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Phylogeographic analysis reveals multiple cryptic species of amphipods (Crustacea: Amphipoda) in Chihuahuan Desert springs

Richard A. Seidel [a,*], Brian K. Lang [b], David J. Berg [c]

[a] Department of Zoology, Miami University, 212 Pearson Hall, Oxford, OH 45056, USA
[b] New Mexico Department of Game and Fish, One Wildlife Way, Santa Fe, NM 87507, USA
[c] Department of Zoology, Miami University, 1601 University Blvd., Hamilton, OH 45011, USA

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ABSTRACT

Biodiversity conservation and the identification of conservation units among invertebrates are complicated by low levels of morphological difference, particularly among aquatic taxa. Accordingly, biodiversity is often underestimated in communities of aquatic invertebrates, as revealed by high genetic divergence between cryptic species. We analyzed PCR-amplified portions of the mitochondrial cytochrome *c* oxidase I (COI) gene and 16S rRNA gene for amphipods in the *Gammarus pecos* species complex endemic to springs in the Chihuahuan Desert of southeast New Mexico and west Texas. Our analyses uncover the presence of seven separate species in this complex, of which only three nominal taxa are formally described. The distribution of these species is highly correlated with geography, with many present only in one spring or one spatially-restricted cluster of springs, indicating that each species likely merits protection under the US Endangered Species Act. We present evidence suggesting that habitat fragmentation, long-distance colonization, and isolation-by-distance have occurred at different temporal and spatial scales within this system to produce the lineages that we report. We show that patterns detected in the *G. pecos* species complex also correlate with endemic fishes (*Gambusia* spp., pupfish) and hydrobiid snails. Our results provide clues important for future biodiversity investigations in geographically isolated aquatic habitats, and shed light on the understudied and underestimated levels of biodiversity present in desert spring systems.

1. Introduction

Biodiversity conservation relies heavily on the identification and description of units of conservation. The determination of these minimal units brings the field of conservation biology into close association with systematic biology, a discipline that seeks to discover monophyletic groups at higher taxonomic levels, and to delineate distinct lineages at lower levels (Dimmick et al., 1999; Wheeler and Meier, 2000). The identification of discrete species as units of conservation continues to present challenges for investigators, but recent approaches represent improvement in analytical detection of discontinuous evolutionary groups. While natural processes including speciation and extinction produce relatively deep divergences between species (depicted by longer branch lengths between species on phylogenetic trees), relatively recent coalescence processes produce a multitude of shallow branches at the population level (Pons et al., 2006). Recently Pons et al. (2006) emphasized the logic and practical application of combining the approaches of phylogenetics and population genetics, the two programs which investigate biological relationships above and below the species boundary (Brower et al., 1996). In conservation practice, genetic investigations have helped identify appropriate conservation units, including geographical locations where appropriate management actions would be most effectively implemented in organisms as varied as Komodo dragons (Ciofi et al., 1999) and southwest Australian plants (Coates, 2000).

One practical challenge in assessing biodiversity involves the difficulty of identifying the units of diversity in the field, which undermines the reliability of estimates of distribution (McNeely et al., 1990). The challenge becomes even more acute when the task focuses on aquatic invertebrates, which often display low levels of morphological distinctiveness (Müller, 2000; Pfenninger et al., 2003; Witt et al., 2003). This might be because the actual cues used by aquatic taxa for conspecific recognition may not involve the same morphological characters used by taxonomists for species determination (Knowlton, 1993). Given such low levels of morphological difference, we would expect traditional taxonomy

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[*] Corresponding author. Present address: Department of Biology, Southern Adventist University, Hickman Science Center, Collegedale, TN 37315, USA. Tel.: +1 423 236 2926; fax: +1 423 236 1926.

E-mail addresses: seidelr@southern.edu (R.A. Seidel), brian.lang@state.nm.us (B.K. Lang), bergdj@muohio.edu (D.J. Berg).

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to underestimate marine and freshwater biodiversity (Thorpe and Solé-Cava, 1994; Gómez et al., 2002). With the advent of molecular techniques, conservation biologists now have additional means for discovering diagnostic characters in organisms that are indistinguishable based on morphology alone. Molecular genetic techniques have revealed substantial hidden diversity within morphologically delimited species (Remerie et al., 2006), and unusually high levels of genetic divergence between cryptic species (Bucklin et al., 1995; Knowlton and Weigt, 1998; Lee, 2000). Identification of species boundaries is particularly crucial in situations involving endangered species assessments. For 38 recent endangered species petitions in the United States, 81% of those showing genetic distinction were granted protection status (Fallon, 2007), which underscores the importance of using genetic markers to reveal important differences among morphologically similar taxa, particularly among aquatic invertebrates.

Amphipods comprising the *Gammarus pecos* species complex (Cole, 1985) are endemic to spring systems associated with the Pecos River of New Mexico and Texas (Fig. 1). It has been hypothesized that these freshwater amphipods are derived from a broadly distributed marine progenitor that became isolated inland upon the recession of the Western Interior Seaway from the North American continent during the Late Cretaceous (Bousfield, 1958; Holsinger, 1976; Baldridge, 2004). Members of this complex likely speciated in response to diverse ecological conditions that developed in various aquatic environments differing in elevation, substrate mineral composition, drainage patterns, and local hydrochemical conditions. This complex consists of three nominal species (*G. pecos* Cole and Bousfield, 1970; *Gammarus desperatus* Cole, 1981; and *Gammarus hyalelloides* Cole, 1976) differentiated by morphology, at least six populations of undetermined taxonomic affinity that may represent several undescribed species, and at least two other populations presumed to be extirpated (Cole and Bousfield, 1970; Cole, 1976, 1981, 1985). All current species designations (Table 1) based on earlier morphological findings are treated as hypotheses which will be evaluated based upon the genetic results we present. Presently, this group of endemic amphipods is confronted with a high rate of imperilment related to habitat modification and groundwater withdrawal (Lang et al., 2003). Loss of spring habitat by groundwater mining and habitat alterations (e.g. diversions, damming, dewatering, channelization) is a major threat to aquatic biodiversity in arid regions of the western United States (Glennon, 2002), where isolated spring systems often harbor unique assemblages of narrowly endemic biota (Minckley and Unmack, 2000; Hershler and Sada, 2002; Sada and Vinyard, 2002; Sada et al., 2005).

In this study, we combine a model-based tree estimation method with a population genetic analysis to investigate the phylogeography and species composition of amphipods comprising the *G. pecos* species complex. We employ the Wiens and Penkrot (WP) method to detect species using a general tree-based inference protocol (Wiens and Penkrot, 2002, Fig. 1) by constructing a phylogenetic tree based upon the mitochondrial cytochrome c oxidase subunit I (COI) and the 16S rRNA (16S) gene fragments. Our objective in this study was to evaluate these high resolution genetic data to clarify the number of species present in a faunal group of conservation concern. While a previous investigation of this species complex using allozymes allowed some degree of resolution in detecting differences among amphipod populations (Gervasio et al., 2004), our sequencing of the partial mitochondrial genome provides greatly increased resolution, revealing the presence of previously undetected species that may merit conservation action. Because similar processes of geographic isolation following diversification are likely to operate on other Chihuahuan Desert aquatic biota (Sei et al., 2009), our results are likely to predict biogeographic patterns for many other taxa.

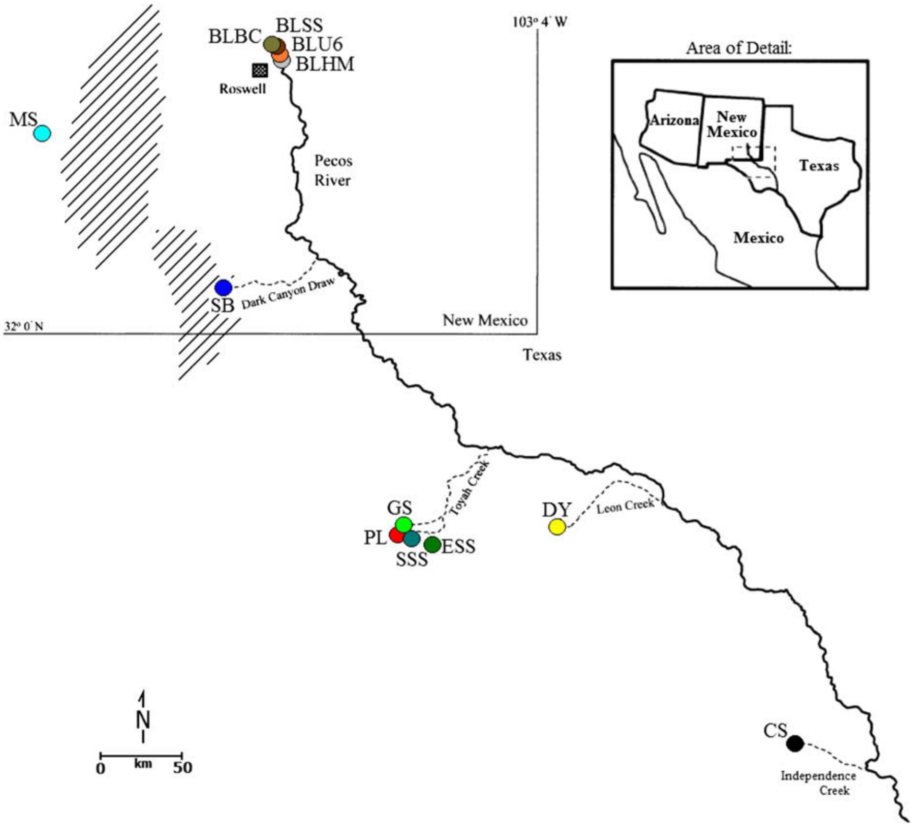

Fig. 1. Collection sites for *G. pecos* complex populations from the Chihuahuan Desert. Mountains are indicated by diagonal lines. See Table 1 for population codes.