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12S DNA sequence data confirm the separation of *Alopecosa barbipes* and *Alopecosa accentuata* (Araneae, Lycosidae)

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Summary

Phylogenetic analyses of DNA sequence data from the 12S ribosomal subunit supported the relationship of *Alopecosa accentuata* as sister species to *A. barbipes*. Other *Alopecosa* species, although more morphologically distinct, were found to have far less variation in their sequence data.

Introduction

The Lycosidae is a morphologically conservative family. In some cases species are separated by slight morphological differences, which have become apparent only after behavioural and/or ecological observations. This has been the case with species in genera such as *Alopecosa* Simon, 1885 (Kronestedt, 1990), *Schizocosa* Chamberlin, 1904 (Stratton, 1991) and *Pardosa* C. L. Koch, 1847 (Kronestedt, 1992, 1999).

Dahlem *et al.* (1987) studied the courtship behaviour of *Alopecosa* spp. and found differences between *Alopecosa barbipes* (Sundevall, 1833) and *A. accentuata* (Latreille, 1817). Subsequently, Cordes & von Helversen (1990) noted morphological, behavioural, and ecological differences between the species and removed *A. barbipes* from synonymy with *A. accentuata. Alopecosa barbipes* is found in regions with an oceanic climate, adults mate in autumn and often in the following spring, and males have an exaggerated courtship posture and have a black tibial hair-brush on the front legs (Cordes & von Helversen, 1990). *Alopecosa accentuata* is found in parts of Central Europe with a warm, continental climate, adults mate in spring, the courtship posture of males is less distinctive and they have no black tibial hair-brush on the front legs (Cordes & von Helversen, 1990). The validity of *A. barbipes* as a species distinct from *A. accentuata* is now generally accepted (e.g. Merrett & Millidge, 1992; Roberts, 1993).

12S DNA sequences have recently been used to infer European lycosid phylogenetic relationships (Zehethofer & Sturmbauer, 1998), which included sequences from *Alopecosa accentuata, A. cuneata* (Clerck, 1757), *A. pulverulenta* (Clerck, 1757), *A. taeniata* (C. L. Koch, 1835), *A. trabalis* (Clerck, 1757) and *A. inquilina* (Clerck, 1757). Zehethofer & Sturmbauer (1998) found that 12S was especially suitable for resolving relationships between more distantly related taxa. In this study, a section of 12S mitochondrial DNA from *A. barbipes* was sequenced and compared with sequences from other species of *Alopecosa*, including *A. accentuata*. Our objectives were to see whether sequence data supported the separation of *A. barbipes* and *A. accentuata* and how these species were related to other species of *Alopecosa*.

Material and methods

A male specimen of *A. barbipes* was collected by CJV and M. A. Hudson on 6 October 1999 in grass at Redgrave and Lopham Fen, Suffolk, (Grid ref. TM 0479, 52°23′N, 01°00′E). The specimen was stored in 95% ethanol and washed in sterile, deionised, distilled water before DNA extraction. DNA was extracted by homogenising one each of legs III and IV using a proteinase-K digestion and high salt precipitation method (White *et al.*, 1990). A 263 base pair segment from the 12S rDNA was amplified and sequenced from *A. barbipes* following the methods in Zehethofer & Sturmbauer (1998). The sequence data have been submitted to GenBank (Benson *et al.*, 2000) (accession number AY028420).

Sequence data of the six *Alopecosa* species studied by Zehethofer & Sturmbauer (1998), and *Trochosa terricola* Thorell, 1856, were obtained from GenBank (accession numbers AJ008022, AJ008024, AJ008025, AJ008026, AJ008027, AJ008030, AJ008017 respectively).

Phylogenetic analyses were performed on the six *Alopecosa* sequences generated by Zehethofer & Sturmbauer (1998) plus *A. barbipes*. It should be pointed out that for this analysis we did not attempt to test the monophyly of *Alopecosa*, but simply rooted the resulting trees on *T. terricola*, since Zehethofer & Sturmbauer (1998) found this species to be distinct, but closely related to *Alopecosa*. The data were analysed with PAUP* (Swofford, 2001) using parsimony (an exhaustive search) and maximum likelihood (a heuristic search with TBR branch swapping and 10 random sequence additions). The general time reversible model (Yang, 1994) was used to estimate the maximum likelihood tree. The rate and among-site heterogeneity parameters were estimated in the search. Support for the parsimony tree was assessed by bootstrap analysis (Felsenstein, 1985).

Results

Maximum likelihood analysis resulted in two trees, one of which is shown here (Fig. 1). The second tree differed only in the placement of *A. inquilina*, which came out in a trichotomy with a second clade of *A. accentuata* and *A. barbipes* and the remaining *Alopecosa* species in a third clade. The parsimony analysis resulted in one most parsimonious tree that had an identical topology to the maximum likelihood tree shown in Fig. 1. Bootstrap analysis (1000 replicates) showed good support (89%) for the clade containing *A. cuneata*, *A. pulverulenta*, *A. taeniata* and *A. trabalis*, and some support (44%) for the clade containing *A. barbipes*, *A. accentuata* and *A. inquilina*. *Alopecosa barbipes* and *A. accentuata* were found to be sister species (bootstrap value of 44%). The sequences of *A. barbipes* (4 unique nucleotide sites) and *A. accentuata* (six unique nucleotide sites) differed by nine transitions and one transversion. Within the seven *Alopecosa* species examined there were 29 variable sites and these are shown in Fig. 1.

Discussion

The ten base pair difference between the same 12S mitochondrial subunit sequence from *A. barbipes* and *A. accentuata* supports the morphological, behavioural and ecological differences found by Cordes & von Helversen (1990). In contrast, *A. pulverulenta* and *A. trabalis*, which are morphologically distinct from each other (in both male and female genitalia) (Heimer & Nentwig, 1991), have identical 12S sequences (Zehethofer & Sturmbauer, 1998). The clade containing *A. cuneata*, *A. pulverulenta*, *A. taeniata* and *A. trabalis* is part of the "*pulverulenta* group" (Lugetti & Tongiorgi, 1969). There are only seven variable sites within this clade, so it would appear that *A. barbipes* and *A. accentuata* have been separate species for at least as long as the divergence of the species in the "*pulverulenta* group". The section of mtDNA sequenced has been found to be highly conserved among many taxa (Kocher *et al*., 1989), so sequencing of other gene regions (e.g. COI (see Garb, 1999), ND1 (see Hedin, 1997)) may more fully distinguish between closely related species such as *A. pulverulenta* and *A. trabalis*.

The topology of the clade containing four species of the "*pulverulenta* group" in the parsimony analysis and the maximum likelihood analysis agrees with the tree produced by Zehethofer & Sturmbauer (1998). The relationship of *A. accentuata* and *A. inquilina* to the other *Alopecosa* species was unresolved in Zehethofer & Sturmbauer (1998), but the addition of *A. barbipes* in this study provides some resolution. *Alopecosa barbipes* is also shown to be sister to *A. accentuata*.

The genus *Alopecosa* contains more than 130 species (Platnick, 2000) and a very small proportion of this genus has been sequenced. However, this study demonstrates that morphological and genetic distinctiveness are not strictly correlated.

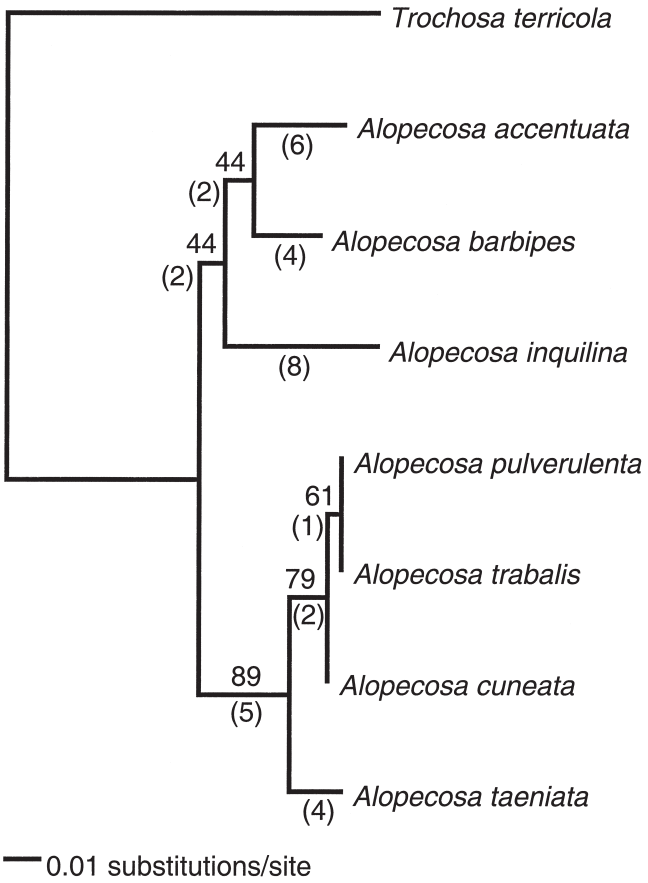

Fig. 1: Maximum likelihood tree of *Alopecosa* spp. Numbers without brackets are bootstrap values (1000 replicates) from the parsimony tree with the same topology. Numbers in brackets show numbers of apomorphies (nucleotide changes).

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Opopaea fosuma, n. sp. from Sumatra, Indonesia (Araneae, Oonopidae)

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Summary

A new species of oonopid, *Opopaea fosuma,* is described from both sexes collected in the Kerinci Seblat National Park in Central Sumatra. Possible relationships with other species of *Opopaea* and the structures of the female genitalia and the embolus are discussed.

Introduction

The oonopid genus *Opopaea* was described by Simon in 1891. The male palp of the type species, *O. deserticola,* is notable for a characteristically enlarged patella (Simon, 1891). The following description of an Indonesian species of the genus *Opopaea* is based on material collected in the Kerinci Seblat National Park in Central Sumatra and provided for description by Dr C. Deeleman. All measurements are in mm.

Opopaea fosuma Burger, n. sp. (Figs. 1–24)

Types: Male holotype, Kerinci Seblat National Park, 800 m a.s.l., Central Sumatra, Indonesia, 21–30 July 1988, leaf litter, Suh. Djojosudharmo leg., deposited in NMBE (Natural History Museum, Bern, Switzerland). Paratypes, leg. Suh. Djojosudharmo at type locality: 1♂ 2♀ (NMBE), 2♂ 2♀ (Coll. Deeleman, Ossendrecht, The Netherlands).

Etymology: The specific name is an abbreviation of "found in Sumatra".

Diagnosis: Males of *Opopaea fosuma,* n. sp. are separated from those of most other *Opopaea* species by the structure of the palp. The cymbium and bulb are only partly fused in *O. fosuma*, n. sp. whereas they are completely fused in most other *Opopaea* species, and the embolus is long and slender in *O. fosuma,* n. sp. The species is similar to the Micronesian *O. foveolata* Roewer, 1963, where, according to the original description, the cymbium and bulb of the male palp are also only partly fused (Roewer, 1963: fig. 6g). Males of *O. fosuma,* n. sp. differ from those of *O. foveolata,* however, by their long and slender embolus, whereas it is rather short and blunt in *O. foveolata*.

Male: *Measurements* ($n=1$): Prosoma length 0.59, width 0.48, height 0.23. Opisthosoma length 0.67, width 0.42, height 0.41. Appendages:

	Fe	Pa	Ti	Mt	Ta	Total
Leg I	0.37	0.23	0.26	0.20	0.15	1.21
Leg II	0.32	0.20	0.22	0.19	0.15	1.08
Leg III	0.29	0.15	0.16	0.17	0.15	0.92
Leg IV	0.41	0.22	0.30	0.26	0.20	1.39
Palp	0.12	0.16	0.06	—	0.16	0.50

Colour (alcohol-preserved material): Prosoma and chelicerae orange; legs and palps light orange. Opisthosoma: ventral scutum orange; dorsal scutum and sclerite that partially surrounds spinnerets light orange; spinnerets pale yellow; soft areas white. *Carapace*: Ovoid; narrowed in eye region (Fig. 1), slightly ascending behind PME, then almost horizontal and posterior 1/3 steeply descending (Fig. 2). Tiny hairs forming U-shaped band; three areas of tubercles near lateral borders; additional hair-bearing tubercles along lateral borders, some in posterior area (Fig. 1). *Eyes*: Six; almost circular, with roughly same diameter; AME lacking; eye group occupies slightly more than 1/2 width of head; posterior row slightly recurved (Fig. 1); PME almost contiguous; ALE separated from PME and from PLE by 1/2 and from each other by one diam. of ALE; PLE separated from PME by 1/2 diam. of PLE (Fig. 3). Exact position and size of eyes somewhat variable. *Clypeus* (Fig. 3): Two diam. of ALE high; slightly extended between chelicerae in middle. *Sternum and*