

An unusual palaeobiocoenosis of subfossil spiders in Colombian copal

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Summary

Inclusions in amber and copal provide us with a unique insight into terrestrial palaeocommunities because they represent a palaeobiocoenosis: a naturally co-occurring group of organisms that perished at the same point in time and in the same place. We report the first (sub)fossilized example of a spider population, preserved in Colombian copal, which has been dated back as far as 1736 +/- 35 years. The specimen contains 26 spiders belonging to the *Euryopsis/Emertonella* genus complex (Theridiidae). Such subfossils in copal provide exciting new research opportunities in molecular palaeobiology for investigating changes in genetic variation within a group at the threshold of ecological and evolutionary timescales.

Introduction

Organisms preserved in fossil resins provide us with a unique insight into extinct communities because it is evident, in most instances, that all inclusions in a single piece of amber or copal perished at the same point in time and in the same place. Hence, they most often represent a palaeobiocoenosis (a portion of a biocoenosis preserved in fossil form), rather than a thanatocoenosis or taphocoenosis in which, although many organisms may be preserved together, it is unclear whether they co-existed (and died simultaneously) in space and time; the latter situation is more often observed with fossils preserved in sediments than in resins. As an example, a highly unusual piece of Cretaceous amber (5 × 3 × 2.5 cm) from Archingeay, France, contains a total of 274 syninclusions, as follows: 86 arthropods (19 families in 13 orders), 181 microbes and 7 feathers (Perrichot & Girard 2009).

Subfossil spiders, by definition, are relatively young in geological terms, but nonetheless have the potential to be highly informative at many different levels (Scott 2003; Penney & Preziosi 2010). One source of these spiders is as inclusions preserved in copal, the subfossilized tree resin

precursor of amber. Copal deposits occur around the world, with particularly large deposits in Santander, Colombia, which were recently radiocarbon dated at 1736 +/- 35 years old (D. Grimaldi pers. comm. 2011), although younger ages have also been obtained using this method. Here we report on a remarkable specimen from this locality that contains a palaeobiocoenosis consisting predominantly of adult (both male and female) spiders and discuss the potential of such specimens for molecular palaeobiological research.

Material and Methods

The Colombian copal sample is an ovate, clear specimen of 65 × 36 mm and 19 g (Fig. 1A) and is held on display in the museological department at Lyme Regis Fossils, Lyme Regis, UK. The copal was examined using a Leica stereomicroscope and light microphotographs were assembled from a stacked series of digital images recorded by a Nikon Coolpix 4500 camera mounted on a Leica M10 stereomicroscope with 0.63× and 1.6× planapochromatic objectives (Green 2005).

Results

The spider inclusions, which consist of 26 mature individuals (18 males and 8 females) with a body length of approximately 2 mm, all belong to the extant *Euryopsis* Menge, 1868/*Emertonella* Bryant, 1945 complex (family Theridiidae: Hadrotarsinae *sensu* Agnarsson 2004), identified based on the genitalia of both mature males and females (Fig. 1C,D); the pedipalp being of rather simple configuration and the epigyne consisting of a small semi-sclerotized opening and two pairs of spermathecae. In addition, the general somatic morphology (Fig. 1B), such as absence of a colulus, relative leg lengths, the presence of weak spines on the tibiae and patellae, anterior median eyes largest above a high clypeus, and the abdomen shape tapering posteriorly confirm placement in this genus complex. It was not possible to assign the inclusions with certainty to any of the known extant species, although there is no reason to suggest they represent an extinct species. Co-occurring inclusions (syninclusions) in this sample include various Diptera, Psocodea, Hymenoptera (ants and wasps), Trichoptera, Collembola, insect frass, and a leaf.

Discussion

The obvious thought on first examination of this specimen was that it probably contains a sample of a population of social spiders. The epitome of social behaviour in arthropods is seen in the highly diverse eusocial Hymenoptera (ants, bees and wasps) and the Isoptera (termites), in which different castes have developed to perform different roles at the expense of their own reproduction. The presence of some of these groups in Cretaceous ambers (Penney 2010) and other deposits (Martínez-Delclòs & Martinell 1995) suggests that this behaviour had already evolved by the Mesozoic. In contrast, communal/eusocial spider species

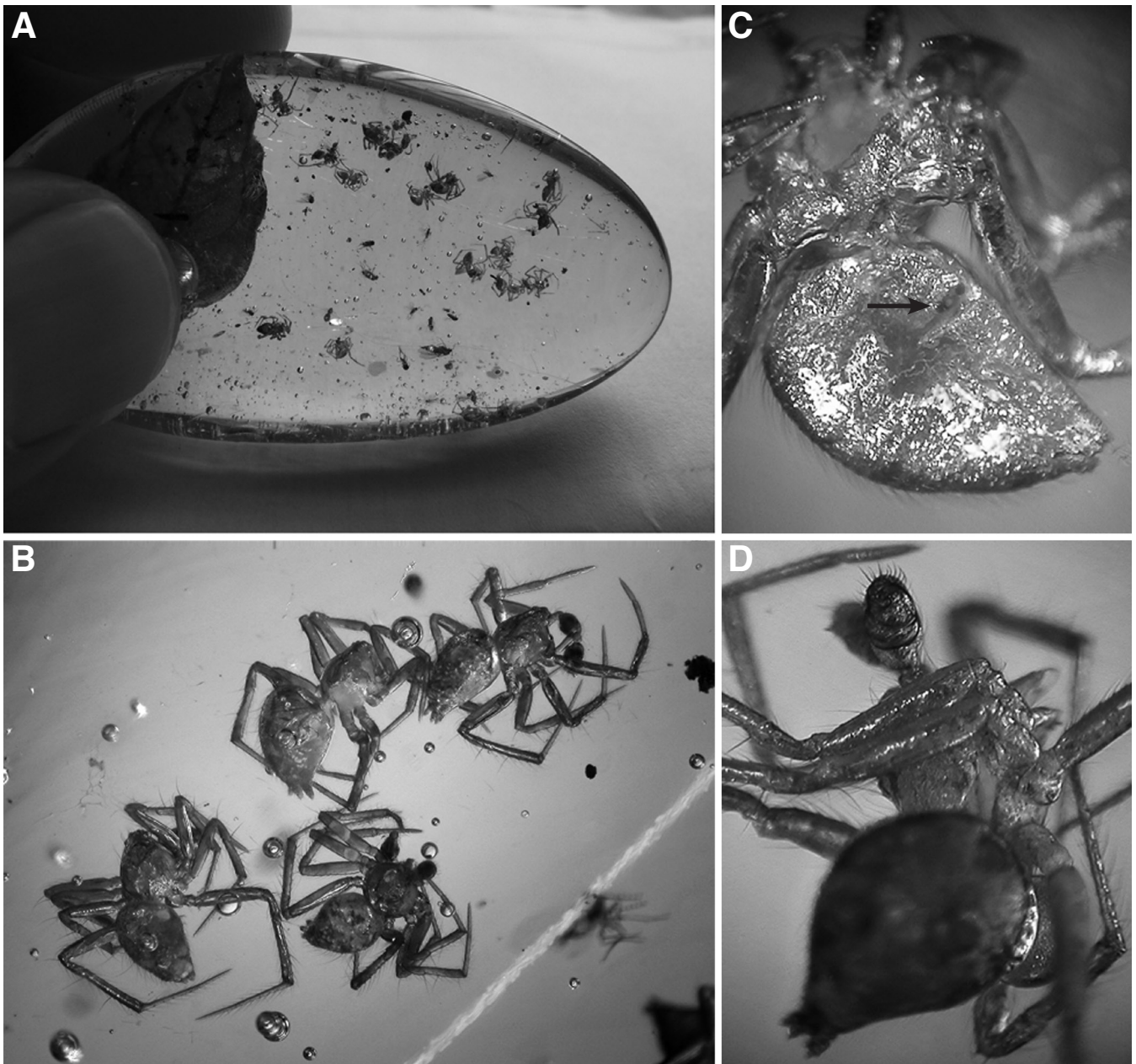


Fig. 1: A palaeobiocoenosis dominated by subfossil *Euryopsis/Emertonella* spiders (Theridiidae) in copal from Colombia. **A** Whole specimen; **B** Close-up of the spider inclusions; **C** Female genitalia (arrowed); **D** Male genitalia. Body length of spiders \approx 2 mm.

are uncommon today and are unknown in the fossil record (Selden & Penney 2010; Penney & Selden 2011). Indeed, most spider species are aggressive towards conspecifics and given the absence in the fossil record of genera known to be social today, social behaviour in spiders may be a relatively recent phenomenon in geological terms. Possibly the most familiar and best studied social spiders belong to the extant family Theridiidae (e.g. the genus *Anelosimus* Simon, 1891), the same family to which the spiders in our specimen also belong. However, closer scrutiny of the specimen and the available literature provide an alternative explanation for the co-occurrence of these inclusions.

Euryopsis includes 74 species with a global distribution (Platnick 2012) (although some species listed here are considered to belong in *Emertonella*); they are hitherto known in the fossil record from four species in Tertiary Baltic (Eocene) and Bitterfeld (?Oligocene) ambers

(Wunderlich 2008; Dunlop *et al.* 2012), described on the basis of well preserved male spiders, although none of the amber pieces containing these spiders preserves more than one individual. The New World *Euryopsis* were revised in the mid twentieth century (Levi 1954, 1963) but the delimitation of this genus, especially with regard to its putative sister taxon *Emertonella* (and *Dipoena* Thorell, 1869), is equivocal. This is evident from the large number of systematic transfers in the literature (Platnick 2012), and a single cladistic analysis of all taxa from these genera is required in order to resolve this issue to modern standards.

It is impossible to determine whether or not the spiders preserved in the specimen described were engaged in any degree of co-operative behaviour, but based on what little is known about the behaviour of extant species this is unlikely. *Euryopsis/Emertonella* species do not construct webs and most are thought to prey on ants (five worker ants from two

different species are present as syninclusions). The predatory behaviour of *Emertonella* (*sensu* Agnarsson 2004) *funnebris* (Hentz, 1850), and how these spiders immobilize their ant prey by throwing adhesive-laden silk which traps them to the substrate, has been described in detail (Carico 1978). *Euryopsis/Emertonella* spiders are found most often on vegetation, under bark or moss, or on the ground under stones (Levi 1954), and in some species they can occur together in large numbers (Carico 1978; Porter & Eastmond 1982). Although this facet of their ecology is poorly documented, it serves to explain the spider-dominated palaeobio-coenosis observed in this subfossil.

Individuals that live in very close proximity to one another, and reproduce under such circumstances, have the potential to cause a dramatic shift from an outbred, panmictic population structure to one which consists of a strongly inbred population. For example, social individuals tend to be genetically homozygous with only slight intra-population variation compared to individuals belonging to subsocial or solitary species. Such inbred populations can be highly susceptible to sudden environmental changes so, while they may benefit in the short term (ecological time), they may fail to diversify in the long term (geological or evolutionary time) (Agnarsson 2006). Testing the idea of disparate success between these different time scales would normally rely on indirect evidence based on population genetics, and phylogenetic research based on both morphological and molecular data derived from extant populations. However, the discovery of subfossil specimens as described herein, in which all individuals co-existed in both space and time, provides an alternative potential research approach to this issue: molecular palaeobiology.

While molecular techniques have been applied successfully to extract ancient DNA from a variety of sources, previous claims of arthropod DNA extraction from amber using traditional PCR techniques appear to have been in error (Austin *et al.* 1997) with the DNA sequences that were obtained probably deriving from modern non-insect DNA of fungal and/or vertebrate origin that had contaminated the samples. However, the problems with extracting DNA from amber specimens many millions of years in age cannot necessarily be extrapolated to copal samples, most of which are probably Pliocene–Recent (2.5 Ma–200 a) in age (Ragazzi *et al.* 2003). In other areas of ancient DNA research, many of the limitations of the conventional PCR approach to sequence retrieval have been sidestepped by switching to next-generation sequencing methods (Callaway 2011). These methods are ideal for ancient DNA because they provide sequences for all the DNA molecules in an extract regardless of their length, and avoid contamination-prone targeted PCRs. Theoretical and empirical data indicate that ancient DNA fragments >50 bp are present in well preserved materials up to at least 100,000 years in age (Hebsgaard *et al.* 2005). Given their excellent preservation and relatively young geological nature, it is not unreasonable to expect successful extraction of DNA from copal inclusions in the very near future. Unfortunately, the sampling technique used for potential DNA harvesting from subfossil resins currently requires gaining physical access to the body cavity of the inclusion and so is destructive.

Such studies would open up previously unexplored research avenues. They would provide data on intra- and interspecific genetic diversity to pre-date any current information based on museum collections, creating the opportunity to investigate changes in genetic variation within a group at the threshold of ecological and evolutionary time scales. Genetic information from copal specimens would bridge the gap between contemporary/museum biodiversity and the fossil record, and would directly inform phylogenetic and evolutionary genetic research. Perhaps most importantly, copal inclusions could yield information on how genetic variation within species (a less familiar but important component of biodiversity) has changed over the scale of a few thousand years. Another exciting possibility is obtaining sequence information for species dichotomies that are not at the tips of phylogenetic branches and the resulting potential for more accurate DNA sequence substitution rate calculations and, hence, a better calibrated molecular clock. We must stress that working with molecular data of such a relatively young age requires the precise assignment of the inclusions to modern species (not just genera or lineages) and highlight that even very small errors in dating of the copal would strongly affect the estimated age of origin of lineages in deep time (as a result of how dating programs actually work).

Rather than considering subfossils in copal as too young to be of any significance, as seems to be the consensus of many palaeontologists, we believe that molecular palaeobiological investigations employing next generation sequencing techniques may be very rewarding.

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