

Molecular data can help to unveil biogeographic complexities since the Miocene: lessons from ameronothroid mites and isotomid springtails

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Summary The diversification of Antarctic springtails and mites is likely to have occurred as a result of the glaciation and isolation of the Antarctic continent completed by ~10 million years ago, and not by the sequential break-up of Gondwana (completed by ~32 million years ago). More recently, population level structuring is likely to have been driven by the repeated glacial cycles of the Pleistocene (<2 million years ago) or common circum-polar corridors. It is clear that current taxonomic designations based on morphology for these springtails and mites are in conflict with our molecular topologies. These taxonomic inconsistencies are clear given the large number of paraphyletic species presented here. Our data show clear inconsistencies between the contemporary taxonomy and molecular evidence and we propose a full taxonomic revision for the ‘*Cryptopygus*’ group, the *Halozetes* genus, and indeed the larger ameronothroid group where detailed morphological investigations may help to resolve “unexpected” relationships when taking traditional classifications into account.

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Antarctic biogeography

The biogeography of the Antarctic is both complex and contentious with much of this complexity deriving from the compound history of the continent and its surrounding islands. Antarctica itself is an amalgam of a large, eastern Antarctic block and a western complex of accreted terranes (Vaughan and Storey, 2000; Clarke, 2003). Likewise, its surrounding islands have a variety of geological origins and histories, ranging from relatively young volcanic islands like Heard Island and the Prince Edwards (McDougall et al., 2001), to more complex older islands and archipelagos such as South Georgia (Peck et al., 2007) and Îles Kerguelen (Wallace et al., 2002). There is much debate in the literature concerning the origins of terrestrial Antarctic biotas (Brundin, 1970; Udvardy, 1987; Marshall and Pugh, 1997; Morrone, 1998; Greve et al., 2005; Allegrucci et al., 2006) and whether they are a consequence of dispersal or of vicariance.

Perhaps the most important reason for the current situation is the absence of molecular work for most terrestrial species. To date, most molecular work has concerned population structure of specific species within given areas of the Antarctic or sub-Antarctic, in plants (reviewed in Skotnicki et al., 2000) and arthropods (reviewed in Stevens and Hogg, 2006a). Exceptions are one recent investigation of the moss *Ceratodon purpureus* (Skotnicki et al., 2004), and several studies of seabirds (e.g. Nunn, 1996; Burg and Croxall, 2001, 2004). This situation is understandable given the large size of Antarctica and the difficulty of reaching its isolated fragments of ice-free land, as well as the wide distribution and isolation of the Southern Ocean islands. However, recent collaborative efforts within the “Evolution and Biodiversity in Antarctica” (EBA) programme of the Scientific Committee on Antarctic Research (<http://www.scar.org/researchgroups/lifescience/eba/>) have enabled cross-regional sampling to be undertaken across the sub-Antarctic and continental Antarctic regions. We therefore present here the first comparative broad-scale molecular phylogenetic study of terrestrial organisms, the ameronothroid mites and isotomid springtails, as a basis for understanding the evolution and biogeography of terrestrial invertebrate taxa in the Antarctic region. Such information will also provide further understanding of the evolutionary basis for species' distributions and genetic diversity

in the Southern Hemisphere. We focused on these groups since they often feature in biographical studies due to their limited dispersal capabilities and their status as ancient groups (see for example Wise, 1967; Wallwork, 1973; Stary and Block, 1998; Stevens and Hogg, 2006a,b). They are also abundant and well represented in the South Polar Region (Wise, 1967; Wallwork, 1973, Stary and Block, 1998). Lastly, the mites are unique considering their ability to occupy a diverse array of habitats (they extend to the marine intertidal fringes; Marshall and Convey, 2004) allowing us to test several biogeographical and evolutionary hypotheses.

The ameronothroid mites

The taxonomy of the superfamily Ameronothroidea (Oribatida) is very uncertain with various proposed classifications. In general, it is thought that the superfamily contains approximately 40 peri-Antarctic species partitioned into three genera namely *Podacarus* (Grandjean, 1955), *Alaskozetes* (Hammer, 1955) and *Halozetes* (Berlese, 1917). In short, we included eight *Halozetes* species (*H. fulvus*, *H. crozetensis*, *H. belgicae*, *H. marinus*, *H. marionensis*, *H. intermedius*, *H. macquariensis* and *H. capensis*) incorporating four subspecies (*H. m. marinus*, *H. b. mickii*, *H. b. devilleirsii* and *H. b. brevipilis*) as well as representatives of *Podacarus auberti* and *Alaskozetes antarcticus*. Several outgroup taxa were also included amongst them more distantly related oribatid taxa such as *Aquanothrus montanus* (Ameronothroidea) believed to be an ancient species (Norton et al., 1996), *Magellozetes antarcticus* (Ceratozetoidea) and *Macquarioppia striata* (Liacaroidea). The islands included are Marion, Possession, Heard, Kerguelen, Macquarie and South Sandwich and South Georgia as well as localities from the Antarctic Peninsula and South Africa. Our phylogeny is based on the protein coding mitochondrial cytochrome oxidase I gene as well as the nuclear histone-3 gene, and we applied a variety of molecular and biogeographical analyses to our data.

Several striking results emerged from our study, and these are discussed in more detail below. First, our findings indicated that there was a transition from intertidal ameronothroid mites to terrestrial/supralittoral mites and it would appear that niche occupation may be a possible driver of diversification. Our findings support the proposal that sub-Antarctic intertidal ameronothroid mites are more primitive than those from other biotopes (Wallwork, 1973; extensive further work on this topic have been done, see for example Proches and Marshall, 2001) and subsequently radiated to the younger terrestrial areas. In a broader sense, when we consider the outgroups, especially *Aquanothrus montanus*, which is considered to be the ancestor to these mites, we might also speculate that the ancient origin for these mites were terrestrial (it should, however, be noted that *Aquanothrus* is capable of surviving under water for extended periods). A transition from terrestrial to marine and then back to terrestrial might have occurred for the ameronothroid mites. Secondly, molecular data suggest that this group is relatively recent and post dates large scale continental drift. In this respect, contemporary biogeography has largely been sculpted by dispersal. This is supported by a congruent pattern in both marine (Antarctic notothenioid fish and bivalve fauna; Bargelloni et al., 2000) and terrestrial taxa (the moss *Ceratodon purpureus*; Skotnicki et al., 2004), despite the considerable differences between these two environments (Peck et al., 2007). For all these taxa, multiple independent dispersal events occurred across the APFZ with concomitant diversification within it. To further substantiate our findings of a more recent history for the Ameronothroidea, we applied a conventional molecular clock as well as a relaxed Bayesian clock to our data. Molecular clocks are dependent on the genes used; therefore these dates should only be seen as estimates, especially since no fossil data are available for calibration. The estimates of both clocks indicated that some Antarctic ameronothroid mites may be very young (<10 million years old), a notion that is contrary to the belief that they comprise an ancient group. Our estimates are also consistent with molecular clock calculations for *Limatula* (for 16S: 1.36-8.03 million years; for 18S:6.81-19.12 million years; for ITS-1:0.24-2.87 million years) and most of the notothenioid fishes (5-12 million years). Thus, the molecular data indicated that ameronothroid mites together with several other groups, diverged after the APFZ was formed since the Drake Passage opened ~45–30 million years ago (Livermore et al., 2007).

The isotomid springtails

The genus *Cryptopygus* (Collembola, Isotomidae) is relatively speciose and one of the few springtail genera that includes extant continental Antarctic species (Wise 1967; Stevens and Hogg 2006a). Like the ameronothroid mites, the taxonomy of the Isotomidae is constantly being revised. The *Cryptopygus* group has several interesting elements, namely the apparent circum-polar

distribution of *Cryptopygus antarcticus antarcticus* combined with a number of subspecies, as well as the presence of closely related species in continental Antarctica (Wise, 1967). Here, collections were made from four continental Antarctic sites along the Trans-Antarctic Mountains (Ross Sea Region) to obtain *Antarctophorus subpolaris* (from Beardmore Glacier), *Neocryptopygus nivicolus* (McKay Glacier), *Gressittacantha terranova* (Terra Nova Bay), and *Cryptopygus cistantarcticus* (Cape Hallett). In addition, taxonomically related *Cryptopygus* species (*Cryptopygus a. antarcticus*, *C. a. maximus*, *C. a. reagens*, *C. a. travei*, *C. dubius*) were obtained from three islands along the Antarctic Peninsula, several sub-Antarctic islands (Îles Kerguelen, Îles Crozet, Marion Island, Macquarie Island, Heard Island), Tasmania and mainland Australia, New Zealand, and Patagonia (see Stevens et al., 2006 for complete details). We used the mtDNA (COI) gene to examine the diversity and relationships (maximum likelihood and bayesian models) of sub-Antarctic and Antarctic springtails throughout the higher latitudes of the Southern Hemisphere (>35°S). We used five outgroup taxa, including *Desoria klovstadi* from Cape Hallett.

We identified high levels of divergence between *Cryptopygus* species and related genera, which may indicate divergences for this group in the middle to late Miocene (~23-5 million years ago). However, the range of within-species sequence diversity was remarkable. For example, the paraphyletic lineages and high sequence divergence levels greater than 14% among *Cryptopygus a. antarcticus* populations indicate that these lineages have not shared a common evolutionary history for ~ 23 to 5.4 million years. Likewise, comparisons between the *Cryptopygus* spp. and related genera from the Antarctic continent indicate that *C. a. antarcticus* is a complex of undescribed species, but are also paraphyletic with some having closer affinities to the Antarctic genera than to *C. a. antarcticus sensu stricto* (type locality: Gerlache Straits; Antarctic Peninsula). This species now appears restricted to the Antarctic Peninsula and offshore islands (maritime Antarctica) (with low divergence, 1.5-6.3%). The level of sequence divergence found among the described *Cryptopygus* subspecies (*travei*, *reagens*, *maximus*) indicate that they are species, for example the level of sequence divergence (12.3-17.4%) of *C. a. maximus* (from Îles Kerguelen and Macquarie Island) is consistent with springtail species elsewhere (e.g., >14%; Hogg and Hebert, 2004). In contrast, low levels of sequence divergence revealed several dispersal pathways in common with the ameronothroid mites. In particular, among *C. a. antarcticus* populations from the Antarctic Peninsula (isolation during the Pleistocene). Similarly, Macquarie Island has been above sea-level for ~700-500 ky and colonized recently by at least three lineages (1) undescribed species (previously *C. a. antarcticus*) around 550 to 300 thousand years ago from Heard Island; (2) less than 1.6 million years ago from Îles Kerguelen (*C. a. maximus*); and (3) undescribed species of unknown origins. Such closely related lineages exist only where suitable ocean currents can act as “dispersal corridors” for springtails; these corridors appear to have played a similar role for the ameronothroid mites, particularly for islands with a relatively recent origin which now provide suitable molecular clock calibrations. The deep divergences for the four endemic continental Antarctic species to each other and to other circum-Antarctic lineages suggest that their origins are likely to be during the Miocene (~21-11 million years ago). The molecular evidence here indicates that the continental Antarctic species were completely isolated from surrounding sub-Antarctic and temperate landmasses once sea/glacial ice was sufficient to restrict successful oceanic dispersal (~23-10 million years ago) (see Stevens et al., 2006). Our molecular dates correlate well with glacio-geological dates of fossils for the isolation and persistence of now-extinct Gondwanan flora and fauna in the Trans-Antarctic Mountains (e.g., Ashworth and Cantrill, 2004).

Summary

Our results convincingly show that speciation as well as population structuring in Antarctic springtails and mites were predominantly driven by glaciations and concomitant isolation. As a result, we argue that these taxa are evolutionary young groups (isolation of the Antarctic continent was completed ~ 10 million years ago). In addition, we propose a full taxonomic revision for the ‘*Cryptopygus*’ group, the *Halozetes* genus and indeed, the larger ameronothroid group.

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