *Malé Karpaty m.* Bratislava, r. Devínska Kobyla (1♂ 5.VII.–26.IX.1979, 1♀ 24.V.1980, P. Gajdoš, PG; 1♀ 10.VI., F. Miller, NMPC); Pezinok near Chrastina (1♂ 17.VII.–15.XII.1994, P. Gajdoš, PG); Stupava, Vrchná hora h. (1♂ 19.IX.–17.X.1999, 1J 17.VII.–18.VIII.1999, 4♂ 18.VIII.–19.IX.1999, O. Mazjan, PG). *Považské podolie:* Trenčianske Bohuslavice, r. Turecký vrch (3♂ 22.X.1978, J. Svatoň, JS). *Považský Inovec m.* Lúka, r. Tematínske vrchy (1J 1978, V. Thomka, VMH). *Slovenský kras a.–Plešivecká planina plateau:* Gočaltovo, Železné vráta rocks (4♂ 13.IX. 1983, J. Svatoň, JS); Rožňavské Bystré, r. Gerlachovské skaly (1♂ 14.IX.1983, J. Svatoň, JS); Kunova Teplica, Vel'ký vrch h. (1♂ 5.IX.1984, 6♂ 16.X.1984, J. Svatoň, JS). *Slovenský kras a. — Silická planina plateau:* Jablonov nad Turňou, Hradište h. (4♂ 6.IX.1984, 9♂ 16.X.1984, J. Svatoň, JS); Hrušov nad Turňou, r. Hrušovská lesostep (1J 28.VI.1984, 1♂ 23.VIII.1984, 2♂ 16.X.1984, J. Svatoň, JS). *Tríbeč m.* Nitrianska Streda, r. Hrdovická (1♂ 31.VII.–9.X.1986, 2♂ 9.X.–10.XII. 1986, P. Gajdoš, PG); Nitra, r. Zobor (1♂ 14.X.1992, P.J. van Helsdingen, RMNH). *Štiavnické vrchy m.* Počúvadlo, r. Holík (1♂ 16.IX.1985, LPG, PG).

SLOVENIA: Korytnice (1♂ 21.IX.1988, 1♂ 26.IX.1988, S. Polak, UL; 1♀ 23.V.1995, S. Polak, MR); Podtabor (1♂ autumn 1995, S. Polak, MR; 1♀ 17.IX.1995, M. Řezáč, MR); Bepše near Logatec (1♂ IX. 1934, Dolar, NMPC).

SPAIN: Sierra Alta, Teruel (1♂ 15.IX.1953, K. Schnellb, SMF).

SWITZERLAND: (1♂ X.19?22, MNHN). *Wallis:* (2♂ 26.VIII.1922, Stalden, BMNH); (3♂, NMBE); Stalden (1♂, NMBE); Fiesch (23♂, NMBE); Salvan, Valais, Pente de Chippis à Niouc, Sierre (2♂ 25.VII., M. Weber, MHNG); Salvan (13♂1J, R. de Lessert, MHNG); Valère s/Sion, Valais (1♂ X. 1955, A. Comellini or Valése, MHNG), Leuk (1♂, NMBE); Savièse (2♂2J, R. de Lessert or Jullien, MHNG); Lens (1♂ 25.VII.1964, J.C. Regnier, MHNG); Leytron (5♂, NMBE).

*Diagnosis.* Males of this species are distinguished from *E. moravicus* sp. n. and *E. sandaliatus* by proximally red and distally black hind legs (Fig. 4A, Table 1) and a conductor with a small terminal tooth and small groove (Fig. 5A). Females are recognized by an epigyne in which the proximal part is divided by a wide longitudinal bar (see an arrow in the Fig. 4G) and by a vulva with spermathecae reaching only as far to the sides as the copulatory ducts (Fig. 4J).
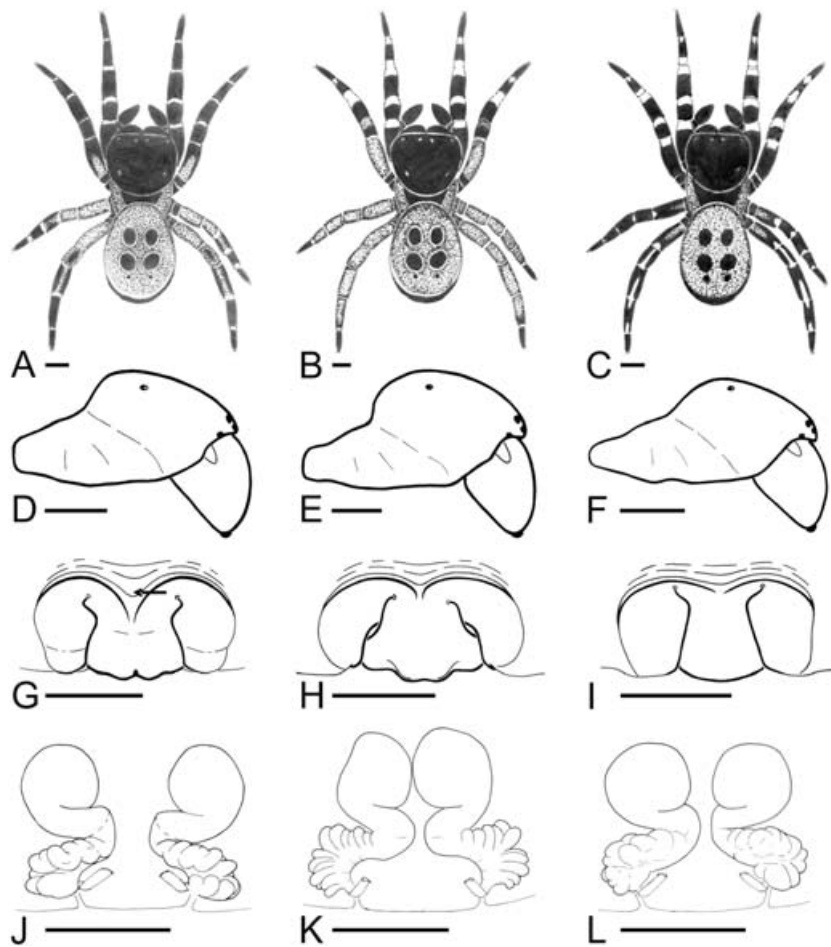
**Fig. 4** A–L. Characters of three *Eresus* species. —A, D, G, J. *Eresus kollari*. —B, E, H, K. *Eresus moravicus* sp. n. —C, F, I, L. *Eresus sandaliatus*. —A to C. Colour pattern of males, black–black colouration, white–white colouration, dotted-red colouration. —D to F. Male prosoma, lateral view. —G to I. Epigyne, ventral view. —J to L. Vulva, dorsal view. According to specimens from the Czech Republic (paratypes in case of *E. moravicus* sp. n.). — Scale bars 1 mm (A–F) or 0.5 mm (G–L).

### Description

*Male. Prosoma* (Fig. 4AD): 2.6–4.2 (mean 3.6) mm long. The cephalic part is black with scattered white hairs, distinctly prominent, slightly wider than the thoracic part, slightly extending to the front, and the area between the PME and PLE is almost flat. The thoracic part is black-brown and bordered with a band of red hairs. *Chelicerae:* Black with long black hairs. *Legs* (Fig. 4A): Legs I and II are black-brown with mainly black hairs, the dorsal sides of Fe II have a few red hairs, and distal extremities of Fe, Pt, Ti and Mt have rings of white hairs. Legs III and IV are ferruginous-brown, with the proximal podomeres (segments) (Fe, Pt, dorsal side of Ti) having red hairs, and the distal segments having black hairs. The distal extremities of Ti and Mt had a few white hairs on the dorsal side. *Opisthosoma* (Fig. 4A): Dorsally red with scattered white hairs and with two, rarely three, pairs of black spots of variable size. A line of white hairs usually borders the red area and the black spots, especially posteriorly. The ventral side of the opisthosoma is black, and only

the branchial opercula have red hairs. *Palp:* Conductor wrinkled, much longer than wide in the lateral view (Fig. 5A); terminal tooth small, equal or lower than lamella, straight; groove small, V-shaped. The basal part of the conductor is relatively narrow in the ventral view (Fig. 5D).

*Female. Prosoma:* 3.6–6.1 (mean 4.7) mm long, of the same shape as in males. The cephalic part is brown-black, with long black hairs and scattered short white hairs; the median eyes also have pale rusty hairs around. *Chelicerae:* Brown-black with long black hairs and scattered short pale rusty hairs on the basal half. *Legs:* Brown-black, with dorsal side of Fe, Pt, Ti and Mt of legs I–III having scattered and, distally, clustered short pale rusty hairs. *Opisthosoma:* Violet-black with long black hairs and, anteriorly, with scattered short white hairs. *Epigyne* (Figs 2A–J and 4G): The anterior part is divided by a wide longitudinal bar, and the anterior parts of fissures are remarkably incurvated sidewards. *Vulva* (Figs 3A–J and 4J): Spermathecae are composed of distinct lobes reaching
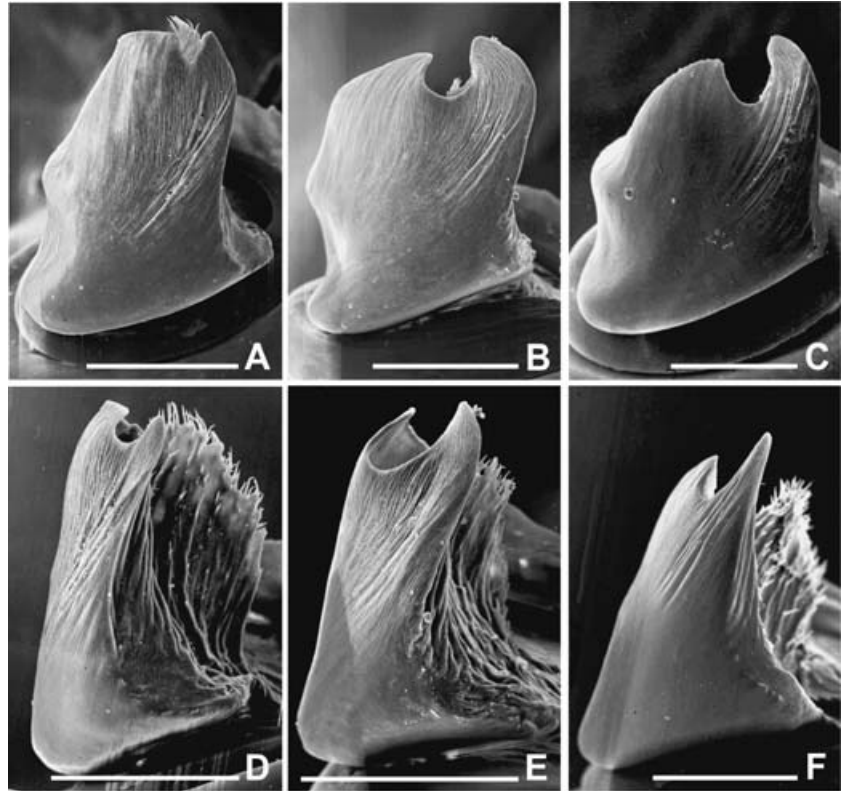
**Fig. 5** A–F. Conductors of three *Eresus* species. —A, D. *Eresus kollari*. —B, E. *Eresus moravicus* sp. n. —C, F. *Eresus sandaliatus*. —A to C. Lateral view. —D to F. Ventral view. Specimens from the Czech Republic. Scale bars 0.1 mm.

approximately as far to the side as the copulatory ducts. The copulatory ducts constitute heavily sclerotized elliptical organs in the anterior part of vulva.

*Remark*

This is probably an aggregate of at least two cryptic taxa that can be distinguished only at the molecular level. The first *E. kollari* taxon is known to be from western Germany, Switzerland and France. The second taxon is likely of hybrid origin, evolving from the first *E. kollari* taxon and *E. sandaliatus*, and was detected in eastern Germany and the Czech Republic. The genetic identity of the other populations is unknown.

The phenotype of this aggregate was recently redescribed as *E. cinnaberinus* (Ratschker & Bellmann 1995). However, the only conductor illustrated in Ratschker & Bellmann (1995: fig. 3, drawn after the SEM photo in Ratschker 1992: fig. 131) has an unusually large terminal tooth and groove, and may represent a different species. Roberts (1998) provided exact drawings of the copulatory organs of this species, which are also under the name *E. cinnaberinus*. The type material of *E. cinnaberinus*, described from Florence, probably does not exist any more. Several *Eresus* species may occur in central Italy, all of which fit the brief original description. In addition, the type material of other early *Eresus* nominal species,

namely *E. moniligerus*, *E. quatuorguttatus*, *E. ater* and *E. illustris*, probably no longer exists. These species were described from regions where several *Eresus* species may occur. For *E. illustris*, the type locality is unknown. Therefore, the correct usage of these names will only be solved if representative samples from the specific type localities become available in the future. Presently, they should be considered *nomina dubia*. The oldest name undoubtedly related to the studied species is *Eresus kollari* Rossi 1846. Only two species occur in the region of its type locality (Baden near Vienna), the analysed one and the *E. moravicus* sp. n.; the description unambiguously refers to the analysed species. Moreover, we probably identified its original type specimen.

In the southern part of the distribution area of *E. kollari*, there are some aberrant populations of vague taxonomic value. Specimens from Slovenia, Croatia and Italy are markedly larger, the male rear legs have red hairs on only the dorsal side of Fe III and IV (males with a similar colour pattern were described as *E. illustris* C. L. Koch 1838), the conductor is relatively larger and the terminal tooth stronger (as in Kulczynski 1903; pl. 1, figs 1–2). Specimens from Kyrghyzstan are also clearly larger, with conductors of a similar shape. Moreover, females possess spermathecae reaching further to the side. Males from the Sierra Alta in Spain and Abruzzo in Italy have a markedly wide conductor base.

### Phenology

*Eresus kollari* matures in July, copulates in August–October and lays eggs in May (populations in the Czech Republic).

### Habitat

This species occurs mainly in rocky steppes (*Festucion valesiacae, F. pallentis*) in the Czech Republic. Less often, it occurs at the edges of heathlands (*Euphorbio-Callunion, Genistion*) and xerophilic acidophilous grasslands (*Koelerio-Phleion phleoidis*). In the southern part of its distribution area, it also occurs in alpine grasslands at 2000 m a.s.l. (French Alps, Kyrgyz Plateau), pine forests on sand (Novosibirsk area in Russia), saline steppes (south Ural) and birch woods (north Kazakhstan).

### Distribution

The distribution area of *E. kollari* is unusually large compared to the distributions of other *Eresus* species, ranging from the Iberian Peninsula to Novosibirsk in central Russia. *Eresus kollari* has expanded relatively far to the North (only *E. sandaliatus* has expanded further), but is probably missing from the hottest and driest parts of the Palaearctic region.

*Eresus moravicus* sp. n. Řezáč (figs 2K–Q, 3K–Q, 4BEHK, 5BE, 6).
*Eresus* sp. Ratschker 1995: D♂♀ (723–725), B (fig. 2), V (fig. 1), Austria: Lavamünd.

**Misidentifications**

*Eresus ruficapillus* Herman 1879: D♀ (293), Hungary.
*Eresus cinnaberinus* Herman 1879 (variety γ): D♂ (292), Hungary; Sauer & Wunderlich 1997: D♂♀ (34), C♂ (35), C♀ (35); Bellmann 2001: C♀ (39, second figure from the top on the left); Johannesen & Veith 2001: specimens from Bojnice in Slovakia; Thaler & Knoflach 2002 (*E. cinnaberinus* s.l.): D♂ (425), C♂ (fig. 41). C♀ (figs 43–44), B (figs 19–20), Austria: Fließ, Dürnstein.
*Eresus niger* Chyzer & Kulczyński 1891: D♀ form '*frontalis*' (152–153), B (pl. 6, fig. 22b), Hungary: S. a. Ujhely, Budapest; Rumania: Gherla; Croatia: Rijeka, Bakar, Crikvenica, Velebit; Roewer 1928: is.D♂♀ (127), B (443, probably after Chyzer & Kulczyński 1891); Ratschker 1992: B (figs 133–134), Austria: Lavamünd.
*Eresus niger* variety *frontalis* Lessert 1910: D♀ (32), Switzerland.
*Eresus niger* variety *ruficapillus* Loksa 1969: D♀ (20), Hungary. The following references formerly attributed to *E. cinnaberinus* in a broad sense (Platnick 2007) could possibly refer to *E. moravicus*, however, they do not allow for undoubted identification.
*Aranea cinnaberina* Walckenaer 1802: D♂ (249, diagnostic characters red rear legs and June sexual activity are mentioned), Paris: Menil–Montant (currently the urban part of the city).

*Eresus fulvus* Rossi 1846: D♀ (17) (not ♂ as stated by the author). Only the identity of the female syntype from Dalmatia in Croatia, which was not found in NMW, remains doubtful.
*Eresus tricolor* Simon 1873: D♀ (348–349. Only the identity of the female syntypes from Basse Alpes (Mélan) and Hautes Alpes (Briançon, le Monétier), which was later excluded from the typeset by Simon (1914); remains doubtful. It was not found in MNHN).
*Eresus niger* Lehtinen 1967: is.B (fig. 467); Azheganova 1968: is.D♂ (21), is.B (fig. 24), former USSR.
*Eresus cinnaberinus* Roberts 1995: C♂ (pl. 1, fig. 2a).

*Holotype.* CZECH REPUBLIC: *Moravia:* Tišnov, r. Květnice (1♂ 4.IV.2004, M. Řezáč, CRI).

*Paratypes.* We decided to designate material from only the south-eastern part of the Czech Republic as paratypes, to minimize the possibility of including more taxa in the type material (frequent presence of morphologically very similar, allopatrically distributed species in the genus *Eresus*). CZECH REPUBLIC: *Moravia: Brněnsko a.* Dolní Kounice (1♀ X.1966, F. Miller, NMPC); Brno (1♂, F. Miller, NMPC). *Podyjí a.* Havraníky, r. Údolí Dyje, Šobes (1♂ 2.V.2003, J. Vilímová, CRI); Čížov, r. Údolí Dyje, Sloní hřbet hill (1♂ 1.VI.2007, M. Škorpík, CRI). *Pálava a.* Horní Věstonice, r. Děvín-Kotel-Soutěska (4♂ 13.V.1993, 4♂ 26.V.1993, 1♂ 8.VI.1993, J. Svatoň, JS; 1♀ 26.V.1998, M. Řezáč, CRI). *Tišnovsko a.* Tišnov, Čebínka (3♂ 20.V., 1♂1♀ 30.V., F. Miller, NMPC); Tišnov, r. Květnice (1♂ 25.IV., F. Miller, NMPC; 2♂1♀ 20.III.2004, 3♂3♀ 4.IV.2004, 7♀ 27.VI.2006, M. Řezáč, CRI). *Znojemská pahorkatina hilly country:* Mohelno, r. Mohelenská hadcová step (1♀ VII. 1941, 1♂ 1950, F. Miller, NMPC; 1♀ 20.V.2005, M. Řezáč, CRI).

*Other localities and material examined.* AUSTRIA: Dürnstein (1♂ 31.V. 1997, Kopf, BT); north Dunkelstein Wald (1♂ 22.V. 1967, W.D. Oberhammer, BT).
   HUNGARY: Szásanlyó h., Villány (1♂ 8.V.1999, C. Szinetár, CS); (1♂, ZMHB).
   SLOVAKIA: *Beskydské predhorie m.* Ptíčie, r. Humenský Sokol (1♂ 30.IV.1994, V. Thomka, VMH). *Biele Karpaty m.* Babina (1♀ 13.VIII.1992, R. Prídavka, JS). *Borská nížina lowland:* Sekule (1♂ 12.V.1993, O. Jakeš, MR); Moravský Svätý Ján (1♂ 26.V.2002, J. Erhart, MR). *Cerová vrchovina m.* Fil'akovo, Červený vrch h. (1J 7.VI. 1990, R. Prídavka, JS). *Kremnické vrchy m.* Budča, r. Boky (1♂ 1976, V. Thomka, VMH). *Krupinská planina plain:* Plášt'ovce (1J 4.VIII.2001, 1♂1♀ VI.2006, J. Šejna, MR). *Malé Karpaty m.* (1♂ 20.V.1950, F. Miller, NMPC). *Muránska planina plateau:* Tisovec, r. Hradová (1J 14.VIII.1979, J. Svatoň, JS). *Popradská kotlina:* Hranovnica, r. Hranovnicka dubina (1♂1J 12.V.2006, 1♂ 18.V.2006, 1♂3J 22.5.2006, S. Korenko, MR). *Považský Inovec*

*m.* Luka, r. Javorničky (1♂ 9.V.2003, photo M. Ducháček). *Slovenský kras a.* Horný vrch plateau, Zádiel (1♂ 26.V.1978, F. Miller, NMPC); Plešivecká planina plateau, Lúčka h. (1♂ 1 J 11.VI.1983, J. Svatoň, JS). *Starohorské vrchy m.* Panský diel m., Banská Bystrica, r. Jakub (1♂ 7.V.2003, S. Korenko, SK). *Strážovské vrchy m.* Bojnice, Kalvária h. (1J 28.VIII.1992, S. Pekár, MR); Bánovce nad Bebravou, Uhrovec, Jankov vršok h. (1♂ end of V.1985, photo M. Kopeček). *Tríbeč m.* Nitra (1♀ 1891, HNHM). *Vihorlatské vrchy m.* Vinné, r. Vinniansky hradný vrch (1♂ 2.IV.–24.V.1993, V. Thomka, VMH); Krivošťany (1♂ 19.V.1993, V. Thomka, VMH). *Východoslovenská rovina lowland:* Svätuše (1♂ 6.V.1989, J. Prouza, JP); Kráľovský Chlmec, Veľký Kamenec h. (2♂ 8.VI.1960, J. Buchar, NMPC). *Zvolenská kotlina basin:* Vígľaš (1♂ 1.VI.1978, V. Jánsky, JS).

*Etymology.* Named after the eastern part of the Czech Republic (Moravia), the area where the type locality is situated.

*Diagnosis.* This is the largest central European *Eresus* species (Table 1), characterized by a markedly prominent (Fig. 4E) and wide (Fig. 4B) cephalic part of the prosoma. Males are distinguished from the other two central European species by almost entirely red hind legs (Fig. 4B) and by a conductor having a strong terminal tooth that is markedly incurvated towards a round groove (Fig. 5B). Females are characterized by the compact cover of orange hairs on the cephalic part of the prosoma and the basal part of the chelicerae, by an epigyne with the anterior parts of fissures slightly incurvated centrally (Fig. 4H), and by a vulva with remarkably lobed spermathecae (Fig. 4K).

Females of *E. moravicus* sp. n. can be distinguished from the Mediterranean species, which also possess orange hairs on the prosoma, as follows: females (subadult) of *E. ruficapillus* have the cephalic part of the prosoma remarkably tapered backwards. Females of *E. fulvus* differ by greater size, having yellow hairs that are relatively longer and finer covering almost the entire frontal sides of the basal cheliceral segments, having spermatheca that are less lobed, and having copulatory ducts that are almost horizontal in the centre of the vulva. Females of *E. ignicomis* are larger and have a relatively flat area between the PME and PLE. The cover of orange hairs is less consistent, tending to reduce to spots. The epigyne is larger and the spermathecae are less lobed. Females of *E. tricolor* differ by the presence of white spots on the opisthosoma, orange hairs on the prosoma grouped into isolated spots, and a small and shallow epigyne.

### Description
*Male.* *Prosoma* (Fig. 4B,E): 3.5–5.6 (mean 4.6) mm long. The cephalic part is black-ferruginous with scattered white hairs, is very prominent, much wider than the thoracic part,

and extends considerably to the front. The area between PME and PLE is gibbous. The thoracic part is ferruginous, either bordered or entirely covered with red hairs. *Chelicerae:* Black with short black hairs, and with scattered white hairs on the frontal side of the basal part. *Legs* (Fig. 4B): Legs I and II are brown with black hairs, Fe II and Pt II are ferruginous with red hairs, and the distal extremities of Fe, Pt, Ti and Mt, and often also the proximal extremities of Pt, Mt and Tà, have rings of white hairs. Legs III and IV are orange, and almost all segments (podomeres) are covered with red hairs. Only Ta and part of Mt have black hairs. *Opisthosoma* (Fig. 4B): Dorsally red with scattered white hairs and with two, rarely three, pairs of black spots of variable size. A line of white hairs usually borders the red area and the black spots, especially posteriorly. The ventral side of the opisthosoma is black and only the branchial opercula have red hairs. *Palp:* Conductor wrinkled, approximately as long as wide in the lateral view (Fig. 5B); terminal tooth strong, slightly higher than lamella, markedly incurvated toward the groove. The groove is usually large and round. The basal part of the conductor is relatively narrow in the ventral view (Fig. 5E).

*Female. Prosoma:* 5.9–9.9 (mean 7.5) mm long and same shape as in males. The cephalic part is rusty, with a compact cover of orange hairs, especially around the median eyes. *Chelicerae:* Rusty with a compact cover of orange hairs on the frontal side of their basal third. *Legs:* Brown-black, dorsal side of Fe, Pt, Ti and Mt of legs I–III with scattered and, distally, with clustered short pale rusty hairs. *Opisthosoma:* Violet-black with long black hairs and, anteriorly, with scattered short white hairs. *Epigyne* (Figs 2K–Q and 4H): Anterior part with a negligible longitudinal bar, anterior parts of fissures slightly incurvated centrally. *Vulva* (Figs 3K–Q and 4K): Spermathecae composed of very distinct lobes, reaching further to the sides than the copulatory ducts. Copulatory ducts constitute heavily sclerotized elliptical organs in the anterior part of the vulva.

### Remark
Due to the remarkable cover of orange hairs on the cephalic part of the prosoma, females of *E. moravicus* sp. n. were recognized early, but erroneously identified as *E. ruficapillus* (Herman 1879; Loksa 1969) or *E. cinnaberinus frontalis* (Chyzer & Kulczyński 1891; Lessert 1910). Type specimens of *E. ruficapillus* Koch, 1846 belong to a different species, probably endemic to Sicily. The type material of *E. cinnaberinus frontalis* Latreille, 1819 probably no longer exists as we did not find it in MNHN. However, this form was described in south-western Europe, where *E. moravicus* sp. n. do not seem to occur. We revised type specimens of other species having females that are characterized by the presence of orange prosoma hairs, particularly *E. fulvus* Rossi, 1846, *E. tricolor* Simon, 1873 and *E. ignicomis* Simon, 1914, which also represent

different species. *Eresus moravicus* sp. n. has recently been considered an *E. cinnaberinus* variety (Sauer & Wunderlich 1997; Bellmann 2001; Johannesen & Veith 2001). Ratschker (1995) and Thaler & Knoflach (2002) recognized that individuals corresponding to *Eresus moravicus* sp. n. might belong to a separate taxon, but they did not resolve the nomenclature. Because the names mentioned above relate to different species, we have given a new name to this species: *Eresus moravicus* Řezáč.

*Comparative material on related species. Eresus ruficapillus* Koch, 1846: ITALY: Sicily, Escurial, Seidlitz (3J, coll. C. L. Koch in BMNH, syntypes).
*Eresus* sp. n.: GREECE: Pelp, Peleponees (2♂, J. Johannesen, MR).
*Eresus fulvus* Rossi, 1846: YUGOSLAVIA: Syrmien (Srem) 2♀, 19th century, NMW, probably syntypes, in the description erroneously referred to as Syrien. ITALY: Etrurien, Toscana (2♀1J, 19th century, coll. Koelbel in NMW, 1896.VIII.467, 1896.VIII.469, probably syntypes). UKRAINE: Crimea, Taurien (1♀, 19th century, coll. Koelbel in NMW, 1896.VIII.468, probably syntype).
*Eresus ignicomis* Simon, 1914: FRANCE. CORSICA: Patrimonio (1♂ IX.1907, F. Major, BMNH); Corte (1♂ 15.IX.1952, L. Kahmann, SMF); Corte and Vivario (1♂ 12.IX.1952, L. Kahmann, SMF); Luri (1♂ 4.IX.1952, L. Kahmann, SMF); Moncale (1♂2J 8.IX.1952, L. Kahmann, SMF); Asco (1♀ 11.IV.1952, L. Kahmann, SMF); Campile (1♀ VI.1999, N. Verneau, MR); St Florent (1♂ 28.VII.1957, MHNG); Vizzavona? (1♀, 19th century, coll. T. Thorell in NHRS, *E. ruficapillus* — det. E. Simon, probably syntype); Iles Sanguinaires, Bonifacio (7♀8J, MNHN, syntypes), Bonifacio (1♀ VI.1921, MNHN); Evisa (2♂, MNHN). ITALY, SARDINIA: Tüla (1♀ IV.1952, SMF).
*Eresus tricolor* Simon, 1873: FRANCE, CORSICA: Calacuccia (1♂ 6.IX.1952, 2♂ 7.IX.1953, L. Kahmann, SMF); Mt. Asto near Lama (1♂ 18.IX.1952, L. Kahmann, SMF); Quenza (4♂ 12.IV.1952, L. Kahmann, SMF); (1♀, E. Simon, coll. T. Thorell in NHRS, *E.tricolor* — det. E. Simon, probably syntype); (5♀ 1J, MNHN, syntypes); (2♂, MNHN, *E. niger* — det. E. Simon); (1♂, MNHN, *E. tricolor* — det. E. Simon).

### Phenology
*Eresus moravicus* sp. n. matures in April, copulates in April–June and produces an egg sac in June (populations in the Czech Republic).

### Habitat
In the Czech Republic, *E. moravicus* sp. n. occurs mainly in rocky steppes with the dominant grasses *Festuca valesiaca, Stipa dasyphylla, S. capillata, Botriochloa ischaemum* or *Carex humilis* (*Festucion valesiacae*, less often *F. pallentis*). More rarely, it occurs on sandy soils with steppe grasslands characterized by the grass *Stipa borysthenica* (*Plantagini–Festucion ovinae*).

### Distribution
This species occurs in the Pannonian region, Balkan Peninsula and warm regions of the Austrian Alps.

The northern border of *E. moravicus* sp. n. distribution probably runs through the Czech Republic.

*Eresus sandaliatus* (Martini & Goeze, 1778) (figs 2S–AA, 3S–AA, 4CFIL, 5CF, 6).
*Aranea sandaliata* Martini & Goeze in Lister 1778: D♂ (287).
*Eresus sandaliatus* Ratschker & Bellmann 1994: D♂♀ (217), B (fig. 3), V (fig. 4), Germany?; Ratschker & Bellmann 1995: D♂♀ (807–809), B (fig. 5), V (fig. 6), after Ratschker & Bellmann 1994; Bellmann 2001: D♂♀ (40), C♂ (1, 41), C♀ (1, 41), B (40, after Ratschker & Bellmann 1994), V (40, after Ratschker & Bellmann 1994), Germany?; Almquist 2005: D♂♀ (41), C (fig. 73a), B (fig. 73b–c), Sweden.

**Junior synonyms**
*Aranea purpurata* Panzer 1804: D♂ (47), C♂ (pl. 32, fig. 20).
*Eresus purpuratus* Ratschker 1992: B (figs 93–98), E (fig. 79), V (fig. 80), Germany: Schernfeld near Koblenz, Breitenfurt near Nuremberg, Fridingen.
*Eresus annulatus* Hahn 1821: C♂ (pl. 5, fig. B); Hahn 1832: D♂ (not ♀ as stated by the author) (47–48), C♂ (fig. 36), Germany, Bavaria: Eichstätt, Kelheim an der Donau; Koch 1846: D♂ (14), C♂ (087); Simon 1873: D♂ (341–342), Paris.

**Misidentifications**
*Eresus sandaliatus* Topçu *et al*. 2005: E (fig. 4), Turkey.
*Eresus cinnaberinus* Blackwall 1861: D♂ (46), C♂ (pl. 3, fig. 23), England?; Herman 1879 (variety β): D♂ (292), Germany?; Roberts 1995: D♂♀ (78), C♀ (pl. 1, fig. 2b, perhaps subadult), England?; Roberts 1998: D♂♀ (80), B (80), E (80, one subadult), V (80, one subadult); Řezáč *et al.* 1999: C♀ (208), Czech Republic; Johannesen & Veith 2001: specimens from Denmark, England, and the localities near the Danube river in south-eastern Germany; Thaler & Knoflach 2002 (*E. cinnaberinus* s.l.): D♂ (425), C♂ (fig 40, 42), B (figs 21–22), Austria: Bazallerkopf.
*Eresus niger* Braendegaard 1966: D♂ (36–37), D♀ (36), B (fig. 22), E (fig. 21), Denmark?; Roberts 1985: D♂ (46), is.B (fig. 12b), is.E (fig. 12b, subadult), England? (doubtful identity).

*Type specimens. Eresus annulatus:* Germany: Eichstädt (1♂ summer, repository unknown, not examined); Germany: Kelheim an der Donau (1♂ autumn 1811, repository unknown, not examined). *Eresus purpuratus:* Germany: Regensburg (♂, repository unknown, not examined). *Eresus sandaliatus:* Germany: Regensburg (♂, 18th century, repository unknown, not examined).

*Localities and material examined.* AUSTRIA: north Tirol, Bazallerkopf (1♂ 8.VI.2000, K. Thaler, BT).

CZECH REPUBLIC: Numerous materials from this country will be listed elsewhere.

DENMARK: west end of Mossö l. (1♀ 4.IX.2005, M. Řezáč, MR); Rye Sönderskov, between towns Gl Rye and Salten (1♀ 4.IX.2005, M. Řezáč, MR); Enebærbakken (1♂1♀ 26.IV.2007, J. Lissner, MR).

FRANCE: Paris (1♂, E. Simon, MNHN; 1♂, T. Thorell, NHRS, *E. purpuratus* — det. E. Simon); (1♂, HUJ).

GERMANY: *Bavaria:* Kallmünz (1♂, J. Johannesen, MR); Altmühltal (1♂, J. Johannesen, MR).

NETHERLANDS: Gelderland, Caitwickerzand (1♂ 21.I.2004, J. Noordijk, RMNH).

SWEDEN: Eriksdal (5♂ 3.–15.V.2006, R. Gerell, MR).

*Diagnosis.* Males of this species are distinguished from the other two central European species by almost entirely black hind legs (Fig. 4C, Table 1), opisthosoma with typically three pairs of black spots that are usually not bordered with white hairs, and by a conductor with a strong, almost straight terminal tooth and large, U-shaped groove (Fig. 5C). Females are recognized by an almost rectangular epigyne (Fig. 4I), by indistinctly lobed spermathecae, and by copulatory ducts constituting usually circular organs (Fig. 4L).

### Description

*Male. Prosoma* (Fig. 4CF): 2.9–4.1 (mean 3.6) mm long. The cephalic part is black with scattered white hairs, is slightly prominent, is almost as wide as the thoracic part, neglible extension to the front, and the area between PME and PLE is almost flat. The thoracic part is black-brown and is either bordered or entirely covered with red hairs. *Chelicerae:* Black with short black hairs. *Legs* (Fig. 4C): Black with wide rings of white hairs around the joints. The dorsal sides of Fe III and IV with a few red hairs basally. The dorsal sides of Pt II–IV, and partly Ti IV, often have a longitudinal band of white hairs. *Opisthosoma* (Fig. 4C): Dorsally red with three, rarely two, pairs of black spots of variable size. Usually neither the red area nor the black spots are bordered with white hairs. The ventral side of the opisthosoma is black, and only the branchial opercula have red hairs. *Palp:* The conductor is almost smooth, slightly longer than wide in the lateral view (Fig. 5C); terminal tooth strong, distinctly higher than the lamella, and slightly incurvated towards the groove. The groove is large and U-shaped. The basal part of the conductor is distinctly wide in the ventral view (Fig. 5F).

*Female. Prosoma:* 4.2–7.2 (mean 5.4) mm long, of the same shape as in males. The cephalic part black has long black hairs and scattered short white hairs. It also has pale rusty hairs around the median eyes. *Chelicerae:* Brown-black with long black hairs and scattered short pale rusty hairs on the basal half. *Legs:* Black, dorsal side of Fe, Pt, Ti and Mt of legs I–III with scattered and, distally, with clustered short pale rusty hairs. *Opisthosoma:* Violet-black with long black hairs, sometimes with scattered short white hairs anteriorly. *Epigyne* (Figs 2S–AA and 4I): Almost rectangular, anterior part with a negligible longitudinal bar, anterior parts of fissures incurvated side-wards. *Vulva* (Figs 3S–AA and 4L): Spermathecae composed of indistinct lobes, reaching further to the sides than the copulatory ducts. Copulatory ducts constitute finely sclerotized circular organs in the anterior part of the vulva.

### Remark

Thanks to good colour paintings and arachnologically well-explored type localities, the names *E. purpuratus* and *E. annulatus* can be unambiguously identified as synonyms of *E. sandaliatus*, even though the type specimens probably no longer exist. Simon (1873) recognized and recorded this species from France, but later rejected it (Simon 1914). Since then, this species has been neglected by many authors. Recently, Ratschker & Bellmann (1995) and Roberts (1998) redescribed the species. However, Johannesen & Veith (2001) refer only to *E. sandaliatus* as a colour variety, whereas Thaler & Knoflach (2002) refer to it as *E. cinnaberinus* s.l. (in a broad sense).

### Phenology

*Eresus sandaliatus* matures in August, copulates in April–June and produces an egg sac in June (populations in the Czech Republic).

### Habitat

This species occurs in xerothermic grasslands (*Koelerio-Phleion phleoidis*, less often *Festucion pallentis* and *F. valesiacae*) charac-terized by the grasses *Festuca rupicola*, *Avenochloa pratensis*, *Nardus stricta* and *Carex humilis*, and heath *Calluna vulgaris*. It often builds its nests in tufts of moss, terrestrial lichens or grasses. In Northern Europe, it occurs in dry heathlands, and on sandy slopes with the heath *C. vulgaris* and the grass *Avenella flexuosa*.

### Distribution

*Eresus sandaliatus* has expanded the furthest North of all *Eresus* species (Fig. 6). It occurs in Northern Europe (Denmark, southern Sweden, southern England, Schleswig-Holstein in Germany) as well as the western part of central Europe (Czech Republic, Danube region in Bavaria, Austrian Alps, northern France). The eastern distribution border runs through the Czech Republic. Records from the Pyrenees (Ratschker & Bellmann 1995) and Turkey (Topçu *et al.* 2005) refer to other species with unsolved nomenclature.

### Molecular phylogenetics — allozymes

Allozyme analyses clustered Czech *E. kollari* with eastern German *E. kollari*, and Czech *E. sandaliatus* with the Danube
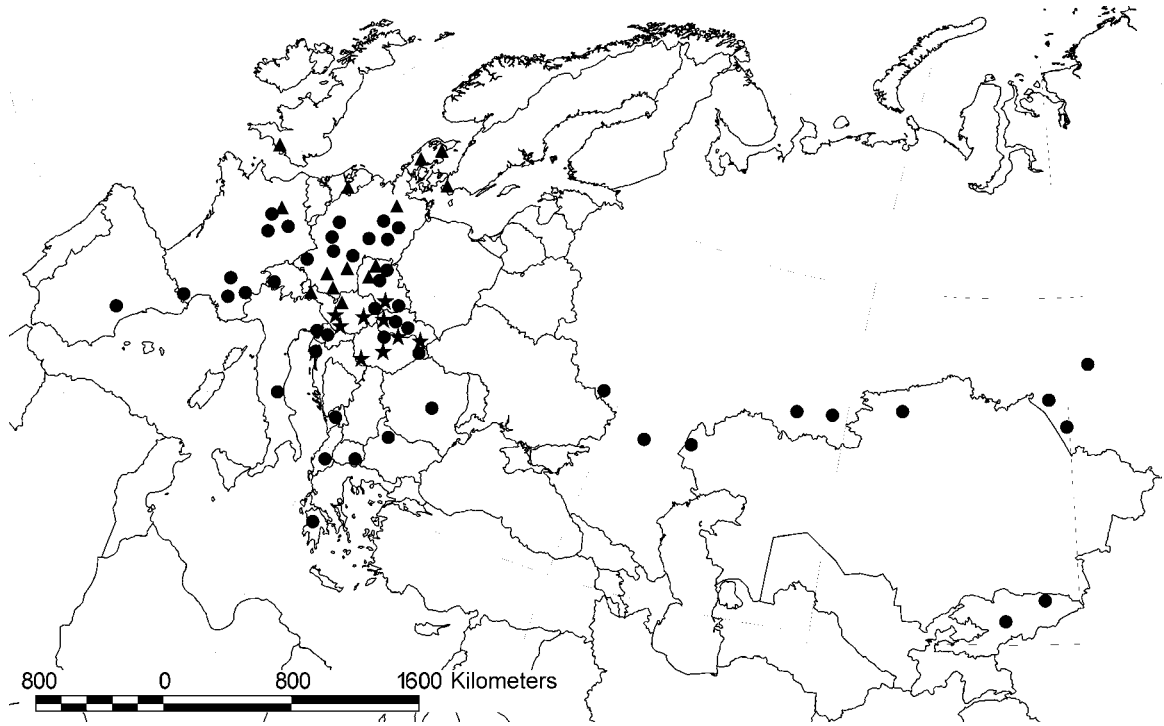
**Fig. 6** Distribution map of the three *Eresus* species. Circle, *Eresus kollari*; asterisk, *Eresus moravicus* sp. n.; triangle, *Eresus sandaliatus*.
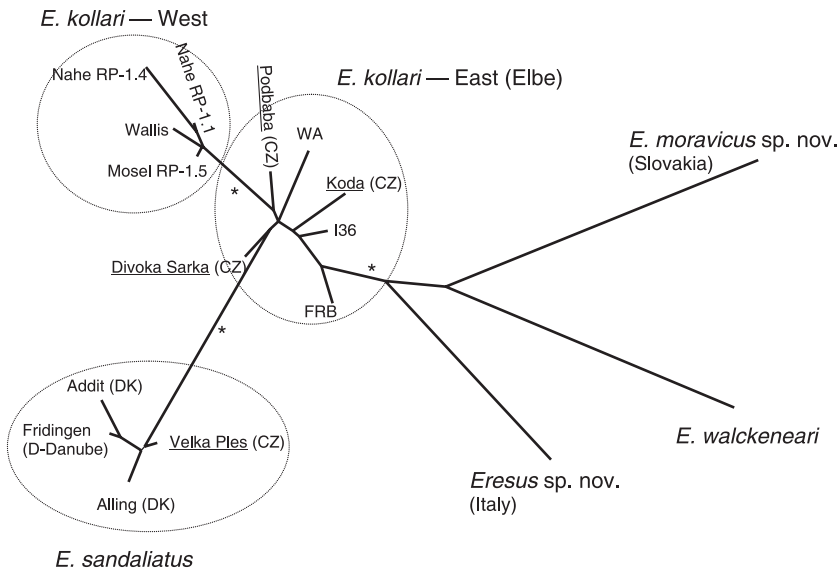


**Fig. 7** Maximum-likelihood allozyme tree. Underlined populations are from the Czech Republic. Asterisks (*) indicate significant (95% confidence interval) internal branch lengths. Note that Czech *Eresus kollari* populations do not occupy an independent branch.

and Danish populations of this species. These results confirmed previous findings that clearly separated *E. sandaliatus*, *E. kollari* (west), *E. kollari* (east) and *E. moravicus* sp. n. The division between western *E. kollari* and *E. sandaliatus* was caused by allelic fixation at three loci (*Gpd, Hbdh, PepB1*). Introgression was suggested for central European *E. kollari*,

which had a mixture of these specific alleles. The western *E. kollari* alleles dominated in the central European samples, leading to greater *E. kollari* affinity. The intermediate status of central European *E. kollari* was confirmed by the unrooted ML tree (Fig. 7); central European *E. kollari* did not cluster on an independent branch. Introgression involving *E. moravicus*

**Table 3** Nomenclature and distribution of haplotypes found in *Eresus* ordered by major- and sublineage divergence. Haplotypes based on alignment of 548 characters of the 16S and ND1 mtDNA genes. Lineage divergence between the three major lineages (west, east, south) was 6.1%–8.2%. Lineage divergence between sublineages was 3.0%–4.4%. Nomenclature example: W (major lineage West)-a (sublineage a)-1 (haplotype 1). Haplotypes were named in the order they were found. (—) designates new haplotypes in this study.

| Lineage | Haplotype name | Old name (Johannesen & Veith 2001) | Distribution | Genbank accession numbers |
|---|---|---|---|---|
| West | W-a-1 | West1 | Western-D, CH | AF374171 |
| East | E-a-1 | East1 | Eastern-D, CZ | AF374172 |
| | E-a-2 | East2 | Eastern-D | AF374173 |
| | E-a-3 | — | CZ | EU442183 |
| | E-a-4 | — | CZ | EU442184 |
| | E-a-5 | — | CZ | EU442185 |
| | E-a-6 | Danube | D (Danube), CZ | AF374180 |
| | E-a-7 | DK1 | DK, GB, S | AF374177 |
| | E-a-8 | DK2 | DK | AF374178 |
| | E-b-1 | Bojnice | CZ, SK | AF374174 |
| | E-c-1 | Greece1 | GR | AF374176 |
| | E-c-2 | Greece2 | GR | AF374184 |
| | E-c-3 | Greece3 | GR | AF374185 |
| | E-d-1 | DK3 | DK | AF374179 |
| | E-d-2 | — | CZ | EU442186 |
| | E-d-3 | — | CZ | EU442187 |
| South | S-a-1 | South1 | I (Lago di Garda) | AF374175 |

D, Germany; CZ, Czech Republic; SK, Slovakia; DK, Denmark; CH, Switzerland; GB, Great Britain; I, Italy; GR, Greece; S, Sweden.

sp. n. cannot be ruled out because some common *E. moravicus* sp. n. alleles (*Fum* allele 4, *Got1* allele 1; *PepB3* allele 3) were found in a few central European *E. kollari* populations and the *Gpd* and *Hbdh* alleles are identical to western *E. kollari*. However, the *E. moravicus* sp. n. specific *Hk* allele 1 was not found in neighbour *E. kollari* populations. An allele frequency table of Czech and companion populations used in this study is given in Appendix A.

### Molecular phylogenetics — mtDNA

Sequence analysis based on 548 bp of the mtDNA genes 16S, tRNA$_{Leu}$ and ND1 from 32 individuals from the Czech Republic and Slovakia revealed nine haplotypes, of which five were new to this study (Tables 2 and 3). The new haplotypes belonged to two sublineages of the eastern Balkan lineage (Johannesen & Veith 2001). The haplotypes from Johannesen & Veith (2001) have been renamed to take into account the interaction between geography and lineage subdivision. New and old haplotype names, accession numbers and haplotype distributions are shown in Table 3.

A partition-homogeneity test that included outgroups showed similar evolution of the 16S-tRNA$_{Leu}$ and ND1

genes, $P > 0.90$, thus the genes were analysed together. BIC estimated the Tamura–Nei + I distance model (TrN + I) as the best model, –ln $L$ = 1163.0929. The best trees found by NJ based on TrN + I and LogDet distance models had minimum evolution scores = 0.35550 and 0.27703, respectively (Fig. 8). The phenogram confirmed the division of *Eresus* into three major lineages (uncorrected $P$-distances: 0.061–0.082). The major lineage E was further divided into three sublineages (uncorrected $P$-distances: 0.030–0.044). The MP heuristic search resulted in three most parsimonious trees (tree lengths 148, CI = 0.764, RI = 0.685, HI = 0.236), of which 59 variable characters were parsimony-uninformative and 48 characters were parsimony-informative. The three trees all resolved the major- and sublineages, but differed in the relationship among haplotypes within sublineages. ML analysis found one most like tree, –ln = 1353.6799. ML analysis rooted *E. walckenaeri* within the E-a lineage. This did not change the phylogenetic distinctness or relationship of any lineage and explained the lack of high bootstrap scores at the base of the phylogenetic tree (Fig. 8).

Two sublineages (E-a and E-d) showed admixture of haplotypes with respect to *E. sandaliatus* and central European (Elbe) *E. kollari*: (i) *E. sandaliatus* lineage E-d, previously found only in Denmark (DK3), was observed in one *E. kollari* and one *E. sandaliatus* in the Czech Republic. (ii) Haplotype E-a-1, found previously only in eastern *E. kollari*, was observed in Czech *E. sandaliatus* in the population from Křivoklátsko — Velká Pleš. In all cases, the individuals had allozymes of the correct morphospecies. To resolve specific and geographical haplotype relationships and to understand the extent of possible introgression within clade E-a, we performed a gene tree analysis (Fig. 9). Discrete partitions of Danish and Czech haplotypes in the gene tree indicated that hybridization involved only Elbe spiders. At the syntopic site Koda in the Český kras area, mtDNA haplotypes were discretely distributed between *E. kollari* and *E. sandaliatus*. Here, the Koda *E. sandaliatus* population had the same haplotype as German Danube *E. sandaliatus* (Johannesen & Veith 2001).

### Discussion

The revision of *Eresus* from central Europe yielded two forms, which we recognized as *E. kollari* and *E. sandaliatus*, and a new form, which we named *E. moravicus* sp. n. These three taxa are morphologically very similar, but differ in the shape of the copulatory organs and prosoma, the body size, and the colour pattern. No intermediate morphological forms were detected. The three species further differ in phenology, habitat preferences and genetics. However, genetic data indicated that populations of *E. kollari* in central Europe were putative hybrids of *E. kollari* and *E. sandaliatus*. Therefore, the morphospecies *E. kollari* is genetically paraphyletic. Cladistically, species are monophyletic entities.
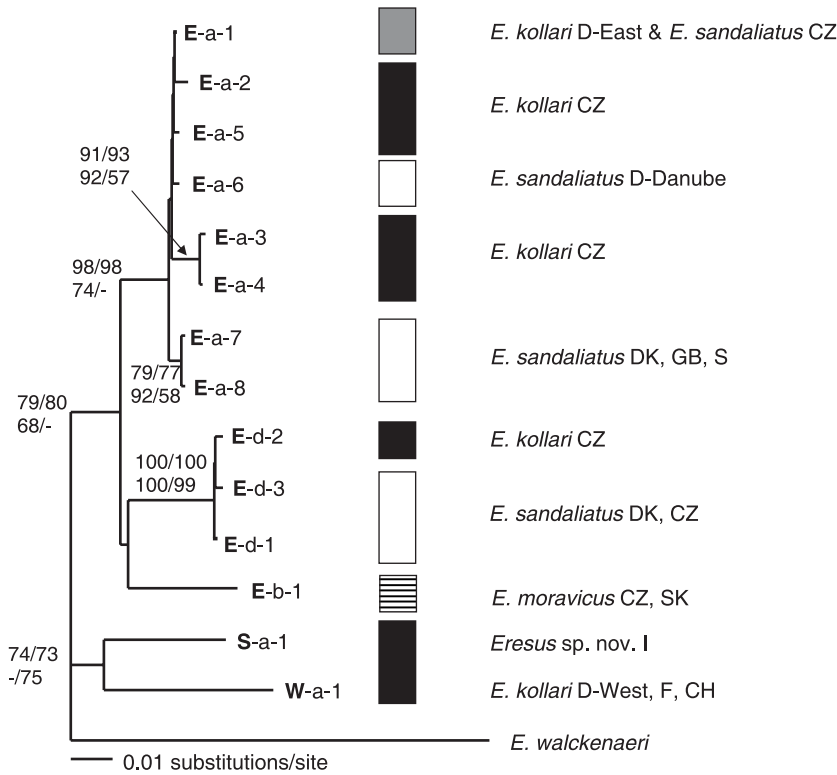
Fig. 8 Heuristic neighbor-joining phylogram (LogDet distance) of *Eresus* mtDNA sequence evolution. Bootstrap scores represent LogDet – NJ, Tamura – Ne + I – NJ, MP and ML analyses, respectively. ML analysis rooted the tree at the base of the E-a-lineage (x), whereas NJ and MP rooted the tree between the three major lineages. Country of origin: D, Germany; CZ, Czech Republic; SK, Slovakia; DK, Denmark; CH, Switzerland; GB, Great Britain; I, Italy; S, Sweden.
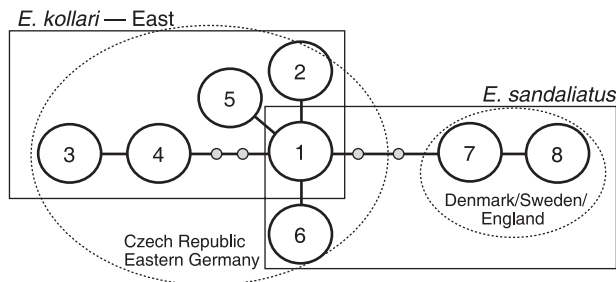


Fig. 9 Gene tree of the E-a lineage showing species-specific and geographic distributions of haplotypes in eastern *Eresus kollari* and *Eresus sandaliatus*. Solid lines enclose haplotypes found within species. Dotted lines encircle the geographic distribution of haplotypes. Note that only Czech spiders show haplotype admixture between species. The haplotype numbers (1–8) refer to their numbers within the E-a(–*x*) lineage as shown in Fig. 6 and Tables 2 and 3.

However, this approach rules out unfinished lineage sorting in new species or that taxa experiencing introgressive hybridization may effectively function as one parental species. Below, we discuss differences among the species and implications for and against the status of the species.

## Morphology

The morphology of the studied *Eresus* species is uniform. It is in accordance with their ecological uniformity: all three species exhibit the same life style and occur in very similar habitats. Even the most complex macro-characters, such as the colouration and shape of the copulatory organs, are not as useful as in other groups of spiders. The usage of colouration for species discrimination in *Eresus* is constrained by its intraspecific variability. In some groups of spiders, for example, representatives of the families Salticidae and Lycosidae, where colouration plays an important role in recognition of conspecific partners, it is highly species specific (e.g. Kronestedt 1990; Azarkina 2002). Eresids, on the other hand, dispose of poor vision (Bristowe 1958), they have evolved a varicolored pattern as protection against predators (Cloudsley-Thompson 1995). The copulatory organs of *Eresus* are simple, providing little space for diversification. Nevertheless, their shape (Figs 2–4G–L and 5) is still the most explicit diagnostic character among the studied taxa. Concerning this character, *E. sandaliatus* and *E. moravicus* sp. n. are similar, while *E. kollari* differs. We were not able to morphologically distinguish pure *E. kollari* from populations of *E. kollari* × *E. sandaliatus* hybrid origin, despite putting great effort into analysing as many specimens of both taxa as possible. The hybridogenic taxon possesses no intermediate traits of the details studied. The

morphology of *E. kollari* is extraordinarily homogenous: the same morphotypes occur throughout a vast geographical area ranging from Spain to Novosibirsk in central Russia. Therefore, only molecular analyses can answer whether the hybrid taxon is restricted to central Europe or has expanded its range into central Asia.

### Distributions and habitat preferences

Distribution areas of *E. kollari* and *E. moravicus* sp. n. overlap in the Czech Republic, and areas of *E. sandaliatus* and *E. moravicus* sp. n. overlap in the Austrian Alps (cf. Thaler & Knoflach 2002). Overlapping distributions imply that these taxa are not mere subspecies, which are the product of reduced gene flow between geographically isolated populations (Kraus 2002). Subspecies are predicted to lose morphological specificity due to renewed gene flow upon contact (Grant *et al*. 2005).

### Genetic evidence for species and hybrid speciation

In general, molecular data corroborate morphological divergence among the central European *Eresus*. Both mtDNA sequence divergence and allozyme allele frequencies support monophyly of *E. moravicus* sp. n. and west European *E. kollari*. *Eresus sandaliatus* is monophyletic for allozymes, but consists of two mtDNA lineages (E-a and E-d) that have not been monophyletically resolved within the eastern (E) mtDNA lineage. Nevertheless, *E. sandaliatus* possesses different chromosome numbers from *E. kollari* and *E. moravicus* sp. n. (J. Král, unpubl. data). The level of divergence between the two *E. sandaliatus* mtDNA sublineages (E-a and E-d) and the finding that they share diagnostic allozyme alleles suggest that *E. sandaliatus* is an ancient species. In contrast to the findings above, central European *E. kollari* are genetically complex, showing mtDNA belonging to the *E. sandaliatus* lineage and an admixture of western *E. kollari* and *E. sandaliatus* enzyme alleles. This implies that central European *E. kollari* are introgressed, that is, hybrid, individuals (Johannesen & Veith 2001). These authors cautioned that: 'cohesive factors such as post-glacial secondary contacts [i.e. gene flow] (may) prevent the budding off of new fully independent species'. However, the extended genetic data set presented here show that species specificity (and hybrid specificity) is upheld in areas of range contact between eastern *E. kollari* and *E. sandaliatus*. We therefore argue that the three *Eresus* species are separate evolutionary units, even though hybridization may occasionally occur.

At present, we do not know whether central European *E. kollari* functions as a distinct hybrid species or rather should be considered an allopatric (and introgressed) part of the *E. kollari* species assemblage. Speciation caused by introgressive hybridization is common in plants (Stebbins 1959; Arnold 1997), but its significance in animal evolution remains

controversial. Nevertheless, Mallet (2005) showed that hybridization on a per species basis is much more common in animals than anticipated. In fact, speciation via introgressive hybrididization (DeMarais *et al*. 1992; Taylor *et al*. 1996; Salzburger *et al*. 2002; Smith *et al*. 2003) and/or the establishment of viable hybrids (F2 and backcrosses) (Sperling & Harrison 1994; Langor & Sperling 1997; Weisrock *et al*. 2005; Johannesen *et al*. 2006) is suggested for a wide range of taxa, often involving very divergent lineages. Hybrid species are often morphologically intermediate to the parent species. However, morphology is not a predictor of genotype in *E. kollari*. Reasons for this may be manifold and include unidentified cryptic species, elevated mutation rates or lack of Dobzansky–Muller incompatibility during hybridization of old lineages. Interestingly, a decoupling of the morphological change from lineage divergence may be common in spiders (Hedin 1997; Bond *et al*. 2001; Hedin & Wood 2002; Johannesen *et al*. 2007).

Hybrid speciation in *E. kollari* would involve homoploid hybrid speciation (J. Král, unpubl. data). Detecting homoploid hybrid speciation and distinguishing it from introgression without speciation is difficult. Simulations show that homoploid hybrid speciation requires ecological and spatial isolation (Buerkle *et al*. 2000). Ecological isolation results from adaptation to environments not occupied by the parent species, whereas any form of spatial isolation contributes to genetic isolation. Seehausen (2004) argued that hybridization might drive some adaptive radiations, and specified a geographical test to examine hybrid radiations (for *E. kollari* this considers a two-species scenario): 'the geographical distribution … should be significantly associated with contact zones between closely related species or divergent populations of allopatric origin.' These expectations fit with most aspects of the Czech *Eresus* populations. Although the two species are associated with post-glacial environments, probably with different refugia and spatial isolation related to psychrotolerance, similar habitat requirements indicate that competition, and not only adaptations to new habitats, are also responsible for discrete distributions. The evolution of putative hybrids in *Eresus* suggests that alternating ranges during and after the glacial periods resulted in isolation and secondary contact interactions that might have reshuffled the genomes.

The eastern central European *E. kollari* form a coherent genetic group, which is distributed throughout the Elbe drainage system. The combined genetic and geographical coherensy supports a single historical origin of this lineage. If gene flow happens on a regular basis, sympatric/parapatric populations of the parental species should merge (Grant *et al*. 2005), and local hybridization events would create nonspecific combinations of enzyme alleles. This was not found. Furthermore, within the *E. sandaliatus* E-a lineage, the slightly divergent Danish *E. sandaliatus* E-a-7/8 haplotypes were not

involved in hybridization (Fig. 9). This suggests that the hybrid event was related to range alterations and/or dispersal phases of isolated *E. sandaliatus* populations (i.e. Czech populations), perhaps during the most recent ice age. Nevertheless, we also found evidence for a second introgression event: in one individual with an *E. kollari* phenotype, we found *E. sandaliatus* mtDNA, sublineage E-d. Again, nuclear genes delimited all E-d spiders to the correct morphological species. This event resembles the previously described hybrid event: *E. sandaliatus* mtDNA is found in *E. kollari* (never vice versa), and the putative hybrid expressed *E. kollari* morphology and a dominance of *E. kollari* nuclear genes. Hence, hybridization involves female *E. sandaliatus* and male *E. kollari*. This mating direction is in agreement with the phenology of the species: male *E. kollari* always have the first opportunity to mate.

## Summary

Morphological, behavioural and genetic data support at least three *Eresus* species in central Europe. Morphological conservatism and lack of distinct morphological characters makes it difficult to delimit *Eresus* species systematically, particularly hybrids (Mallet 2005). To our present knowledge, evidence for hybridization has been found only in central Europe, where post-glacial re-colonizations have brought diverged lineages into secondary contact. Hybridization must be rare because lineages were genetically well-defined. Presently, we cannot distinguish central and western European *E. kollari* morphologically. We therefore retain them as one species. However, detailed future research may divide *E. kollari* into *E. kollari* (as an eastern 'hybrid' lineage) and *Eresus* sp. n. as a western lineage. This research should include population-based analyses (Maddison & Knowles 2006) of western *E. kollari*. It should be designed to delimit specificity relative to introgressive status in contact zones of *Eresus* and to take local morphological anomalies into account.

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**Appendix A** Allele frequency table used for revision of central European *Eresus*.

| Locus | Allele | *E. kollari* East | | | | | | *E. kollari* West | | | | *E. sandaliatus* | | | | *E. moravicus* | *Eresus* sp. | *E. walkenaeri* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | D | D | D | CZ | CZ | CZ | D | D | D | CH | D | DK | DK | CZ | SK | I | TR |
| | | I36 | WA | FRB | Podbaba | Div. Sarka | Koda (syn) | RP-1.5 | RP-1.1 | RP-1.4 | Wallis | Fridingen | Allinge | Addit | Velka Ples | Bojnice | Lago di Garda | Hurma |
| Aat-1 | 1 | 0 | 0 | 0 | 0.07 | 0.06 | 0 | 0 | 0 | 0 | 0 | 0.05 | 0 | 0 | 0.10 | 0.33 | 0 | 0 |
| | 2 | 0.80 | 0.90 | 0.50 | 0.93 | 0.89 | 0.65 | 1.00 | 0.96 | 1.00 | 1.00 | 0.95 | 1.00 | 1.00 | 0.90 | 0.67 | 1.00 | 0.94 |
| | 3 | 0.20 | 0.10 | 0.50 | 0 | 0.06 | 0.35 | 0 | 0.04 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.06 |
| Aat-2 | 1 | 0 | 0 | 0 | 0.07 | 0.17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 2 | 1.00 | 1.00 | 1.00 | 0.93 | 0.83 | 1.00 | 1.00 | 1.00 | 1.00 | 0.83 | 0.10 | 0.36 | 0.39 | 0.40 | 1.00 | 1.00 | 1.00 |
| | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.17 | 0.90 | 0.64 | 0.61 | 0.60 | 0 | 0 | 0 |
| Acon-2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.06 | 0 | 0 | 0 | 0.50 |
| | 3 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.94 | 1.00 | 0 | 1.00 | 0.50 |
| | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.00 | 0 | 0 |
| Adh | 1 | 0 | 0 | 0 | 0 | 0 | 0.03 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 2 | 0.05 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 3 | 0.45 | 0.40 | 0.30 | 0.25 | 0 | 0.43 | 0 | 0.39 | 0.18 | 0 | 0 | 0 | 0.05 | 0 | 0 | 0.20 | 0.64 |
| | 5 | 0.50 | 0.60 | 0.70 | 0.75 | 1.00 | 0.55 | 1.00 | 0.61 | 0.82 | 1.00 | 0.70 | 0.97 | 0.70 | 1.00 | 1.00 | 0.80 | 0.36 |
| | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.30 | 0.03 | 0.25 | 0 | 0 | 0 | 0 |
| Ak-1 | 1 | 0 | 0 | 0.05 | 0 | 0 | 0.18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 2 | 0.70 | 0.95 | 0.95 | 1.00 | 1.00 | 0.82 | 1.00 | 0.64 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 3 | 0.30 | 0.05 | 0 | 0 | 0 | 0 | 0 | 0.36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Apk-1 | 2 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Apk-2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.30 | 0 |
| | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.70 | 0 |
| | 3 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0 | 1.00 |
| Fum | 1 | 0 | 0 | 0.25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 3 | 1.00 | 0.80 | 0.75 | 0.93 | 0.94 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.33 | 1.00 | 0 |
| | 4 | 0 | 0.20 | 0 | 0.07 | 0.06 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.67 | 0 | 1.00 |
| G-3-pdh | 1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| G-6-pdh | 1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Gpd | 1 | 0.05 | 0.10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 2 | 0.25 | 0 | 0.05 | 0.21 | 0.17 | 0.05 | 1.00 | 1.00 | 1.00 | 1.00 | 0 | 0 | 0 | 0.05 | 1.00 | 1.00 | 0 |
| | 3 | 0.70 | 0.90 | 0.95 | 0.79 | 0.83 | 0.95 | 0 | 0 | 0 | 0 | 1.00 | 1.00 | 1.00 | 0.95 | 0 | 0 | 1.00 |
| Hbdh | 1 | 0 | 0.50 | 0 | 0.14 | 0.11 | 0.08 | 0 | 0 | 0 | 0 | 1.00 | 1.00 | 1.00 | 1.00 | 0 | 0 | 0 |
| | 2 | 1.00 | 0.44 | 1.00 | 0.86 | 0.89 | 0.63 | 0.95 | 1.00 | 0.75 | 1.00 | 0 | 0 | 0 | 0 | 1.00 | 0.95 | 1.00 |
| | 3 | 0 | 0.06 | 0 | 0 | 0 | 0.30 | 0.05 | 0 | 0.25 | 0 | 0 | 0 | 0 | 0 | 0 | 0.05 | 0 |
| Hk | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.17 | 0 | 0 |
| | 2 | 0 | 0 | 0.05 | 0 | 0 | 0 | 0.05 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.83 | 0 | 0 |
| | 3 | 1.00 | 1.00 | 0.95 | 1.00 | 1.00 | 1.00 | 0.95 | 0.82 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0 | 1.00 | 1.00 |
| | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.04 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Appendix A** *Continued*.

| Locus | Allele | *E. kollari* East | | | | | | *E. kollari* West | | | | *E. sandaliatus* | | | | *E. moravicus* | *Eresus* sp. | *E. walckenaeri* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | D | D | D | CZ | CZ | CZ | D | D | D | CH | D | DK | DK | CZ | SK | I | TR |
| | | I36 | WA | FRB | Podbaba | Div. Sarka | Koda (syn) | RP-1.5 | RP-1.1 | RP-1.4 | Wallis | Fridingen | Allinge | Addit | Velka Ples | Bojnice | Lago di Garda | Hurma |
| Idh-1 | 3 | 0.15 | 0.30 | 0.70 | 0.07 | 0.44 | 0.15 | 0 | 0 | 0 | 0 | 0.15 | 0.42 | 0.11 | 0.05 | 1.00 | 0.95 | 1.00 |
| | 4 | 0.85 | 0.70 | 0.30 | 0.93 | 0.56 | 0.85 | 1.00 | 1.00 | 1.00 | 1.00 | 0.85 | 0.58 | 0.89 | 0.95 | 0 | 0.05 | 0 |
| Idh-2 | 2 | 0.90 | 1.00 | 0.80 | 0.93 | 1.00 | 0.95 | 1.00 | 1.00 | 1.00 | 1.00 | 0.95 | 0.92 | 0.97 | 0.95 | 0 | 0 | 1.00 |
| | 3 | 0.10 | 0 | 0.20 | 0.07 | 0 | 0.05 | 0 | 0 | 0 | 0 | 0.05 | 0.08 | 0.03 | 0.05 | 1.00 | 1.00 | 0 |
| Ldh | 2 | 1.00 | 1.00 | 1.00 | 1.00 | 0.89 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.61 | 1.00 | 0.70 | 1.00 | 1.00 | 1.00 |
| | 3 | 0 | 0 | 0 | 0 | 0.11 | 0 | 0 | 0 | 0 | 0 | 0 | 0.39 | 0 | 0.30 | 0 | 0 | 0 |
| Mdh-1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.18 | 0 | 0 | 0 | 0 |
| | 3 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.85 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.82 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 4 | 0 | 0 | 0 | 0 | 0 | 0.15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mdh-2 | 1 | 0 | 0 | 0 | 0 | 0.17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 2 | 1.00 | 0.80 | 1.00 | 1.00 | 0.83 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.97 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 3 | 0 | 0.20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.03 | 0 | 0 | 0 | 0 | 0 |
| Mpi | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.00 |
| | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.29 | 0 | 0 | 0.05 | 0.08 | 0.11 | 0 | 0 | 0.10 | 0 |
| | 4 | 0.90 | 0.90 | 0.80 | 1.00 | 1.00 | 1.00 | 1.00 | 0.71 | 1.00 | 1.00 | 0.95 | 0.92 | 0.89 | 1.00 | 1.00 | 0.80 | 0 |
| | 5 | 0.10 | 0.10 | 0.20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.10 | 0 |
| Pep-B1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.31 | 0.10 | 0 | 0 | 0 | 0 |
| | 2 | 0 | 0 | 0.25 | 0 | 0 | 0.03 | 0 | 0 | 0 | 0 | 1.00 | 0.69 | 0.88 | 1.00 | 0 | 0.35 | 0 |
| | 3 | 1.00 | 0.90 | 0.75 | 1.00 | 0.89 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | 0 | 0 | 0.02 | 0 | 1.00 | 0.65 | 1.00 |
| | 4 | 0 | 0.10 | 0 | 0 | 0.11 | 0 | 0 | 0 | 0 | 0 | 0. | 0 | 0 | 0 | 0 | 0 | 0 |
| Pep-B2 | 2 | 0 | 0 | 0 | 0 | 0 | 0.18 | 0 | 0 | 0.09 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.05 | 0 |
| | 4 | 1.00 | 1.00 | 1.00 | 0.58 | 1.00 | 0.79 | 1.00 | 1.00 | 0.91 | 1.00 | 1.00 | 1.00 | 0.70 | 1.00 | 1.00 | 0.95 | 1.00 |
| | 6 | 0 | 0 | 0 | 0.42 | 0 | 0.03 | 0 | 0 | 0 | 0 | 0 | 0 | 0.30 | 0 | 0 | 0 | 0 |
| Pep-B3 | 3 | 0 | 0 | 0 | 0.14 | 0 | 0 | 0 | 0 | 0 | 0.17 | 0 | 0 | 0 | 0 | 0.67 | 0 | 1.00 |
| | 4 | 0.90 | 0.80 | 1.00 | 0.86 | 0.83 | 0.85 | 1.00 | 1.00 | 1.00 | 0.67 | 1.00 | 1.00 | 1.00 | 1.00 | 0.33 | 1.00 | 0 |
| | 5 | 0.10 | 0.20 | 0 | 0 | 0.17 | 0.15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pgi | 1 | 0 | 0 | 0 | 0 | 0 | 0.20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 2 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.80 | 1.00 | 0.79 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pgm | 2 | 0 | 0 | 0 | 0.07 | 0.06 | 0 | 0.05 | 0.50 | 0.41 | 0.33 | 0 | 0.06 | 0 | 0 | 0 | 0.05 | 0 |
| | 3 | 1.00 | 1.00 | 1.00 | 0.93 | 0.94 | 1.00 | 0.95 | 0.50 | 0.59 | 0.67 | 1.00 | 0.94 | 1.00 | 1.00 | 1.00 | 0.95 | 1.00 |
| N | | 10 | 10 | 10 | 7 | 9 | 20 | 11 | 14 | 11 | 3 | 10 | 18 | 33 | 10 | 3 | 10 | 8 |

D, Germany; CZ, Czech Republic; SK, Slovakia; DK, Denmark; CH, Switzerland; I, Italy; TR, Turkey.