SPECIES DELIMITATION OF THE NATIVE NORTH AMERICAN FIRE ANTS (FORMICIDAE: *SOLENOPSIS*): RESULTS AND APPLICATIONS TO LIFE SCIENCES EDUCATION

by

PABLO CHIALVO

(Under the Direction of KENNETH G. ROSS)

ABSTRACT

The native North American fire ants (*Solenopsis*) comprise a difficult group taxonomically that has undergone multiple revisions in the past century yet remains in a state of taxonomic uncertainty. In this study, we utilized a large set of microsatellite markers to conduct the first robust genetic analysis of the valid nominal species. Our approach used a variety of methods to test operational criteria commonly employed in species delimitation, including genotypic clustering, reproductive isolation/cohesion, and monophyly. We conclude from our results that the recognized North American fire ant species represent evolutionarily independent entities and, moreover, we confirm the presumed sister status of the desert fire ants, *S. aurea* and *S. amblychila*. However, the presence of at least two genetically divergent populations within the valid nominal species boundaries, including a western form of *S. xyloni* and a distinct population of *S. aurea* endemic to the Salton Trough, suggests that the current taxonomy does not fully capture the species-level diversity in this group. Based on morphological analyses, we proposed raising *S. maniosa*, junior synonym and western counterpart of *S. xyloni*, to full species status and, moreover, described a new species of desert fire ant, *S. arieli*, collected from the Salton Trough. Finally, we developed two active learning modules: a narrative-focused approach to teaching the theory of natural selection, and a data-driven activity that uses data collected in our delimitation study to explore species concepts.

INDEX WORDS: *Solenopsis*, fire ants, alpha taxonomy, species delimitation, population genetic structure, transformative learning

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DEDICATION

For Clare, still.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Taxonomic history of native North American fire ants

The genus *Solenopsis* Westwood 1830 (Formicidae: Myrmicinae) is a cosmopolitan taxon with 195 species worldwide (Bolton, 2014). Of particular interest to entomologists is a small subgroup of species collectively referred to as the “fire ants” (formerly the subgenus *Solenopsis;* Trager, 1991), which, unlike their congeners, have large polymorphic workers and reside exclusively in the New World. Due to their complex morphology, the fire ants have undergone numerous taxonomic revisions and, consequently, have garnered a level of notoriety amongst myrmecologists (Trager, 1991; Pitts *et al*., 2005). The native North American fire ants in particular have changed substantially within the last 150 years. Nominal taxa have shifted repeatedly between species and subspecies ranks – as well as between valid and invalid junior synonym status – over a short time period, reflecting continued disagreement among researchers.

The study of native North American fire ant taxonomy began in earnest with the placement of *Atta geminata* Fabricius 1804 into the genus *Solenopsis* by Mayr (1863). *Solenopsis geminata* quickly became a “sink” for other taxa within the subfamily Myrmicinae; between 1863 and 1991, over 20 species, subspecies, and varieties were synonymized with *S. geminata*, including what would become the other valid nominal North American species (Trager, 1991; Bolton, 1995). Early taxonomic revisions by Wheeler (1906, 1915) recognized *S. geminata geminata*, *S. geminata xyloni*, *S. geminata* *maniosa*, and *S. aurea amblychila*. Creighton (1930) later elevated *S. xyloni* back to species status, but designated all other previously described native forms (other than *S. g. geminata*) as variants of *S. xyloni*. In his follow-up revision, he returned *S. aurea* to species status, demoted *S. amblychila* to a subspecies of *S. aurea*, and synonymized *S. maniosa* under *S. xyloni* (Creighton, 1950). This arrangement only lasted a few years. Strangely, T.W. Cook (1953) – who had never formally published any work regarding ants – reverted to Creighton's earlier (1930) taxonomy. Though his book was considered deficient by many (Brown & Wilson, 1953; Francoeur & Snelling, 1979; Ward, 2005), the taxonomy of the native North American fire ants did not change substantially for another decade. In 1963, however, Snelling published an updated revision in which he reinstated Creighton's latter (1950) treatment, with the exception of *S. amblychila*, which he synonymized under *S. aurea*. He (along with George, 1979) later suggested that *S. maniosa* should be considered a separate, western counterpart to *S. xyloni*, citing differences in venom chemistry (Brand *et al.*, 1972; Blum *et al.*, 1985). Wheeler & Wheeler (1986) agreed with the assessment and raised *S. maniosa* from synonymy.

Finally, in 1991, Trager completed an exhaustive morphological revision of the entire *S. geminata* species group, combining elements of previous investigations with his own findings to produce what we now consider to be the most modern or updated taxonomy (though see Pitts *et al.*, 2005 for minor organizational changes). Currently, only four species are recognized: *Solenopsis geminata* Fabricius 1804, *S. amblychila* Wheeler 1915, *S. aurea* Wheeler 1906, and *S. xyloni* McCook 1880. Trager fully acknowledges, however, that a more thorough taxonomic assessment is required to determine the true number of the species in this group and that genetic data may provide more conclusive results (J. Trager, personal communication).

Original contributions to the taxonomy of *Solenopsis*

In Chapter 2, we follow up on Trager's suggestion and employ a large set of highly variable genetic markers (microsatellites) to test several common operational criteria for species delimitation, including the presence of genotypic clusters, reproductive isolation or cohesion, and monophyly of populations. Such an integrated approach to taxonomy (Dayrat, 2005; Schlick-Steiner *et al*., 2010) has successfully been used in many other insect groups, including moths (Yang *et al*., 2012), bumblebees (Lecocq *et al*., 2015), fruit flies (Krosch *et al*., 2013), and ants (Blaimer, 2012). The goal of this investigation is threefold: 1) to conduct the first comprehensive population genetic analysis of the native North American fire ants; 2) to test whether the valid nominal species correspond to genetically distinct groups and/or contain unrecognized population structure (potentially cryptic species); and 3) to provide a strong body of genetic evidence to frame future morphological, ecological, and behavioral investigations of this group, including the preliminary taxonomic revision found in Chapter 3 of this dissertation.

Original contributions to life sciences education

Chapter 4 is a slight departure from ant taxonomy and explores the fascinating topic of transformative learning theory as proposed by Mezirow (1978, 1991, 2006). This pedagogical theory represents the unique fusion or intersection of several distinct lines of philosophical thinking, including strong influences by Thomas Kuhn, Paulo

Freire, and Jürgen Habermas. It seeks to engender or otherwise promote changes in perspective via the utilization of critical reflection (understanding of inherent, internal assumptions regarding a question or situation) and critical discourse (sociolinguistic validation of perspective). Although somewhat hampered by certain conceptual limitations (*e.g*., the true nature of experience, the assumption of positivity, *etc*.), it has the potential to be an incredibly powerful tool for adult educators in both the social and physical sciences. At the end of the chapter, I propose two novel teaching activities. The first utilizes narrative inquiry (Schwandt, 2007) to explain the development of natural selection and evolution as a theory. The second module uses genetic data collected in Chapter 2 as the basis for teaching species concepts and delimitation.

CHAPTER 2

GENETIC ANALYSES REVEAL CRYPTIC DIVERSITY IN THE NATIVE NORTH AMERICAN FIRE ANTS (HYMENOPTERA: FORMICIDAE: *SOLENOPSIS*)[1]

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Abstract

The native North American fire ants (*Solenopsis*) comprise a difficult group taxonomically that has undergone multiple revisions in the past century yet remains in a state of taxonomic uncertainty. In this study, we utilized a large set of microsatellite markers to conduct the first robust genetic analysis of the valid nominal species. Our approach used a variety of methods to test operational criteria commonly employed in species delimitation, including genotypic clustering, reproductive isolation/cohesion, and monophyly. We conclude from our results that the recognized North American fire ant species represent evolutionarily independent entities and, moreover, we confirm the presumed sister status of the desert fire ants, *S. aurea* and *S. amblychila*. However, the presence of at least two genetically divergent populations within the valid nominal species boundaries, including a western form of *S. xyloni* and a distinct population of *S. aurea* endemic to the Salton Trough, suggests that the current taxonomy does not fully capture the species-level diversity in this group. Our study provides the molecular foundation for future integrated studies of the taxonomy and evolution of this scientifically and economically important group of insects.

INTRODUCTION

The genus *Solenopsis* (Hymenoptera: Formicidae) remains as "extraordinarily intractable in the matter of subdivision" as originally claimed by Creighton (1930), despite almost a century of taxonomic revision, and as such has garnered a degree of notoriety among myrmecologists (Trager, 1991; Pitts, 2005). Recent revisionary attempts to resolve long-standing issues within the portion of the genus comprising the fire ants have been fueled in part by the rapid introduction and spread of invasive species across the globe (*e.g.*, the red imported fire ant, *Solenopsis invicta* [Ascunce *et al*., 2011], and the tropical fire ant, *S. geminata* [Gotzek *et al*., 2015]), as well as by a general interest in the many unique reproductive and social features exhibited by its members, including extensive hybridization within introduced ranges and major behavioral polymorphisms associated with known genetic elements (Shoemaker, *et al*. 1996; Ross & Keller, 1998; Gotzek & Ross, 2007). While these revisions did solve some major problems with the alpha taxonomy of the group, such as distinguishing South and Central American fire ant species from their North American counterparts (Wheeler, 1915; Snelling, 1963; Ettershank, 1966; Buren, 1972), the taxonomic status of the native North American species remains in flux. Nominal taxa have shifted repeatedly between species and subspecies ranks, as well as between valid and junior synonym status, over a relatively short time period (Fig. 2.1), reflecting continued disagreement among researchers. These taxonomic issues stem primarily from a lack of diagnostic morphological characters, as natural variation in traditionally studied traits is quite extensive within the valid nominal taxa (Trager, 1991), and such variation is confounded by extensive size polymorphism in the worker caste of most species (Tschinkel, 2013), the caste from which almost all diagnostic characters are derived (Trager, 1991).

The current classification of fire ants is based primarily on the revisionary and cladistic works of Trager (1991) and Pitts *et al.* (2005). Both authors placed the native North American fire ants into the *Solenopsis geminata* species group, which contains: the tropical fire ant (*S. geminata* Fabricius, 1804), two desert fire ants (*S. amblychila* Wheeler, 1915 and *S. aurea* Wheeler, 1906), and the southern fire ant (*S. xyloni* McCook, 1880) (see Fig. 2.2), as well as two South American species (*S. gayi* Spinola, 1851 and *S. bruesi* Creighton, 1930). However, both authors acknowledge that a more thorough taxonomic assessment is required to determine the true number and boundaries of the species in this group (Pitts, 2002; J. C. Trager, personal communication).

In this paper, we employ a large set of highly variable genetic markers (microsatellites) to test several common operational criteria for species delimitation, including the presence of genotypic clusters, reproductive isolation or cohesion, and monophyly of populations. Such an integrated approach to taxonomy (Dayrat, 2005; Schlick-Steiner *et al*., 2010) has successfully been used in many other insect groups, including moths (Yang *et al*., 2012), bumblebees (Lecocq *et al*., 2015), fruit flies (Krosch *et al*., 2013), and ants (Blaimer, 2012). The goal of this investigation is threefold: 1) to conduct the first comprehensive population genetic analysis of the native North American fire ants; 2) to test whether the valid nominal species correspond to genetically distinct groups and/or contain unrecognized population structure (*i.e.*, cryptic species); and 3) to provide a strong body of genetic evidence to frame future morphological, ecological, and behavioral investigations of this group, one goal of which will be to finally resolve persistent taxonomic and evolutionary issues in the genus *Solenopsis*.

MATERIALS AND METHODS

Taxon Sampling

Adult workers of each of the four valid nominal native North American fire ant species were obtained from museums and personal collections (*n* = 327). Samples spanned much of the known recorded distributional ranges of these species (see Figs. 2.2, 2.3), from Georgia to the California coast and into northern Mexico. While taxon sampling was dense across much of the southwestern United States, where most of the native fire ant diversity occurs, material from the southeastern United States proved more difficult to obtain. The introduction and spread of the red imported fire ant, *S. invicta*, during the latter half of the 20th century led to widespread extinction of *S. xyloni*, restricting it to the western half of its historical range (Jacobson *et al.,* 2006; Tschinkel, 2006). Thus, there are few sources of material from the eastern part of the species range suitable for molecular analyses. Moreover, we avoided sampling from the known *S. xyloni*/*S. geminata* hybrid zone located in southern Texas and northern Mexico (Axen *et al*., 2014). Given the historically unstable taxonomy of native fire ants and variation in dates of the original identifications, we verified the identity of all our specimens by microscope using the most complete diagnostic key (Trager, 1991). A complete list of specimens used in this study can be found in the Appendix 1. Voucher specimens will be deposited at the National Museum of Natural History, Smithsonian Institution.

DNA extraction, amplification, and genotyping

Total genomic DNA was isolated from one individual worker ant per sampling bait or collection site using a modified DNeasy Blood and Tissue kit protocol (Qiagen, Valencia, CA); use of a single such worker minimizes the probability that nestmates or otherwise close kin were included. Prior to extraction, pinned specimens were cut from their points and washed in water to remove excess adhesive, while specimens preserved in ethanol were dried in a 37°C incubator for thirty minutes. Samples were then macerated in 200 µL Buffer ATL using a small plastic pestle prior to overnight (> 12 hours) incubation in a hot water bath (55°C). The final elution of DNA was performed using two consecutive washes of 100 µL Buffer AE. DNA extracts were then diluted to 1:5 (DNA:water) to make working aliquots. A total of 59 fire ant-specific microsatellites were amplified for each individual via 22 multiplex PCR reactions (Asunce *et al*., 2009, 2011; Ross *et al*., 2010). Microsatellites were chosen as genetic markers for this study because they possess an appropriate level of variation in the focal taxa (Gotzek *et al., 2015)* and are relatively robust to the degradation of DNA characteristic of older pinned or ethanol- preserved specimens (Schlötterer & Pemberton, 1998). Moreover, the chosen markers cover much of the fire ant genome (14 of the 16 chromosomes) and are unlinked and thus effectively independent (E. Wade, unpublished). PCR products were visualized on a pre-cast agarose E-gel (Invitrogen, Carlsbad, CA) to verify amplification of target markers. Genotyping of these products was conducted at the University of Florida Interdisciplinary Center for Biotechnology Research. Genotype calls were imported, calibrated, and scored using *Genemarker* (SoftGenetics, State College, PA) and later converted to different software file formats in *Convert* (Glaubitz, 2004). All genotypic data used in this study have been deposited in Dryad (TBD).

Data filtering

The condition of older samples resulted in poor amplification of some microsatellite markers, particularly those with longer amplicons. To test whether the resulting loss of data would adversely affect our results, we partitioned the original dataset into six stepwise subsets based on the number of markers successfully amplified per individual (*e.g.*, ≥10, ≥20, up to 59). Analyses of subsets with less stringent filtering (≥40 markers missing) produced presumably artificial clusters in the *Structure* analyses that appeared to be based primarily on the presence of shared alleles at a subset of loci with smaller and less variable amplicons. As such, we removed from further consideration individuals for which fewer than half of the total panel of microsatellites (30) could be scored. Finally, we excluded *S. geminata* from many analyses because a complete investigation of possible species boundaries within this nominal taxon would entail extensive sampling across its native and invasive pantropical range (Gotzek *et al.*, 2015) and, thus, is outside the scope of the current study. Our final dataset included genotypes of 238 individuals scored at an average of 54 loci.

Genetic data analyses

The genotypic cluster species concept formulated by Mallet (1995) forms a useful starting criterion for the delimitation of species as its places emphasis on the evolutionary formation of distinct genotypic groups with few intermediates, a genetic process assumed in many other concepts (Hausdorf, 2011). To evaluate this criterion, we analyzed our filtered dataset for population genetic structure by utilizing the Bayesian clustering method developed by Pritchard *et al*. (2000) and implemented in *Structure* v2.3.4 (Falush *et al*., 2003; Hubisz *et al.,* 2009). Given the recent common ancestry of the species other than *S. geminata* (~0.4 MYA; D. Gotzek, unpublished) and the possibility of parapatric or sympatric hybridization between some of these, simulations were run using models that allowed for admixture and correlated allele frequencies across populations (Miralles & Vences, 2013). All other parameters were kept at default values. We conducted each independent run for 1,250,000 generations, removing the first 250,000 as burn-in. Five runs were done for each value of *K* (2-10) to ensure convergence upon the most likely model. We then selected the final number of genetic clusters based upon both the log probabilities and the ΔK statistic of Evanno *et al.* (2005) as implemented in *StructureHarvester* (Earl *et al*., 2012). Genotypic clustering schemes initially were produced using a dataset containing all four valid nominal North American fire ant species. To test for lower- level substructure (Evanno *et al*., 2005), we next removed data for *S. geminata*, the most divergent species and presumed sister to the remaining taxa (D. Gotzek, unpublished), then re-ran the analyses using the protocol described above. Multiple *Structure* runs were consolidated and proportions of individual ancestry in each of the proposed genetic clusters were visualized using *Pophelper* (Francis, 2016).

The genetic cluster assignments produced in *Structure* were subsequently imported into the *R* package *adegenet* (R Development Core Team, 2008; Jombart, 2008) and used as the basis for a discriminant analysis of principal components (DAPC; Jombart *et al*., 2010). This multivariate ordination approach describes relationships within and between clusters of genetically similar individuals and thus serves as a useful visual complement to the *Structure* results by revealing relative differentiation between groups.

Other common operational criteria for delimitation of species focus on the magnitude of genetic exchange between populations, as mediated by reproductive isolation (*i.e.*, the Biological Species Concept; Mayr, 1982) or cohesion (Templeton, 1989). To assess the impact of historical and/or recent gene flow as related to isolation or cohesion of our genetically differentiated groups, we employed a number of different techniques. We used *GenoDive* (Meirmans & Van Tienderen, 2004) to estimate relative (F_{ST}; Weir & Cockerham, 1984) and absolute (G''_{ST}; Meirmans & Hedrick, 2011) measures of genetic differentiation between the genetically distinct populations produced by *Structure*. The program *GenoDive* was used as well to calculate allele frequencies for subsets of samples from these populations where they overlapped geographically; the maintenance in sympatry of strong allele frequency differentiation at multiple loci constitutes strong evidence for reproductive isolation (Koffi *et al.*, 2010; Duminal *et al.*, 2012). Significance of allele frequency differences was calculated using the Fisher exact test ($p < 0.05$) and corrected for multiple comparisons using the Bonferroni method. Two estimates of effective gene flow (N_em) were calculated between the genetically distinct populations recognized from the *Structure* results, the first using Slatkin's (1987) formula $N_em = (1/F_{\mathrm{ST}} – 1)/4$ and the second employing the private alleles method (Barton & Slatkin, 1986) implemented in *Genepop* (Raymond & Rousset, 1995; Rousset, 2008). Correlation between the results of these two methods was evaluated via a Mantel test based on the Spearman ranked correlation coefficient and 5,000 permutations of the data. For purposes of comparison, we also calculated F_{ST} and G''_{ST} for *S. geminata* and for a similar dataset derived from three valid nominal South American fire ant species (*S. saevissima*, *S. invicta*, and *S. richteri*; Ross *et al*., 2007, 2010), two of which are believed to contain cryptic species. Contemporary rates of gene flow for the North American populations were calculated using a Bayesian approach implemented in the program *BayesAss* (Wilson & Rannala, 2003). Simulations were run for 10,000,000 generations (of which 1,000,000 were removed as burn-in), with sampling conducted every 1,000 generations. Values for the allele frequency, migration rate, and inbreeding parameters were set to 0.35, 0.33, and 0.17, respectively, as in Ross *et al*. (2010). Measurements of contemporary gene flow rates were averaged across five independent runs. Convergence of these runs was determined by analyzing their MCMC trace files in *Tracer* v1.6 (Rambault *et al*., 2003).

The phylogenetic species concept – unlike those mentioned above – focuses specifically on population lineages and whether they meet the operational criterion of monophyly (Mishler, 1985). To infer the evolutionary relationships of individual specimens (excluding *S. geminata*), we first constructed a neighbor-joining tree (Saitou & Nei, 1987) based on Nei's D_A distance (Nei *et al*., 1983), which has been shown to work particularly well with microsatellite data (Takezaki & Nei, 1996). Genetic distances were calculated with *TreeFit* (Kalinowski, 2009) and imported into the *R* package *ape* (Paradis *et al*., 2004) for tree construction. Nodal support for the final tree was determined using bootstrapping (1000 replicates) prior to visualization in *FigTree* v1.4.2 (Rambaut, 2014). We also conducted this procedure for the genetically differentiated groups recognized following *Structure* analyses (K=7), including data for *S. geminata* to provide an appropriate root.

RESULTS

Genetic diversity

A total of 930 alleles was found across the 59 microsatellite loci (2-49 alleles/locus; mean: 16). Observed heterozygosity (H_O) within valid nominal species ranged widely depending on the locus, from 0.00 (*e.g., i-131* in *S. aurea*) to 0.920 (*C121* in *S. amblychila*). The average H_O within valid nominal species across all loci and samples (0.401) was considerably lower than Nei's corrected total heterozygosity (H'_T; 0.665), the average expected frequency of heterozygotes within populations given Hardy-Weinberg equilibrium (HWE) with a correction for limited sampling of individuals or populations (Nei, 1987). Accordingly, we detected significant deviations from HWE across all loci within each valid nominal species (all $p < 0.05$, Fisher exact test). Such results are expected if cryptic, genetically differentiated populations reside within the recognized fire ant species (Wahlund, 1928).

Genetic clustering

Based on the posterior probabilities and ΔK calculations for our *Structure* output, we consistently recovered K=4 and K=7 as the most probable numbers of genetic clusters for the full dataset and the subset with *S. geminata* genotypes removed, respectively. The assigned ancestry (or apportioned membership) of individuals in these clusters is shown in Figure 2.4. For the full dataset, ancestry of *S. geminata* individuals is nearly exclusively in a genetic cluster distinct from all other clusters. Nominal *S. xyloni* individuals have almost exclusive or predominant membership in two other clusters, while most nominal *S. amblychila* and *S. aurea* individuals (desert fire ants) have exclusive or predominant membership in the fourth cluster. For the subset from which *S. geminata* data were removed, nominal *S. xyloni* comprises individuals with majority ancestry in four clusters (the four populations recognized on the basis of majority ancestry are hereafter designated *xyloni*-1, *xyloni*-2, *xyloni*-3, and *xyloni*-4), and *S. amblychila* and most *S. aurea* now comprise individuals with predominant membership in two distinct species-specific clusters generally not well represented in the ancestry of the other specimens. Notably, a minority of individuals of *S. aurea* with majority assignment to a different cluster than their conspecifics at *K*=4 have predominant membership in a unique, seventh cluster at *K*=7 (the group composed of these individuals is hereafter designated dark-*aurea* because of their unusually dark cuticular coloration relative to other *S. aurea*).

The DAPC analysis based on the *Structure* output (*K*=7) generally showed clear separation between groups of individuals with majority ancestry in the different genetic clusters based on the first two principal components (PCs) (Fig. 2.5). Notably, these projections support an interpretation of meaningful genetic differentiation between the eastern (*xyloni*-1) and western (*xyloni*-2, *xyloni*-3, *xyloni*-4) samples of *S. xyloni*, as well as the separation of dark-*aurea* from the other desert fire ants. These general relationships also held when the data were projected on the second and third PCs (results not shown).

The geographical distribution of samples identified by majority membership according to the *Structure K*=7 clustering scheme (*i.e.*, seven differentiated populations or species) is displayed in Figure 2.3. Most of the seven populations/species occur primarily in the southwestern United States and northern Mexico, although their apparent distributions vary considerably. Groups *xyloni*-2 and dark-*aurea*, for instance, are mostly limited to relatively small regions of California, while others such as *xyloni*-3 and *xyloni*-4 range from southern California and Baja California through New Mexico to western Texas. The desert fire ant group corresponding to *S. amblychila* occupies a similarly broad distribution from southern California to Texas and overlaps substantially with its presumed sister species, *S. aurea*, although the latter is restricted to Arizona and the northern Mexican province of Sonora. The group *xyloni*-1 ranges much farther eastward than any of the other populations, extending from New Mexico to Georgia.

Gene flow and reproductive isolation

To investigate the extent of potential gene flow between the seven differentiated populations discovered (as well as *S. geminata*), we first calculated two common measurements of genetic differentiation, F_{ST} and G''_{ST} (Table 1). Values of F_{ST} between populations of different North American species (interspecific) were 0.110 – 0.312, while those within species (intraspecific) ranged only slightly lower, 0.079 – 0.217. In comparison, F_{ST} values calculated between the three nominal South American fire ant species (interspecific) were 0.271 – 0.442, while those calculated between populations suspected of being cryptic species within species were 0.200 – 0.371. Similar patterns were found in the case of G''_{ST} estimates, with North American values 0.258 – 0.747 (interspecific) and 0.179 – 0.449 (intraspecific), and South American values 0.663 – 0.815 (interspecific) and 0.412 – 0.737 (intraspecific, cryptic species). Thus, some of the newly discovered, genetically differentiated populations within the North American species appear to be as distinct as suspected cryptic South American fire ant species (Fig. 2.6) (see also Ross *et al*., 2010).

We calculated allele frequencies for subsets of our North American samples from areas where the genetically distinct populations overlap geographically. Five fixed allelic differences (*i.e.*, an allele present at a frequency of 1.0 in one group while absent in another) were found between sympatric eastern (*n*=12) and western (*n*=10) *S. xyloni* (*xyloni*-1 and *xyloni*-3/4, respectively); an additional 17 loci displayed highly significant allele frequency differences between the two groups (Fisher exact test; all $p < 0.05$ following Bonferroni correction). Similarly, 26 loci contained alleles occurring at highly significantly different frequencies between sympatric *S. aurea* (*n*=11) and *S. amblychila* (*n*=13) (Fisher exact test; all $p < 0.05$ following Bonferroni correction), although only one locus approached fixed differentiation (allele $i\text{-}137^{173}$, >90% in *S. aurea* but absent in *S. amblychila*). In contrast, only four significant allele frequency differences were found between geographically overlapping samples of *xyloni*-3 (*n*=12) and *xyloni*-4 (*n*=13) (Fisher exact test; all $p < 0.05$ following Bonferroni correction).

Estimates of effective gene flow (N_em) determined via Slatkin's formula and the private alleles method were highly correlated (Fig. 2.7; Mantel test, $P = 0.001$), suggesting that both are useful metrics for inferring degree of gene flow for our samples. Almost all group pairs yielded N_em values above 0.5 using both methods, but fewer than half of the values (42%) exceeded 1.0. This range is of particular interest in species delimitation, as it represents an upper threshold for differentiation, above which gene flow outweighs the effects of drift for neutral genes under a simple island model (Slatkin, 1987; Porter 1990). Group pairs that greatly exceeded this range ($N_em = 2.0 – 4.5$) primarily involved western populations of *S. xyloni* (*xyloni*-2, *xyloni*-3, *xyloni*- 4), although, surprisingly, *S. aurea*/*S. amblychila* also yielded relatively high values of effective gene flow. Estimates of contemporary gene flow between the seven identified populations, calculated using *BayesAss*, are shown in Table 2. Migrants accounted for only 1.2% of individuals, on average, within each population, suggesting generally low levels of current gene flow. Several source/recipient pairs exceeded this value, however, notably *S. aurea*/*S. amblychila* (4.2%) and *xyloni*-4/*xyloni*-3 (2.4%). Thus, these pairs of populations may have experienced elevated levels of recent introgression. Non-immigrant genotypes comprised an average of 92% of individuals in each population. The only group to fall substantially below this average was dark-*aurea* (87%), suggesting the possibility of meaningful immigration into it from overlapping populations (but see Meirmans 2014 for potential inference limitations).

Monophyly of populations

We tested for monophyly of the valid nominal species by constructing neighbor-joining trees based on genetic distances (D_A), both for individuals as well as for the seven groups recognized based on the *Structure* results. In the case of individuals, the analysis was unable to recover a well-supported topology (results not shown); of the 235 internal nodes, only 41 had bootstrap scores >50%. Furthermore, most of these supported nodes were located near the terminals, connecting lineages of only a few, geographically proximate individuals. We note, however, that several of the larger clades recovered correspond well to groups inferred based on the *Structure* output (*e.g.*, *xyloni*-1 and dark-*aurea*). Neighbor-joining analysis conducted on the *Structure*- based groups yielded a fairly well-supported tree with four major lineages or “clades” (labeled A, B, C, D in Figure 2.8). The tree was rooted with *S. geminata* (lineage A), the presumed sister species to the other native fire ants. Groups corresponding to nominal *S. aurea* (excluding dark-*aurea*) and *S. amblychila* comprise clade B. Dark-*aurea* (lineage C) is sister to clade D, which consists of four groups (*xyloni*-1-4), with the eastern *xyloni*-1 sister to the remaining western groups of nominal *S. xyloni* (*xyloni*-2-4).

DISCUSSION

Fire ants have become a model group for the study of complex reproductive and social behaviors and their underlying evolutionary, ecological, and physiological mechanisms (Tschinkel 2006; Hölldobler & Wilson, 2009; Tschinkel & Wilson, 2014). However, to achieve this state of scientific usefulness, the South American taxa required continued major taxonomic treatments (Wheeler, 1915; Snelling, 1963; Ettershank, 1966; Buren, 1972; Pitts *et al.*, 2005; Shoemaker *et al.*, 2006); without a clear understanding of species boundaries and relationships, comparative inference or analysis of complex evolutionary processes within this group would have been greatly limited. Our current taxonomic understanding of the native North American fire ants is in a similar state to that of the South American species prior to the revisionary work of the past century. Members of the North American group can be agricultural/urban pests (Smith, 1936; Holway *et al*., 2002; Field *et al.*, 2007), and they exhibit a range of evolutionarily important reproductive and social behaviors such as hybridization, queen polymorphism, and variation in colony queen number (Adams *et al.*, 1976; McInnes & Tschinkel, 1995; Axen *et al.*, 2014). To place these behaviors in a more coherent evolutionary context, the alpha taxonomy of the group must be investigated using modern methods. The main goals of the present study were to test for evolutionary independence of the recognized species and for cryptic diversity in the native North American fire ants through use of a large set of highly variable genetic markers. We tested several operational criteria that are commonly used for species delimitation to obtain a robust and well-supported set of species hypotheses for the group. In so doing, we lay the taxonomic groundwork for future evolutionary studies of fire ant reproductive and social biology.

Naturalness of recognized species and evidence for cryptic diversity

Initial species hypotheses were developed on the basis of genotypic clustering in our full molecular dataset. *Structure* recovered four distinct genetic clusters as the most probable partitioning of the genetic variation, although evidence of some mixed ancestry/membership between groups was prevalent for many individuals, presumably due to recent population divergence and, perhaps, subsequent gene flow. These four clusters generally correspond well to valid nominal species, presumed sister-species clades, or regional populations (*i.e.*, *S. geminata*, *S. aurea*/*S. amblychila* [the desert fire ants], and eastern and western populations of *S. xyloni*). Although *S. geminata* is considered sister to the remaining native North American fire ants, it is nonetheless highly divergent (D. Gotzek, unpublished) and, as such, its inclusion in the *Structure* analysis predictably yielded only evidence of higher-level genetic structure among the remaining samples (Evanno *et al*., 2005). The separation of nominal *S. xyloni* into two genetically distinct populations, despite the inclusion of *S. geminata*, strongly suggests the existence of at least one morphologically cryptic species in this taxon. We note that it is possible that the differentiated western population corresponds to *S. maniosa*, a junior synonym of *S. xyloni* (Snelling, 1963; Snelling & George, 1979), as the western *S. xyloni* populations and *S. maniosa* share the same general geographic distribution across the Mojave and Colorado Deserts (Wheeler & Wheeler, 1986). We discuss this possibility further below.

Structure analyses revealed additional, lower-level genetic substructure when *S. geminata* was excluded from the full dataset. While one of the previous genetic clusters, corresponding largely to eastern *S. xyloni*, remained essentially intact, the others subdivided into smaller groupings. The western population of *S. xyloni*, for instance, can be separated into two broadly distributed groups (*xyloni*-3 and *xyloni*-4) and one highly localized group in California (*xyloni*- 2). Also, the desert fire ant cluster separates into its constituent nominal species, *S. aurea* and *S. amblychila*. This latter genetic distinction parallels the morphological differences detected by Trager (1991) as well as our current understanding of natural history differences between the two species (*e.g.*, *S. amblychila* is found primarily at higher elevations than *S. aurea*; Mackay & Mackay, 2002). Surprisingly, *Structure* also recovered evidence for a distinct group of *S. aurea* largely confined to the Coachella Valley of California (dark-*aurea*), individuals of which were not assigned to the larger desert fire ant group in the prior analysis including *S. geminata*. In the full dataset, these individuals show a greater genetic affinity to the eastern *S. xyloni* population than either the western *S. xyloni* populations or even the other desert fire ants, despite extensive geographic overlap between dark-*aurea* and several western populations (*e.g.*, *xyloni*-3, -4). There has been long-standing confusion regarding desert fire ants in the Coachella Valley. For instance, Snelling & George (1979) noted that *S. aurea* in this region were observed foraging at midday, although Creighton (1950) reported the species to be nocturnal or crepuscular in its activity. Ward (2005) speculated that *S. aurea* in California might, in fact, be a lightly colored form of *S. xyloni*. Furthermore, the Coachella Valley is a known biodiversity hotspot with a complex biogeographic history (Wood *et al*., 2013; Vandergast *et al*., 2013). As such, it is quite possible that a currently unrecognized species of desert fire ant is endemic to the area.

A known feature of *Structure* is that it can overestimate the optimal number of genetic clusters, particularly in cases with small population sample sizes and large numbers of genetic markers (Latch *et al*., 2006; Kalinowski, 2011). Thus, we conducted further analyses of our genetic groupings based on cluster assignments to determine whether they represent natural entities. Discriminant analysis of principal components (DAPC), a multivariate approach that reveals the magnitude of genetic differentiation between groups, supported the separation and/or distinctiveness of most of the groups inferred using *Structure*. For instance, it provided support for the differentiation between the eastern and western populations of *S. xyloni,* as well as for the split between *S. aurea,* dark-*aurea,* and *S. amblychila*. A notable exception, however, was the observed subdivision of the western *S. xyloni* population inferred from *Structure*. The corresponding genetic groups (*xyloni*-2-4) show a fair degree of overlap in the DAPC projection of the first two principal components, suggesting less meaningful genetic divergence, especially between *xyloni*-2 and *xyloni*-4.

We also examined the operational criteria of reproductive isolation and cohesion by calculating both absolute (G''_{ST}) and relative (F_{ST}) measures of genetic differentiation between groups inferred from the *Structure* output. Estimates of G''_{ST} for pairs of populations within the valid nominal North American species generally were lower than those for populations suspected of being cryptic species within the nominal South

American fire ants *S. saevissima* and *S. invicta* (means of 0.311 and 0.573, respectively), although some estimates (*i.e.*, for *xyloni*-1/*xyloni*-2 and *aurea*/dark-*aurea*) were of similar magnitude to those for the suspected cryptic South American species (Fig. 2.6). Moreover, most of the lowest G''_{ST} and F_{ST} values were between groups of western *S. xyloni* (*xyloni*-2-4), the same groups that showed extensive overlap in the DAPC projection. Such a finding provides further evidence that western *S. xyloni* may have been overdivided in the *Structure* analyses. On the other hand, estimates of G''_{ST} and F_{ST} were also relatively low even for some valid nominal North American species pairs, despite taxonomic recognition of the species in question being supported by other lines of evidence (*e.g.*, morphology and natural history for *S. aurea*/*S. amblychila*). While values of these divergence metrics alone cannot be used to establish some threshold for delimiting species boundaries in this group, in conjunction with other analyses (*e.g.*, *Structure* and DAPC) they highlight group genetic relationships and thus inform decisions regarding species status.

The maintenance of genetically distinct populations/groups within areas of geographical overlap constitutes strong evidence for reproductive isolation and, hence, the presence of multiple species (Koffi *et al.*, 2010; Duminal *et al.*, 2012). As such, we compared allele frequencies for paired subsets of individuals from the *Structure*-based populations where they occurred in apparent sympatry, specifically *xyloni*-1/*xyloni*-3, *xyloni*-3/*xyloni*-4, and *aurea*/*amblychila*. A total of five loci with fixed allelic differences were found between groups *xyloni*-1 and *xyloni*-3, along with numerous other loci displaying strong allele frequency differentiation. This further supports the notion that nominal *S. xyloni* contains a morphologically cryptic western species. It is unlikely, however, that multiple cryptic species exist in this area, as comparisons of allele frequencies between *xyloni*-3 and *xyloni*-4 (which co-occur across much of the southwestern United States) showed few significant differences. Interestingly, comparisons between sympatric *S. aurea* and *S. amblychila* revealed an even greater number of significantly differentiated loci than found between eastern and western *S. xyloni*, although only one locus approached a fixed difference. Paralleling our genetic differentiation measures and DAPC results, the allele frequency estimates in sympatry suggest a single western population of *S. xyloni* that is distinct from the eastern population, as well as a clear separation between *S. aurea* and *S. amblychila.*

Estimates of relative differentiation (F_{ST}) also were used as the basis for calculating levels of effective gene flow between populations ($N_e m$). According to population genetics theory, $N_e m$ values of 0.50 - 1.00 represent a threshold for differentiation above which gene flow outweighs the effects of drift at neutral genes under a simple island model (Slatkin, 1987; Porter 1990). The native North American populations generally exhibited measurements exceeding this threshold, particularly between the three populations of western *S. xyloni* (mean: 2.15) and between *S. aurea* and *S. amblychila* (2.02). Conversely, dark-*aurea* had the lowest $N_e m$ values across most comparisons (excluding *S. geminata*; mean: 0.94), although this result may be affected by our small sample size for this population (Slatkin, 1987). Calculation of effective gene flow between populations using the private alleles method revealed similarly high values in general. However, $N_e m$ values between groups *xyloni*-3 and *xyloni*-4 range even higher than typical under this method (2.91 - 4.13), consistent with these two western *S. xyloni* populations sharing a large number of alleles not found in other populations. Estimates of contemporary gene flow using *BayesAss* confirm above-average migration between these two groups. When viewed with the DAPC, genetic differentiation, and allele frequency analyses, these results again suggest the existence of a single, relatively genetically uniform western population of S. *xyloni* rather than three genetically and evolutionarily separate ones.

In a final analysis, we constructed neighbor-joining trees based on genetic distances between individual samples, as well as between the seven populations recognized using the *Structure* output, to address the issue of monophyly. Although the tree recovered from the inter- individual dataset was poorly resolved, with nodes with marginal or strong support primarily near the terminal branches and consisting of small numbers of geographically adjacent individuals, some larger lineages correspond generally to populations identified using *Structure*. Most internal nodes were not supported, most likely due to the size of the dataset, overall genetic similarity of the specimens, and inherent limitations of bootstrapping. The second neighbor- joining tree based on genetic distances between the differentiated populations was fairly well-supported and generally matched the presumed relationships between valid nominal North American fire ant species (D. Gotzek, unpublished). We note, however, that the lineage corresponding to dark-*aurea* was not recovered within the clade containing *S. aurea* and *S. amblychila*, but instead is sister to nominal *S. xyloni* as a whole. This paraphyletic relationship of nominal *S. aurea* matches the results from the *Structure* analysis, in which individuals with predominantly dark- *aurea* ancestry initially clustered with the eastern *S. xyloni* samples (at *K*=4) rather than the other desert fire ants. Our other analyses also supported the identity of a unique dark-*aurea* population, but they did not recover evidence for a close genetic relationship between it and eastern *S. xyloni*. In the DAPC analyses, for example, dark-*aurea* was more similar genetically to the western *S. xyloni* groups (*xyloni*-2-4) than to the eastern group. Given these findings, more extensive genetic study of *S. aurea* and the other western fire ants is warranted to more fully understand species boundaries and, especially, the relationships of the entities newly discovered.

Taxonomic implications

The results of our genetic study of the alpha taxonomy of native North American fire ants suggest that nominal *S. xyloni* comprises two distinct species: an eastern form that historically ranged from South Carolina to eastern Arizona, and a western form that is found throughout the southwestern United States and northern Mexico. Importantly, the western form may correspond to *S. maniosa*, a junior synonym of *S. xyloni* (Trager, 1991), which was proposed to occur over essentially the same geographic range as our western *S. xyloni* samples. To determine whether this is the case, a thorough morphological comparison is required, although we note that it may not be possible to make fine diagnostic distinctions between relevant populations using the few published morphological descriptions of *S. maniosa* (Wheeler, 1915; Creighton, 1930; Snelling & George, 1979) and its poorly preserved type material.

Our study also suggests the occurrence of a currently unrecognized species of desert fire ant common to the Coachella Valley of California, although this is not as clear as in the previous case given some discrepancies between the findings of different delimitation methods. Expanded collecting and a thorough morphological examination will help determine the ultimate taxonomic fate of this population. It should be emphasized that the current study is only the first step in the taxonomic revision of the native North American fire ants; once morphological analyses are completed and results compared to the molecular data, any necessary changes to the formal taxonomy will be undertaken.

ACKNOWLEDGMENTS

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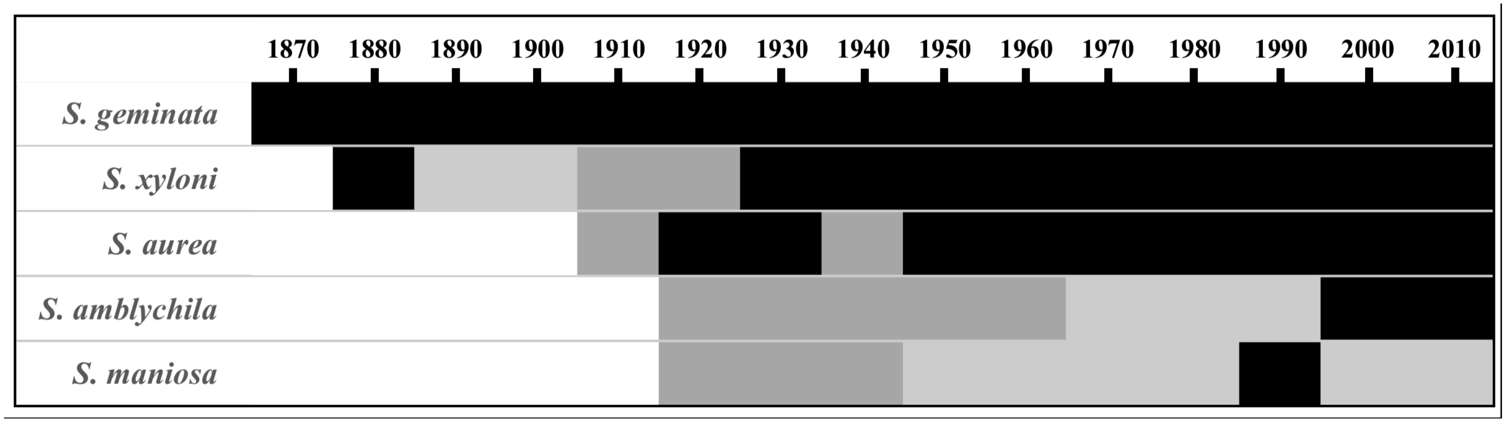

Figure 2.1: History of the taxonomic status of the recognized native North American fire ant species over the past 150 years. Black bars indicate formal species status, dark gray subspecies status, and light gray synonym status. *Solenopsis maniosa*, currently a species synonym of *S. xyloni*, is included due to its potential relevance to future taxonomic changes in the group.

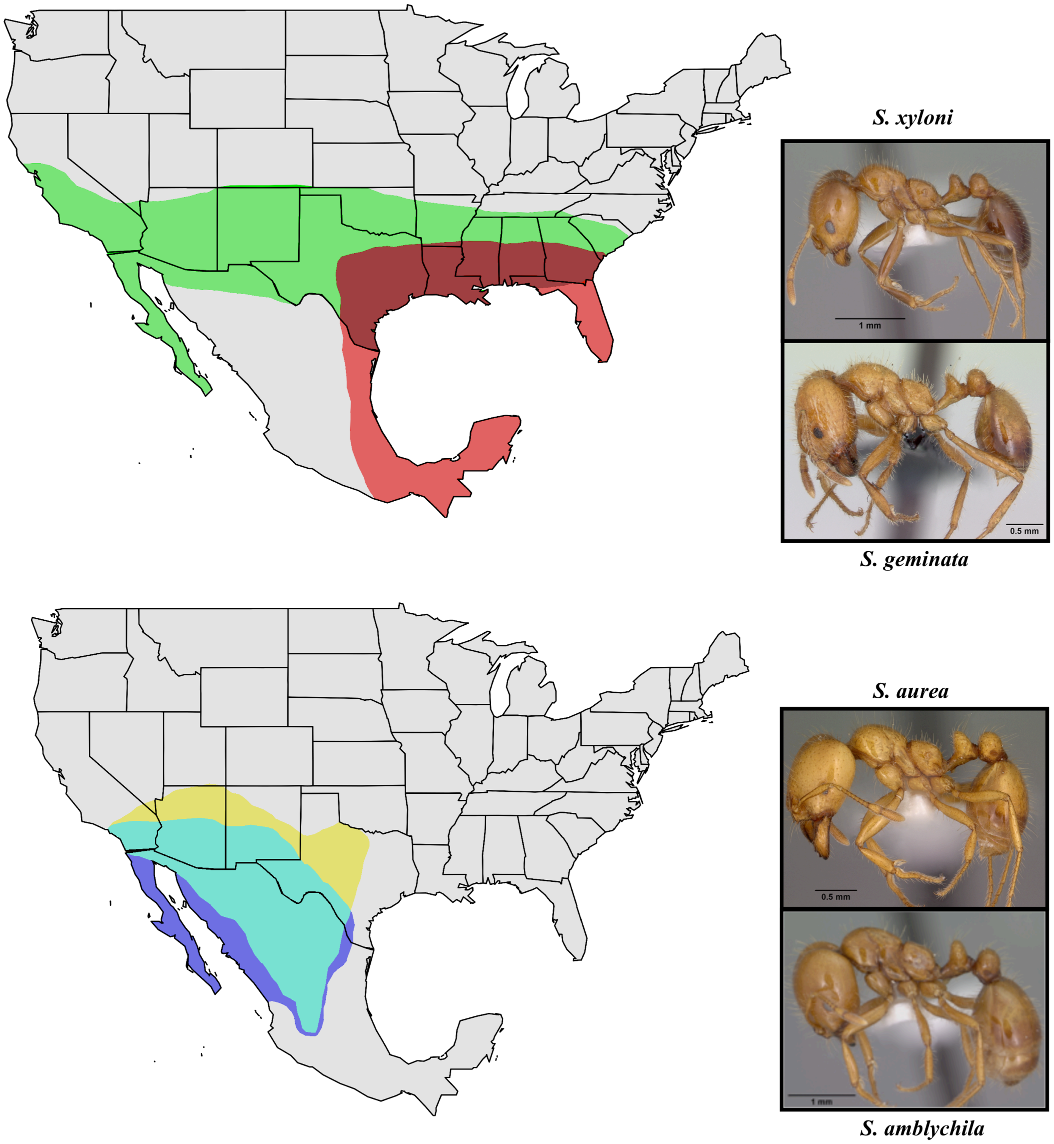

Figure 2.2: Estimated geographical distributions of the native North American fire ant species (adapted from Tschinkel, 2006). Green corresponds to *S. xyloni*, light red to *S. geminata*, yellow to *S. aurea*, and lavender to *S. amblychila*. Dark red and light blue represent overlapping distributions between species pairs. The range of *S. geminata* extends through Central America to northern South America. *Solenopsis xyloni* presently is absent from much of the central and eastern parts of its historical range, presumably because of negative effects on its populations attributable to the invasive congener *S. invicta*. Images by Jen Fogarty and April Noble (from www.antweb.com).

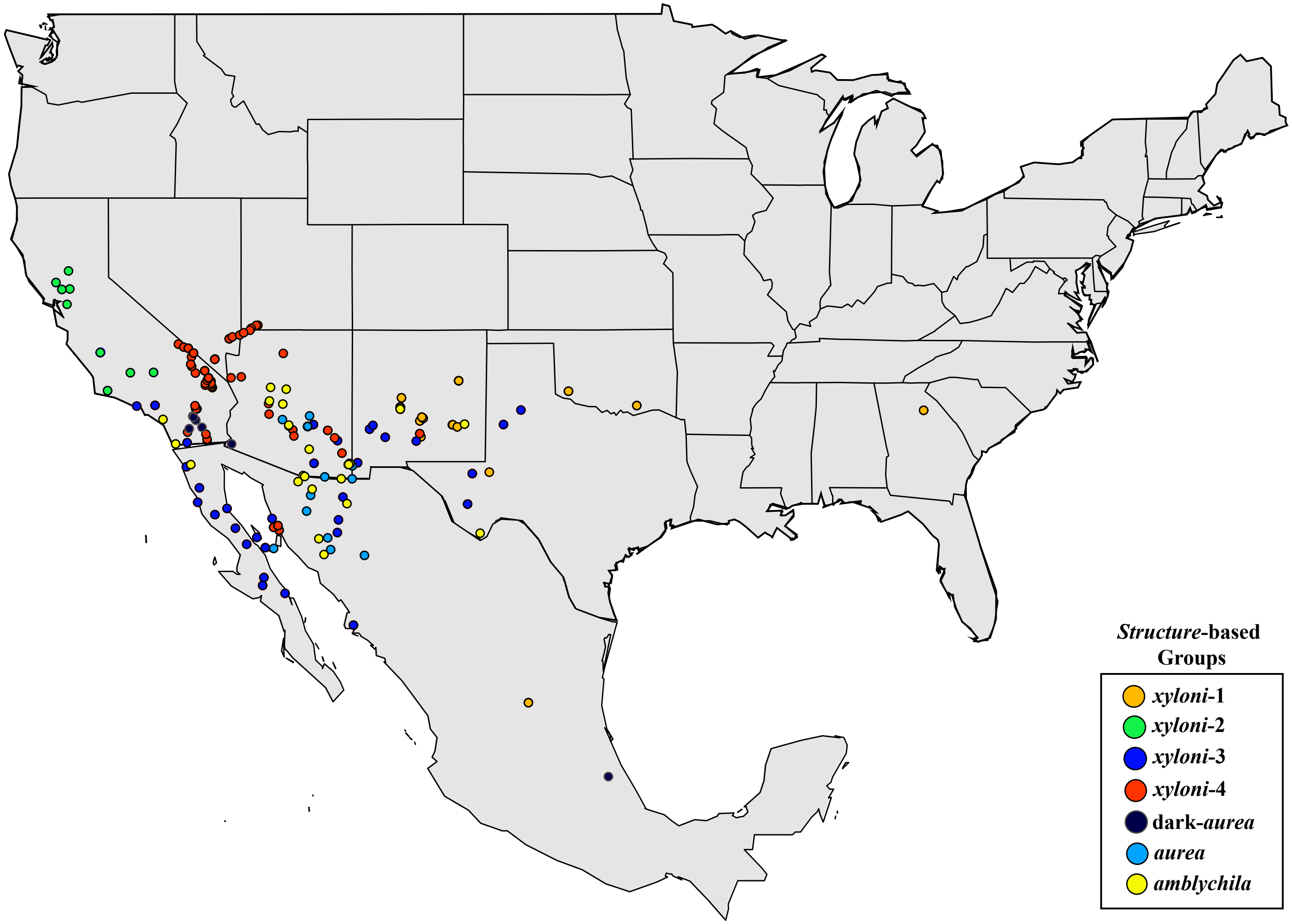

Figure 2.3: Geographical distribution of individual samples used for the filtered dataset (*S. geminata* is excluded from the map). Sample locations are color-coded according to assignment of individuals to a given group based on majority ancestry in a *Structure* cluster (*K*=7 clusters). Specimens of *S. geminata* for this study were obtained primarily from southern Mexico (see Appendix A).

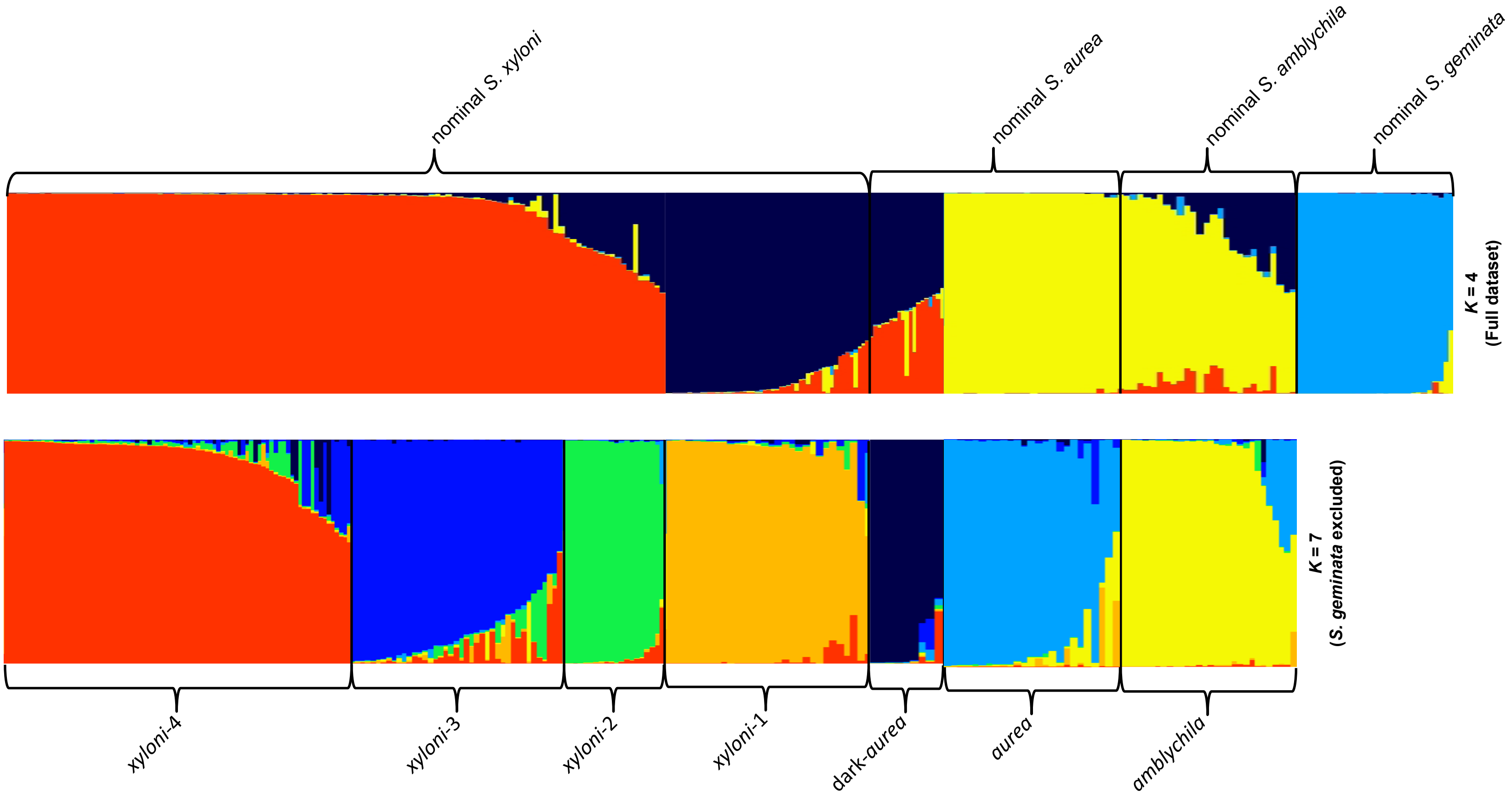

Figure 2.4: Visualization of *Structure* output for both the full dataset (*K*=4 clusters) and the data subset excluding *S. geminata* (*K*=7 clusters). Each individual sample is represented by a vertical bar divided into parts proportional to the individual's ancestry in each proposed genetic cluster. Groups based on individual majority ancestry are demarcated by vertical black lines.

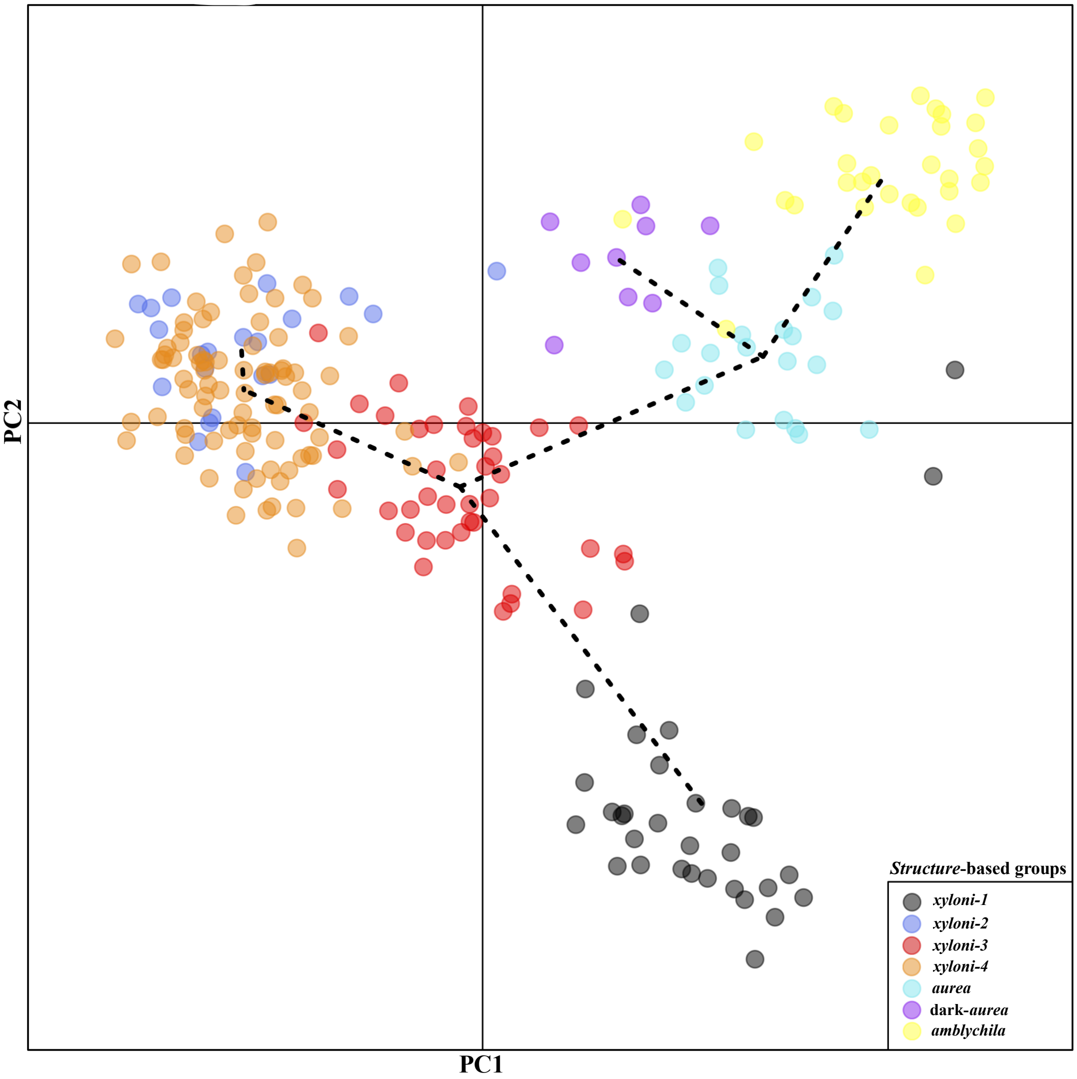

Figure 2.5: Results of the discriminant analysis of principal components (DAPC) based on the majority ancestry groups recognized following *Structure* analyses and projected on the first two principal components (PCs). The first PC explains 11.3% of the total variation, while the second PC explains 5.2%. Group centroids are connected by a minimum-spanning tree (dashed lines).

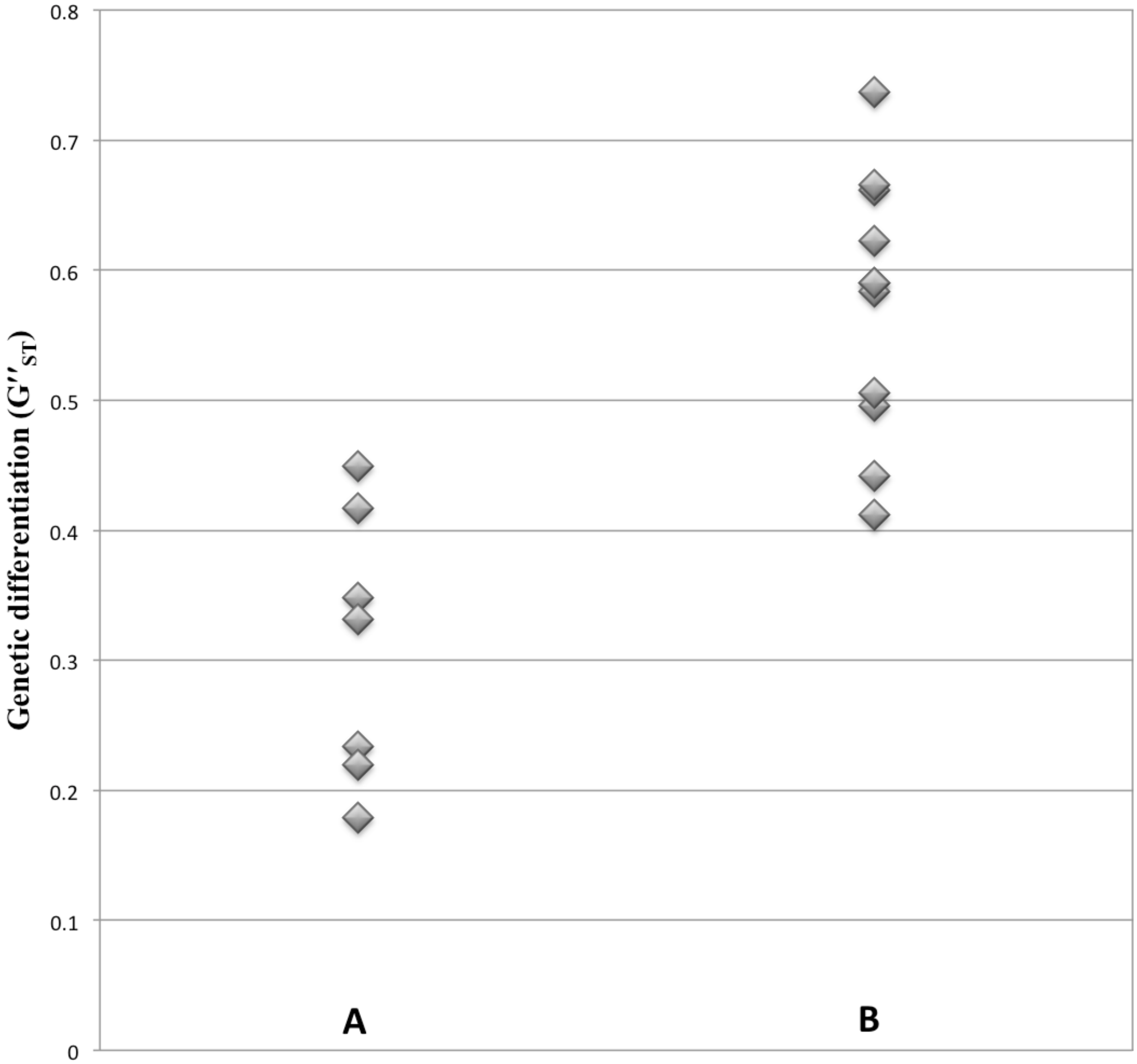

Figure 2.6: Estimates of G''_{ST} between: A) *Structure*-defined groups within valid nominal North American fire ant species; and B) suspected cryptic species within the valid nominal South American fire ant species *S. saevissima* and *S. invicta*. The two highest values in column A represent comparisons involving *xyloni*-1/*xyloni*-2 and *aurea*/dark-*aurea*.

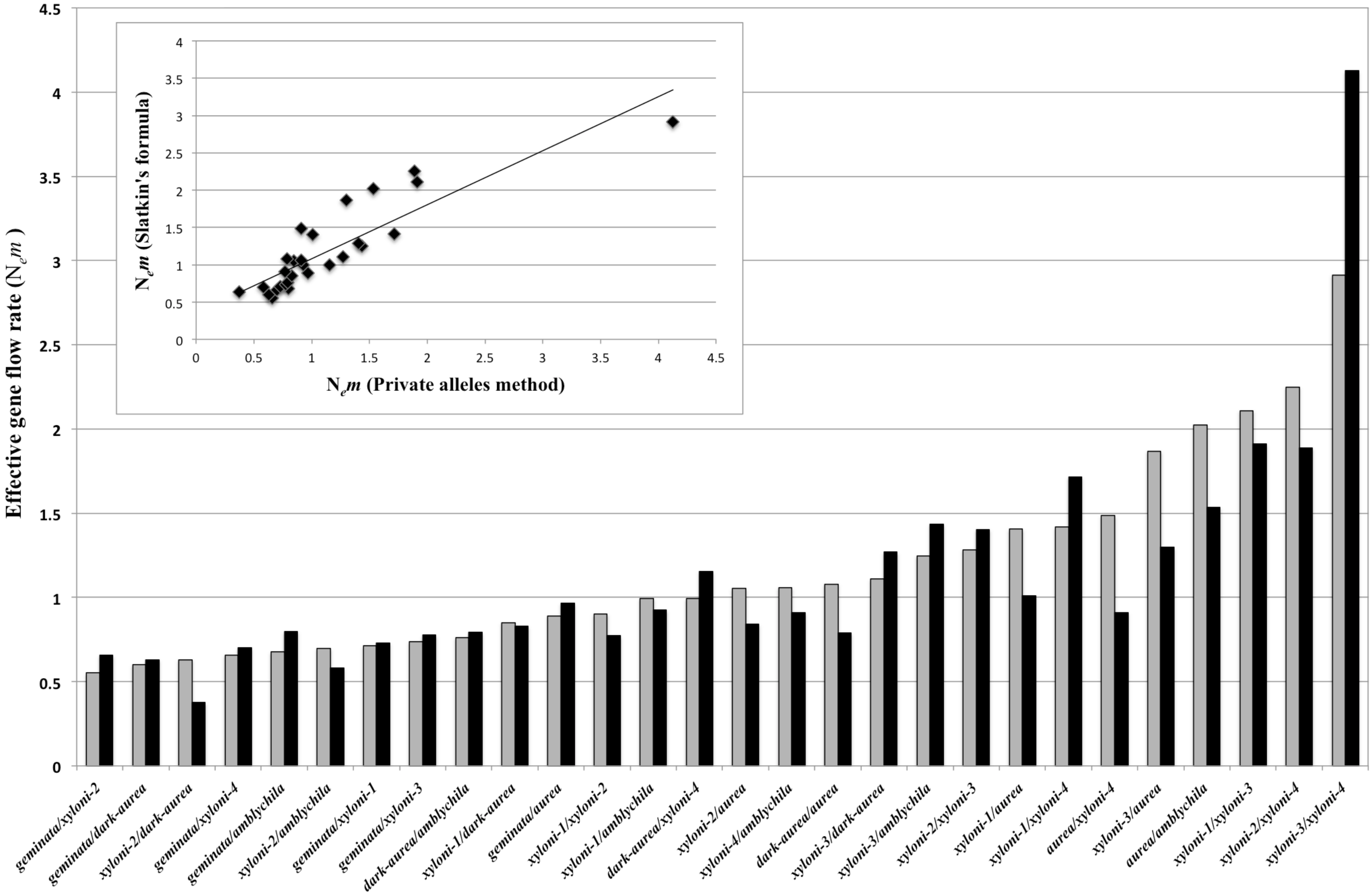

Figure 2.7: Estimates of effective gene flow rate ($N_e m$) between pairs of groups recognized following *Structure* analyses, based on Slatkin's (1987) formula (light bars) and the private alleles method (dark bars). Inset shows the association between the estimates derived from the two methods with the fitted least squares regression line.

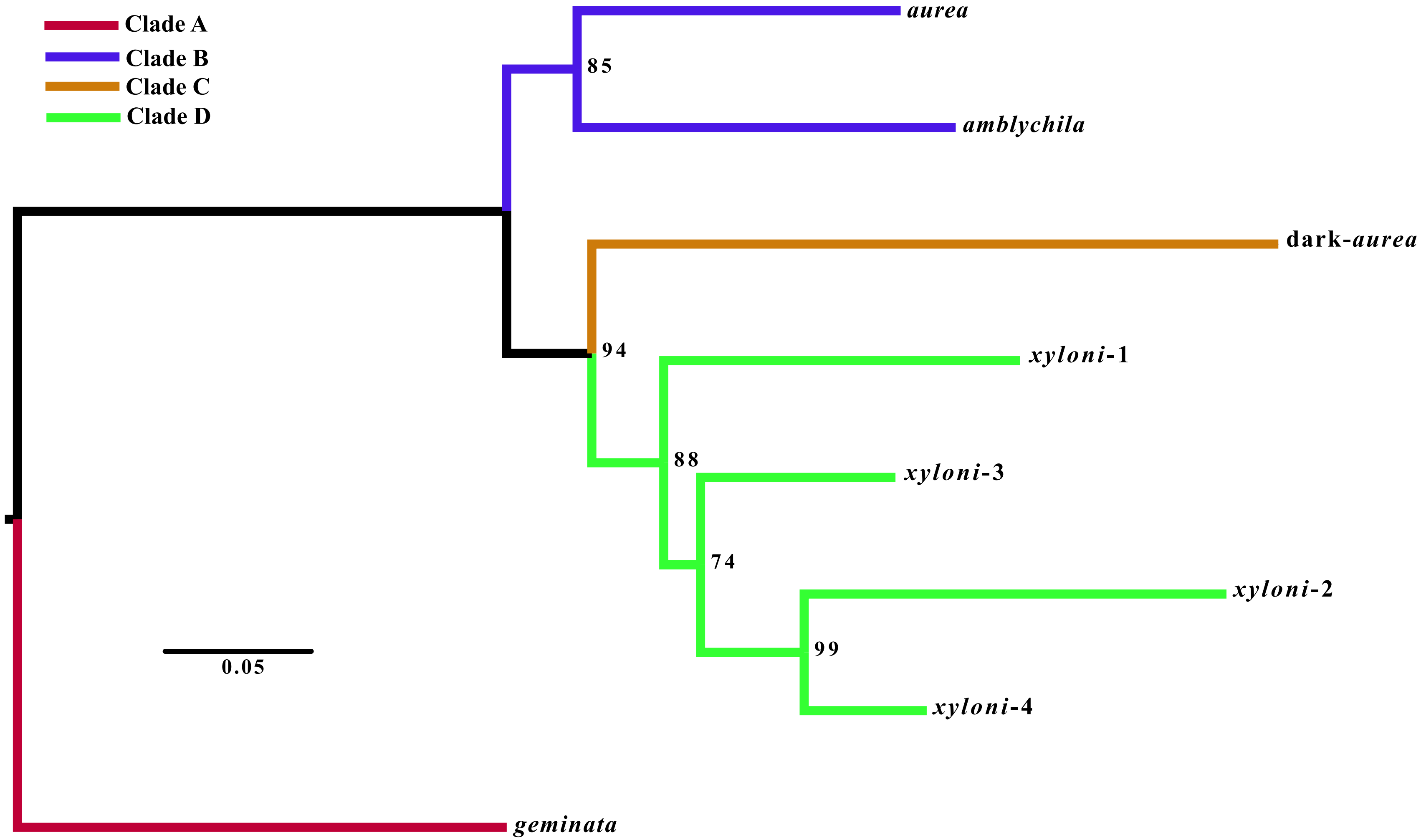

Figure 2.8: Neighbor-joining (NJ) tree depicting relationships of majority ancestry groups recognized following *Structure* analyses. Bootstrap (BS) support values >70 are shown for relevant nodes. Scale bar represents percent distance difference.

Table 2.1: Measurements of genetic differentiation (F_{ST} below diagonal, G''_{ST} above diagonal) between groups of individuals with predominant assignment to seven different genetic clusters inferred using *Structure* (as well as *S. geminata*)

	xyloni-1	*xyloni*-2	*xyloni*-3	*xyloni*-4	*aurea*	dark -*aurea*	*amblychila*	*S. geminata*
xyloni-1	---	0.449	0.234	0.332	0.345	0.462	0.439	0.648
xyloni-2	0.217	---	0.348	0.220	0.427	0.539	0.557	0.757
xyloni-3	0.106	0.163	---	0.179	0.278	0.393	0.374	0.646
xyloni-4	0.150	0.100	0.079	---	0.337	0.435	0.419	0.683
aurea	0.151	0.192	0.118	0.144	---	0.417	0.258	0.615
dark-*aurea*	0.227	0.284	0.184	0.201	0.188	---	0.515	0.718
amblychila	0.201	0.264	0.167	0.191	0.110	0.247	---	0.690

Table 2.2: Estimates of rates of contemporary gene flow, as calculated by *BayesAss*, between groups of individuals with predominant assignment to seven different genetic clusters inferred using *Structure*.

Recipient Populations	Source Populations						
	xyloni-1	*xyloni*-2	*xyloni*-3	*xyloni*-4	*aurea*	dark-*aurea*	*amblychila*
xyloni-1	0.9394 (0.9187 – 0.9602)	0.0087 (0.0002 – 0.0172)	0.0169 0.0050 – 0.0290)	0.0087 (0.0024 – 0.0172)	0.0088 (0.0018 – 0.0173)	0.0087 (0.0001 – 0.0174)	0.0087 (0.0001 – 0.0174)
xyloni-2	0.0123 (0.0004 – 0.0142)	0.9260 (0.9001 – 0.9520)	0.0123 (0.0005 – 0.0241)	0.0123 (0.0004 – 0.0242)	0.0122 (0.0004 – 0.0241)	0.0124 (0.0005 – 0.0243)	0.0124 (0.0005 – 0.0243)
xyloni-3	0.0071 (0.0001 – 0.0141)	0.0007 (0.0003 – 0.0141)	0.9340 (0.9145 – 0.9535)	0.0237 (0.0106 – 0.0368)	0.0070 (0.0000 – 0.0140)	0.0069 (0.0002 – 0.0136)	0.0140 (0.0044 – 0.0236)
xyloni-4	0.0036 (0.0001 – 0.0071)	0.0036 (0.0001 – 0.0071)	0.0076 (0.0061 – 0.0092)	0.9742 (0.9658 – 0.9828)	0.0036 (0.0001 – 0.0071)	0.0036 (0.0000 – 0.0072)	0.0036 (0.0000 – 0.0072)
aurea	0.0132 (0.0006 – 0.0258)	0.0123 (0.0003 – 0.0243)	0.0124 (0.0005 – 0.0243)	0.0123 (0.0052 – 0.0124)	0.9250 (0.8985 – 0.9515)	0.0123 (0.0005 – 0.0241)	0.0123 (0.0005 – 0.0242)
dark-*aurea*	0.0207 (0.0013 – 0.0401)	0.0209 (0.0001 – 0.0406)	0.0209 (0.0013 – 0.0404)	0.0207 (0.0012 – 0.0402)	0.0210 (0.0013 – 0.0406)	0.8748 (0.8359 – 0.9138)	0.0201 (0.0014 – 0.0405)
amblychila	0.0087 (0.0003 – 0.0170)	0.0086 (0.0003 – 0.0170)	0.0086 (0.0003 – 0.0169)	0.0086 (0.0004 – 0.0170)	0.0425 (0.0248 – 0.0602)	0.0086 (0.0002 – 0.0169)	0.9146 (0.8914 – 0.9378)

Confidence intervals (95%) given in parentheses. Non-immigrant rates shaded.

CHAPTER 3

TAXONOMIC REVIEW OF THE NATIVE NORTH AMERICAN FIRE ANTS (HYMENOPTERA: FORMICIDAE: *SOLENOPSIS*)[2]

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[2] Chialvo P, Shoemaker DD, Gotzek DA, and Ross KG. To be submitted to *Zootaxa*.

Abstract

Recent genetic analyses have revealed cryptic diversity in the native North American fire ants (Hymenoptera: Formicidae: *Solenopsis*). *Solenopsis maniosa*, junior synonym and western counterpart of *S. xyloni*, is raised to full status. A new species of desert fire ant, *Solenopsis arieli*, is described from material collected in the Salton Trough. A key to all native North American species within the *S. geminata* species group is provided.

INTRODUCTION

The genus *Solenopsis* Westwood (Formicidae: Myrmicinae) is a cosmopolitan taxon with 195 species worldwide (Bolton, 2014). Of particular interest to entomologists is a small subgroup of species collectively referred to as the “fire ants” (formerly the subgenus *Solenopsis*), which, unlike their congeners, have polymorphic workers and reside exclusively in the New World. Due to their complex morphology, the fire ants have undergone numerous taxonomic revisions and, consequently, have garnered a level of notoriety amongst myrmecologists (Creighton, 1930, 1950; Pitts, 2002). The taxonomy of native North American fire ants in particular has changed substantially within the last 150 years. Currently, only four valid species occur in North America: *S. geminata* Fabricius 1804, *S. xyloni* McCook 1879, *S. aurea* Wheeler 1906, and *S. amblychila* Wheeler 1915. Recent genetic analyses (Chialvo *et al.*, under review), however, have revealed cryptic diversity within the valid North American species. In this paper, we describe *S. arieli*, a new species of fire ant from the Salton Trough, and propose resurrecting *S. maniosa* from synonymy under *S. xyloni*.

MATERIALS AND METHODS

Species concept

For the purposes of this revision, we consider a species to be a separately evolving metapopulation lineage (*i.e.*, the Unified Species Concept; de Queiroz, 2007), with secondary operational criteria (such as reproductive isolation or phenetic distinctiveness) serving as additional evidence for separation.

Specimen information

Specimens used in this study were deposited at the following institutions:

MCZ – Museum of Comparative Zoology, Harvard University, Cambridge, MA.
UGCA – University of Georgia Collection of Arthropods, Athens, GA
NMNH – National Museum of Natural History, Washington D.C.

Genetic markers

Although *S. xyloni* and *S. maniosa* generally can be distinguished by their collection locality, specimens found within their zone of sympatry (*i.e.*, New Mexico and western Texas) can be difficult to identify. As such, we provide a list of microsatellite alleles that are present/absent in one subspecies or the other. For microsatellite PCR and sequencing information, see Asunce *et al*. (2009, 2011) and Ross *et al*. (2010).

Measurements and Indices

Specimen images were taken using a Canon EOS-1 digital camera with a Canon Macro Photo MP-E 65 mm lens (unless otherwise stated). Two Yongnuo Digital Speedlite YN650 III speed flashes were used in conjunction with a Styrofoam diffuser to provide appropriate lighting. Images were taken at varying focal depths using an ML-1000 digital imaging system (Microptics Inc., Ashland, VA) and subsequently combined with Helicon Focus Pro 6.4.2 (Helicon Soft Limited, Kharkov, Ukraine). Measurements follow the approach used by Trager (1991). Holotype material measurements are in parentheses. HL: head length; HW: head width; SL: scape length; EL: eye length; CI: cephalic index; SI: scape index; OI: ocular index; OMD: oculomandibular distance.

***SOLENOPSIS ARIELI*, CHIALVO, SP. N.**

Etymology

In dedication to Dr. Ariel A. Chialvo, renowned scientist and father.

Material examined

Holotype: Worker deposited in NMNH with data: “Holotype: *Solenopsis arieli*\Chialvo (IN-1B) [top label]” “USA: CA: Riverside Co.\Near Indio, 9SEP2013\Collector: SD Porter [bottom label]”

Paratypes: Workers (4) deposited in UGCA with data: “Paratypes: *Solenopsis arieli* \Chialvo (MEC-1) [top label]” “USA: CA: Riverside Co.\Near Mecca, 9SEP2013\ Collector: SD Porter [bottom label]”

Paratypes: Workers (15) deposited in UGCA with data: “Paratypes: *Solenopsis arieli* \Chialvo (DosPalmas-WA) [top label]” “USA: CA: Riverside Co.\Dos Palmas Preserve\ 31.5089 N, 115.8271 W\13MAR2017, W Armstrong [bottom label]”

Distribution (Fig. 3.1)

Salton Trough (Coachella Valley, Anza-Borrego Desert State Park, East/West Mesa, Yuha Desert, Yuma Desert). Colloquially referred to as the Colorado Desert (Brown, 1923; Mulcahy *et al*., 2006)

Worker diagnosis (Fig. 3.2)

Similar in appearance to *Solenopsis aurea*, though differs from the latter as follows: in full-face view, the head appears weakly cordate. Antennal rim reduced and does not surround the antennal socket. Head and thorax concolorous reddish-orange. Metanotal groove darkened. Gaster with distinct transverse black bands at the posterior end of each tergite. Banding pattern particularly evident when specimens placed in ethanol.

Measurements and indices

HL: 1.06-1.37 (1.17), HW: 1.01-1.39 (1.15), SL: 0.76-1.01 (0.84), EL: 0.13-0.19 (0.16), CI: 91-104 (99), SI: 62-77 (73), OI: 11-15 (14). N=39.

Description (Fig. 3.2)

Worker: In full-face view, the head appears weakly cordate; slightly taller than wide. Vertex is indented, but does not bear a deep longitudinal groove. Mandible evenly curved throughout, ending in three mandibular teeth, most posterior tooth fairly blunt. Clypeal carinae present and form conspicuous, triangular ridge. External clypeal margin flat to weakly concave. Eye generally small, with 40-50 facets. Thoracic setae long and abundant. Antennal rim reduced and does not surround the antennal socket. Head and thorax concolorous reddish-orange. Metanotal groove darkened. Gaster with distinct transverse black bands on posterior margins of each tergite.

Comments

Given the unique distribution, morphology, and genetic composition of *S. arieli*, it is unlikely that this new species is a junior synonym of any previously described fire ant (*e.g.*, *S. huachucana*). That said, it is possible that previous accounts of other desert fire ants within the Salton Trough actually referred to *S. arieli*. Snelling & George (1979), for instance, noted nominal *S. aurea* foraging at noon near Indio, CA (the location of the holotype material), despite the fact that *S. aurea* generally is considered a crepuscular forager (Creighton, 1950). Similar behavior has been observed in *S. arieli* found at Dos Palmas Preserve, 30 miles south of Indio (W. P. Armstrong, personal communication).

***SOLENOPSIS MANIOSA*, WHEELER 1915, NEW STATUS**

Taxonomic history

maniosa. *Solenopsis geminata* subsp. *maniosa* Wheeler, 1915:396 (w.q.m.) Variety of *Solenopsis xyloni*: Creighton, 1930:102. Junior synonym of *Solenopsis xyloni*: Creighton, 1950:232. Subspecies of *Solenopsis xyloni*: Cook, 1953:178. Junior synonym of *Solenopsis xyloni*: Snelling, 1963:9. Raised to species from synonymy under *Solenopsis xyloni*: Wheeler & Wheeler, 1986:13. Junior synonym of *Solenopsis xyloni*: Trager, 1991:166.

Material examined

Cotypes (MCZ), workers with data: “San Ysidro, Calif.\6-700 ft. I.6.11.\W.M. Wheeler [handwritten label]” “M.C.Z.\Cotype\22902 [typed red label]” “Syntypes: Solenopsis\ geminata subsp.\maniosa Wheeler [handwritten red label]” “MCZ-ENT\00022902 [white label with QR code]

Distribution (Fig. 3.1)

California, Baja California (Mexico), southern Nevada and Utah, Arizona, New Mexico, western Texas, Sonora (Mexico). May occur as far north as Colorado (Gregg, 1963).

Worker diagnosis

Within the area of sympatry with *S. xyloni* (*i.e.*, New Mexico, western Texas), *S. maniosa* samples can be characterized by the following microsatellite allele frequency patterns: *C259*^{186} fixed, *wharf_rat_PT*^{305} fixed, *C487*^{314} fixed, *Wilco_PT*^{75} present, *Wilco_PT*^{78} present, *i-106*^{87} absent, *i-106*^{89} absent.

Measurements and indices

HL: 0.71 – 1.38, HW: 0.63 – 1.41, SL: 0.46 – 0.91, EL: 0.10 – 0.22, CI: 85 – 105, SI: 57 – 93, OI: 14 – 17. N = 6.

Description (Figure 3.3)

Worker: In full-face view, the head appears slightly cordate with straight posterior border; taller than wide. Vertex noticeably indented, but does not bear deep groove. Mandible gradually curved throughout, ending in three mandibular teeth. Clypeal carinae present. Outer face of mandible bearing coarse, even longitudinal striae. Eye large, with 70-80 facets; OMD 1-1.5 x EL. Antennal scape almost reach posterior corners of head. Head and thorax concolorous reddish-yellow. Gaster often ranging from dark brown to black.

Comments

Although previous morphological studies found no consistent differences between *S. xyloni* and *S. maniosa*, many respected myrmecologists (*e.g.*, Snelling, Buren, George, Wheeler & Wheeler) nevertheless believed *S. maniosa* to be a biologically relevant taxon.

Our previous molecular study (Chialvo *et al.*, in review) further supports this notion, having found significant genetic differentiation between eastern and western populations of *S. xyloni*, even within areas of sympatry.

NATURAL HISTORY

Feeding behavior

S. maniosa is generally considered an omnivore, actively feeding on a variety of floral sources, small live and newly dead arthropods, crops, and household stored foods. This species has also been known to attack ground-nesting birds (Eckert & Mallis, 1937), tend aphids for honeydew (Snelling & George, 1979), and raid the colonies of other ant species (*e.g.*, *Pheidole, Pogonomyrmex, Veromessor;* Wheeler & Wheeler, 1986). Foraging typically occurs during the morning, late afternoon, and evening hours; in the wintertime, however, it can occur at any point during the day.

Nesting behavior

Nests have been located in a range of habitats, including creosote bush scrubland, citrus orchards, and urban areas. Within these areas, they are generally found underneath rocks or at the base of shrubs and trees (Snelling & George, 1979), although these ants have also been known to form large colonies in artificial spaces such as in basements and kitchens (Mallis, 1941; Knight & Rust, 1990). Nest mounds typically are small (3 cm tall, 10-15 cm long), irregularly-shaped, and contain several entrances, although larger nests have been reported (Wheeler & Wheeler, 1973).

Reproductive and social behavior

In California, nuptial swarms can be seen from May to September, usually on a warm evening after a period of cool weather (Mallis, 1938). Some colonies have been found to contain multiple reproductive queens (polygyny; Hooper & Rust, 1997).

Sting and venom chemistry

The sting of *S. maniosa* can be quite painful, often resulting in an intense burning or itching sensation that can last for hours (Snelling & George, 1979). Blum *et al.* (1985) found *S. maniosa* has a similar venom composition to *S. xyloni* (*i.e.*, mostly *cis*- and *trans*- isomers of 2-undecyl-6-methylpiperidine), though it has a much lower *cis*:*trans* ration (1.2:1) than its eastern counterpart (6:1). Moreover, *S. maniosa* lacks the related compound 2-undecyl-6-methyl-1-piperidine, which has only been found in *S. xyloni* (Brand *et al*., 1972, Blum *et al*., 1973).

ACKNOWLEDGEMENTS

The material used in this study was provided by the following museums and individuals: Museum of Comparative Zoology (MCZ) and Sanford Porter. We thank Wayne Armstrong, Ben Gochnour, Tommy McElrath, Joe McHugh, Emma Pletcher, Ted Schultz, Clare Scott Chialvo, James Trager, and Phil Ward for their comments, feedback, and assistance. Funding and facilities for this project were provided by the University of Georgia, United States Department of Agriculture, and grants NSF-DEB#1354479 to K. Ross and D. Shoemaker and NSF- DEB#1020979 to A. Suarez and K. Ross.

Table 3.1: Dichotomous key for the native North American fire ants (*Solenopsis*)

1	Petiole and post-petiole present. Antennae with 10 segments; two most apical segments elongated and form a distinct club. Propodeal spines absent.	2
-	Post-petiole absent. Antennae with fewer or greater than 10 segments. Two segmented apical club absent. Propodeal spines present.	Non-*Solenopsis*
2	Clypeus lacks medial tooth	3
-	Clypeus with a conspicuous medial tooth	Non-native fire ant
3	Vertex deeply grooved. Lack of mandibular teeth. Found in coastal plains of the Carolinas, Georgia, Florida, Texas, and eastern Mexico.	*S. geminata*
-	Vertex indented, but not grooved. Distinct mandibular teeth present. Found in southeastern or southwestern United States.	4
4	Eye contains 40-50 facets	5
-	Eye contains 70-80 facets	6
5	Clypeal teeth and ridge distinct. Found in southern California, Arizona, New Mexico, western Texas, Sonara. Often found at elevations below 1500 meters.	7
-	Clypeal teeth and ridge reduced or absent. Found in southern California, Arizona, New Mexico, western Texas, Sonara. Often found at higher elevations (1500 – 2500 m).	*S. amblychila*
6	Head and thorax concolorous reddish-yellow. Gaster often dark brown or black. Found in California east to western Texas, southern Nevada and Utah, Sonora, Baja. In areas of sympatry with *S. xyloni* (New Mexico and western Texas), the following allelic patterns occur: *C259*^{186} fixed, *wharf_rat_PT*^{305} fixed, *C487*^{314} fixed, *Wilco_PT*^{75} present, *Wilco_PT*^{78} present, *i-106*^{87} absent, *i-106*^{89} absent.	*S. maniosa*
-	Head and thorax red to dark brown. Found in central New Mexico east to central Texas. Historical range further east through Texas to South Carolina. In areas of sympatry with *S. maniosa* (New Mexico and western Texas), the following allelic patterns occur: *Wilco_PT*^{75} absent, *Wilco_PT*^{78} absent, *i-106*^{87} present, *i-106*^{89} present.	*S. xyloni*
7	Head and thorax concolorous reddish-orange. Antennal rim reduced. Gaster with distinct transverse black bands. Found in the Salton Trough (Colorado Desert)	*S. arieli*
-	Head and thorax concolorous yellow or gold. Antennal rim pronounced. Gaster without distinct transverse black bands. Found in southern California, Arizona, New Mexico, western Texas, Sonara.	*S. aurea*

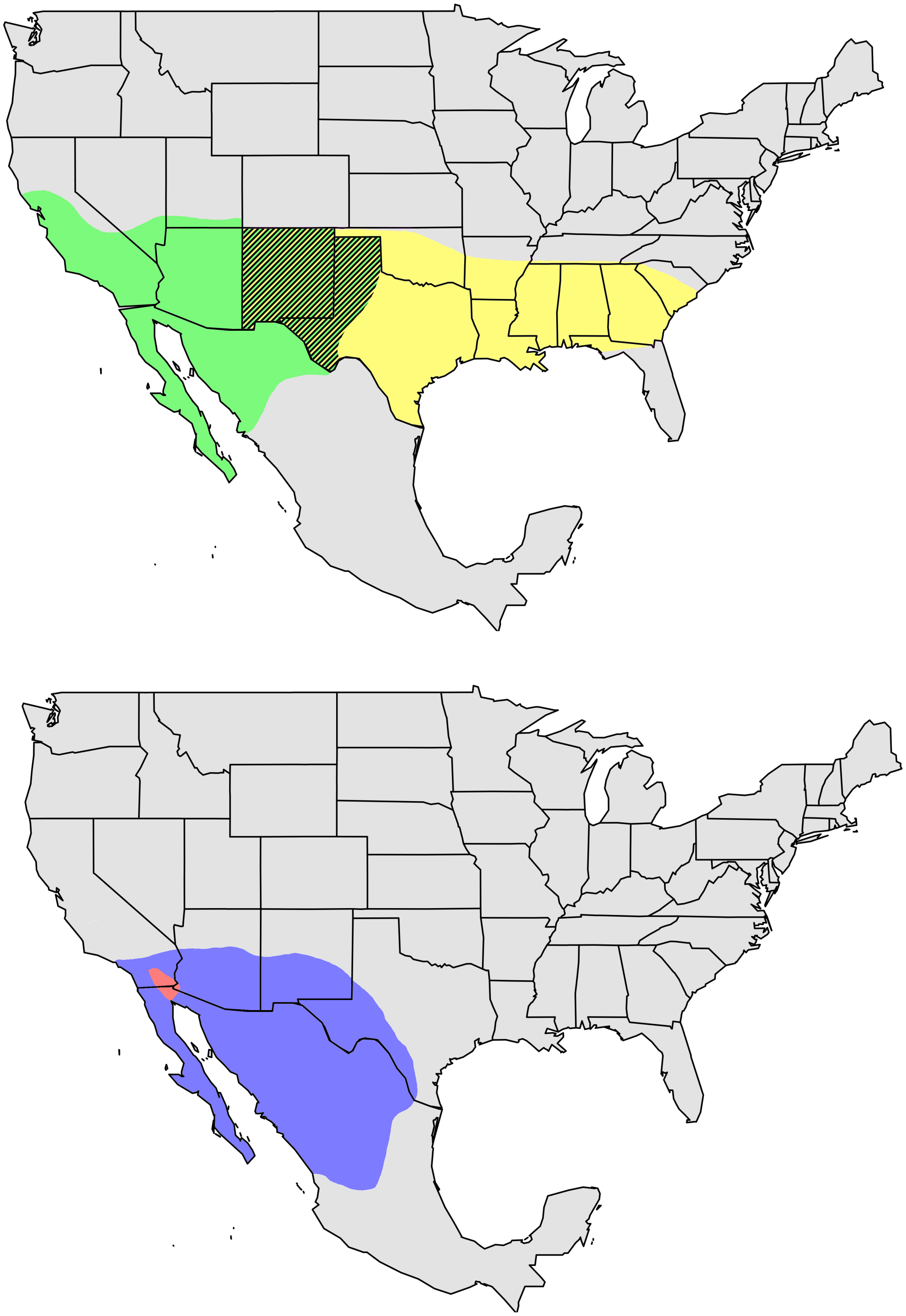

Figure 3.1: Estimated geographical distributions of the new North American fire ant taxa and their closely related congeners. Yellow corresponds to *S. xyloni*, green to *S. maniosa* pink to *S. arieli*, and lavender to *S. aurea*. Hatched lines represent the presumed area of sympatry between *S. xyloni* and *S. maniosa*. *Solenopsis xyloni* is absent from a large portion of its historical range due to the introduction and spread of the red imported fire ant, *Solenopsis invicta*.

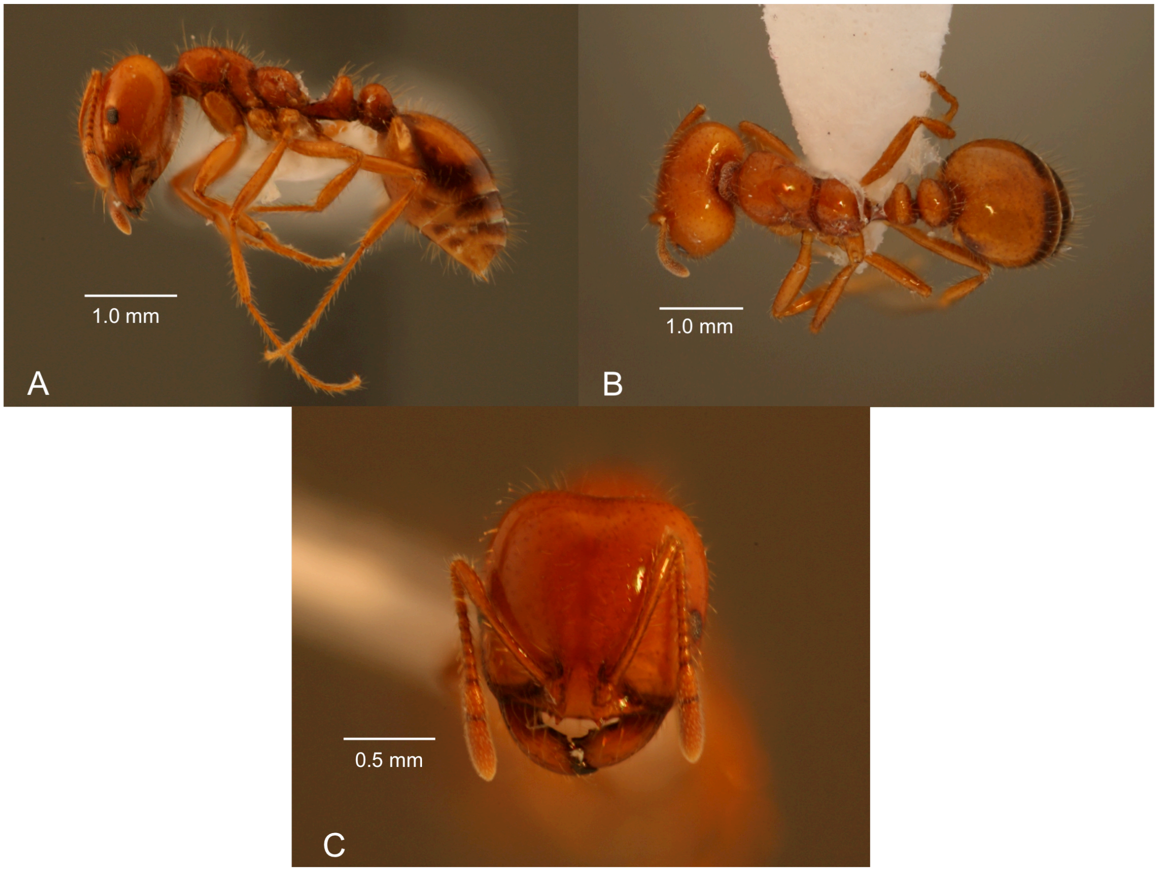

Figure 3.2: *Solenopsis arieli* holotype (IN-1B). Images of specimen in A) profile view; B) dorsal view; and C) full face view.

Figure 3.3: *Solenopsis maniosa* type material (MCZ). Images of specimen in: A) profile view, B) dorsal view, and C) full face view. Images by Stefan Cover.

CHAPTER 4

OVERVIEW OF TRANSFORMATIVE LEARNING THEORY WITH NOTES ON APPLICATIONS TO LIFE SCIENCES EDUCATION[3]

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[3] Chialvo P. To be submitted to *The American Biology Teacher*

Abstract

Transformative learning theory – as interpreted by Jack Mezirow in the late 1970s – represents the unique fusion or intersection of several distinct lines of philosophical and pedagogical thinking, including strong influences by Thomas Kuhn, Paulo Freire, and Jürgen Habermas. It seeks to engender or otherwise promote changes in perspective via the utilization of critical reflection (understanding of inherent, internal assumptions regarding a question or situation) and critical discourse (sociolinguistic validation of perspective). Although somewhat hampered by certain conceptual limitations (*e.g.*, the true nature of experience, the assumption of positivity, *etc.*), it has the potential to be an incredibly powerful tool for adult educators in both the social and physical sciences.

INTRODUCTION

Education, at perhaps its most basic or fundamental level, represents change in an individual; it is the initial construction and continued evolution of knowledge that renders a person wholly different in mind, personality, and attitude. Its transformative nature is apparent across countless learning theories, from Skinner's (1971) behaviorism to the more humanistic concepts of Rodgers (1983) and his contemporaries. There is no theory more evocative of this notion, however, than the appropriately named transformational or transformative learning. Though I had been exposed to the theory before (Merriam & Bierema, 2014), I cannot say I fully understood it. Initially, the idea appeared too nebulous, too abstract to see any practical use or application. Given my extensive background in the life sciences, which oftentimes shun the metaphysical in favor of what is purely empirical and quantifiable, such a conclusion is not all that surprising. However, as I progressed through more advanced courses on pedagogy and began to gain a far better appreciation for adult educational theory, my thoughts constantly returned to the idea of change through learning. It seemed a powerful tool, but could I employ it in my own classes? With this question in mind, I decided to investigate the matter more thoroughly, delving into the theory and influences behind transformational learning, as well as its criticisms. With a more complete background, I then developed two novel teaching activities. The first utilizes narrative inquiry (Schwandt, 2007) to explain the development of natural selection and evolution. The second module uses genetic data collected in Chapter 2 as the basis for teaching species concepts and delimitation.

THEORY OF TRANSFORMATIONAL LEARNING

The theoretical basis underlying transformational learning is certainly complex (Kitchenham, 2008), though its roots can nevertheless be traced back to the work of Jack Mezirow, who initially studied the concept in relation to the postsecondary education of American women. He initially postulated that personal transformations arose in a distinct series of ten phases, beginning with a "disorienting dilemma" or experience that challenges current thinking and ending with the eventual reintegration of oneself via a new perspective (Mezirow, 1978):

> … the transformation cycle was described often involving: (1) a disorienting dilemma; (2) self-examination; (3) a critical assessment of sex-role assumptions and a sense of alienation from taken-for-granted social roles and expectations; (4) relating one's discontent to a current public issue; (5) exploring options for new ways of living; (6) building competence and self-confidence in new roles; (7) planning a course of action; (8) acquiring knowledge and skills for implementing one's plans; (9) provisional efforts to try new roles; and (10) a reintegration into society on the basis of conditions dictated by the new perspective. (p. 12)

To reach this endpoint, however, one must engage in two major activities: 1) critical reflection of assumptions made in a particular perspective and 2) critical discourse that supports one's choice or judgment (Feinstein, 2004; Mezirow, 2006). The two, while sounding quite similar, actually represent entirely different ideas. Critical reflection is one of three major modes leading to transformation, though it focuses primarily on "the nature and consequences of one's actions [and] the related circumstances of their origin," rather than the content or process (Kitchenham, 2008). Likewise, the former, in reflecting on the overall premise of a situation, leads to profound change in perspective, while the latter constitute more simple or straightforward changes of meaning schemes. Critical discourse, however, takes on a much more sociolinguistic facet that is not necessarily found in critical reflection; it is the outward "dialogue involving the assessment of beliefs, feelings, and values" that can lead to some form of transformation (Mezirow, 2003). True meaning may be determined at the individual level, but it is only though a social context that significance is obtained or otherwise constructed (Vygotsky, 1978). Mezirow (1998) further posited that critical reflection of assumptions could be broken down into two basic forms. The first of which, objective reframing, is an action- or narrative-oriented consideration *of* the assumptions made in a particular perspective. Subjective reframing, on the other hand, deals with the distinct narrative, systemic, therapeutic, and epistemic reflections *on* the assumptions themselves.

In the three decades after the original publication of Mezirow's (1978) findings, he provided numerous updates and revisions, expanding his theory in detail while simultaneously narrowing it to a finer scope (for a review, see Kitchenham, 2008). It is of little wonder, then, why Mezirow is often synonymous with the very mention of transformative learning. That is not to say, however, that Mezirow invented the subject or otherwise represents the sole perspective on the matter (Boyd, 1989; Daloz, 1999; Cranton, 2006; Taylor, 2007); while he certainly became one of the theory's most prominent proponents and wrote of it extensively (Calleja, 2014), his work is the synthesis of several fundamental lines of thinking, including the philosophical and pedagogical contributions of Kuhn (1962), Freire (1970), and Habermas (1971, 1984). In order to fully appreciate transformational learning, as Mezirow understood it, it is critical to evaluate – or at the very least mention – the convergence of these seemingly unrelated ideas, as they have immense bearing on the subject itself.

Kuhn: paradigm shifts and frames of reference

Thomas Kuhn's *Structure of Scientific Revolutions* (1962) is arguably the most influential treatise on the philosophy of science in the last century (Mayoral, 2012) and, moreover, represents the first major conceptual building block for Mezirow's theory of transformational learning (Mezirow, 1991). Kuhn, in an attempt to break what he viewed as the "tourist brochure" perspective of scientific inquiry (Kuukkanen, 2013), formulated a powerful idea: the history of science is, in part, influenced by the discovery, maintenance, and replacement of paradigms, or "universally recognized scientific achievements that for a time provide model problems and solutions to a community of practitioners" (Kuhn, 1962). Put more simply, paradigms are discoveries that shake the very foundations of a given field, garnering attention from researchers and pulling them towards not only a new set of problems and solutions, but an entirely different perspective as well. Consider, for instance, the impact Charles Darwin had on the entire subject of biology with the publication of *On the Origin of Species* (1859); it not only birthed the fundamental evolutionary theory – which, in itself, caused a cascade of different fields to arise – but perhaps more importantly called into question the long-established dogma of creation and the tenets of natural theology (Paley, 1803). Truly, it marked a monumental shift in biological thinking and the subsequent 150 years of scientific progress have yet to overthrow its position or robustness as a paradigm. Such revolutions in perspective became the basis for what we now consider transformational learning.

From Kuhn's seminal work, Mezirow obtained a fresh set of ideas that heavily influenced his eventual formulation of transformational learning theory. In particular, he saw paradigms as socioculturally-constructed frames of reference, the boundaries that inform us of "the context of a social situation and how to understand and behave in it" (Mezirow, 1991). These frames are held together by numerous habits of mind, the broad accumulation of significant experiences that change our behavior and thinking, as well as ingrained meaning perspectives or points of view (Mezirow, 1997). When such integral views are challenged or questioned, there is the potential for a transformative act to occur. This potential, however, is heavily affected by – if not totally reliant upon – the individual and his/her ability to approach the situation critically, a point discussed at length by Mezirow's other great influences: Paulo Freire and Jürgen Habermas.

Freire: liberation through transformation

While Kuhn (1962) investigated the transformative nature of viewpoints through the historical and philosophical context of scientific inquiry, Freire approached the matter from a far more personal – and some would say radical – angle. As a Christian Socialist and educator of the poor and destitute in countries across South America, he became intimately familiar with the daily struggles of under-privileged and abused populations. These experiences led him to write the acclaimed *Pedagogy of the Oppressed* (1970), which heavily criticizes the "banking" system of most Western education, the process of simply gifting or depositing information into the student without any further exchange.

Such a system actively prevents personal liberation as it “anesthetizes and inhibits creative power… [and] attempts to maintain the *submersion* of consciousness” (Freire, 1970, italics in original). The solution, Freire claims, is the use of problem-posing educational practices instead, which seek to engender an “ability to analyze, pose questions, and take action on the social, political, cultural, and economic contexts that influence and shape […] lives” (Dirkx, 1998). Such methods seek to raise the individual from a state of local oppression and disempowerment to one that allows for critical reflection of a problem and encourages action or change.

This goal of conscientization (consciousness-raising) can only be achieved, however, by dissolving the “instilled certainty” that instructors and students belong in some form of Hegelian master-slave relationship or that true learning is an act of forced imposition upon a completely naïve subject (Freire, 1974). Education must be conducted in a democratic, bidirectional manner, with both students and instructors engaging each other through critical discourse or dialogue. In doing so, individuals are both freed from the oppressive narrative that previously governed most aspects of their lives and, perhaps more importantly, are allowed to seek a path or position based on their own reflection of the problem.

As with Kuhn, the importance of change or transformation in perspective is highlighted in the work of Freire, though it more greatly emphasizes the individual and his/her use of critical thinking to achieve change. Likewise, the parallels between the sentiments presented in *Pedagogy of the Oppressed* (Freire, 1970) and Mezirow’s theory are quite clear. Both authors acknowledge the presence of powerful cultural and social forces that influence our thoughts, imposing a perspective or frame of reference that may not be entirely our own. This viewpoint, however, can be overcome via critical self-reflection (conscientization) and critical discourse. Truly, language – in its many forms – can act as a powerful transformative or emancipatory force, a notion argued by the likes of Jürgen Habermas and other followers of the continental tradition.

Habermas: communication and domains of learning

Habermas (1984), a proponent of communicative rationality, largely emphasized the importance of sociolinguistic interactions between individuals as a means of establishing validity and coming to a mutual, rational agreement or understanding of a subject. Such communication is, imperative to the general act of learning; it allows an individual to interact with not only their surroundings, but their own internal influences (Calleja, 2014). Among them is an inherent interest to know, to understand and generate knowledge. The purpose of this seemingly instinctual directive, however, is not necessarily the same in all cases. Learning can be placed into three categories based on their relationships to preexisting social contexts: technical, practical, and emancipatory. Technical learning is concerned with "getting the skills and information necessary to construct systems and devise methods for making those systems work" (Jesson & Newman, 2004). It is instrumental in action and generates results via the empirical analysis of causalities. Likewise, practical learning focuses on rules governing social interactions and what specifically defines expected behaviors and outcomes. Finally, emancipatory learning encompasses the principles of self-reflection; it is the process of, "becoming critically aware of how and why the structure of psycho-cultural assumptions has come to constrain the way we see ourselves and our relationships" (Mezirow, 1981).

Mezirow (1985) incorporated Habermas's domains of learning into his formal theory of transformative learning, though with a slight change in terminology. Rather than using the technical, practical, and emancipatory designations, he instead chose the labels of instrumental, dialogic, and self-reflective, though their meanings remained essentially unchanged. Each of the domains share the same basic set of processes: learning within meaning schemes, learning new meaning schemes, and learning through meaning transformation (Kitchenham, 2008). As we have discussed previously, the actions and interactions of these meaning schemes make up the different types of reflection (*e.g.,* content, process, and premise) that can ultimately lead to either straightforward transformations (in the case of content and process reflections) or profound changes in perspective (as with a reflection of premise).

When taken as a whole, it is immensely clear how Mezirow's theory of transformative learning is the convergence point for a huge body of pedagogical and philosophical theory, including the collective work of Thomas Kuhn, Paulo Freire, and Jürgen Habermas. However, as with any theory – educational or otherwise – we must take into consideration the various critiques and criticisms that have been leveled against it by practitioners in its respective field. Understanding a theory and its background is not enough; we must also know its limitations.

CRITICISMS OF TRANSFORMATIVE LEARNING

Though described by some authors (*e.g.,* Kitchenham, 2008) as the "definitive framework for describing how adults learn best," transformative learning has nevertheless garnered a large amount of criticism since its initial development in the late

1970s (Collard & Law 1989; Hart, 1990; Tennant, 1993; Dirkx, Mezirow, & Cranton, 2006), including from Mezirow himself, who has referred to it as a “theory in progress” (Mezirow & Associates, 2000). Much of this critique and research, however, has been fairly redundant, as it has focused too heavily on determining the outcomes of transformative learning in unique settings and has not pushed the conceptual boundaries to any measurable extent. Taylor and Snyder (2012), for instance, have asserted that “researchers seem to be stuck on a treadmill, repeating the same research over and over again, and making less than satisfactory theoretical progress.” Others still have made the bold claim that transformative learning is not even an observable phenomenon and is no different than “good learning” (Newman, 2012). In light of this stagnation, there has been an effort to revitalize the subject of transformative learning by investigating some of the critical gaps that remain in our knowledge. In particular, I wish to examine the contributions of Taylor and Cranton (2013).

Taylor and Cranton: a theory in progress?

Taylor and Cranton (2013) approached the issues surrounding transformative learning from a perspective similar to that of Taylor and Snyder (2012), essentially one of faint optimism for the field in general. However, rather than rehashing the same, tired ideas of countless previous reviews – which, they note, arose from a number of gross oversights, including the lack of participation from European adult educators - they decided instead to focus on a set of five provocative subjects in hopes of spurring future study and discussion: the nature of experiences, role of empathy, assumption of positivity, motivation to change, and expansion of experimental methodology.

The true nature of experiences

Past experiences provide the very building blocks for transformative learning, as they constitute our meaning schemes and overall frames of reference, which, as noted above, can change dramatically through critical reflection and discourse. What we mean by "experience," however, is not exactly clear. Typical definitions, such as those used by Dewey (1938) equate experience to some form of knowledge or skill that can be transferred from one situation to another. Such a definition is oftentimes too vague to be useful. Moreover, it fails to recognize the importance of cultural and social factors that give both context and meaning to that experience as a whole (Clark & Wilson, 1991; Nohl, 2009). Put more simply, experiences cannot be properly interpreted or fully understood without observing them in relation to their original context, as one's current perspective could be highly influenced by external forces (*e.g.,* culture) and internal conflicts (*e.g.,* contradictions).

The roles of empathy

Empathy, whether viewed from a purely cognitive or humanistic viewpoint, plays a number of important roles in certain aspects of transformative learning. Specifically, it: 1) allows individuals to identify different perspectives in others; 2) decreases the probability of being judged prematurely; 3) increases the probability of finding mutual understanding; and 4) "facilitates critical reflection through the emotive valence of assumptions" (Taylor & Cranton, 2013). It is this facilitation that is of particular interest. Critical reflection, as we know, is an integral component of transformative learning; likewise, emotion is inextricably connected to the act of critical reflection given its subjective nature (van Woerkom, 2010). When viewed together, the next question becomes abundantly clear: how and to what extent does empathy support/foster transformative learning as a whole?

The assumption of positivity

In reading much of the background literature for transformative learning, one gets the distinct sense or impression that while the process of transformation may be quite difficult, it is invariably for the better. Such a reaction is not unusual given the theory's strong humanistic and constructivistic influences. Indeed, what could necessarily be wrong about fulfilling one's true potential or constructing a greater understanding of personal experiences? The answer lies in the "romantic notion" that perspectives chosen through critical reflection are "never harmful, and that [their] benefits… always outweigh the injuries inflicted by more coercive alternatives" (Baptise, 2008). This assumption is based largely on the ingrained Western values of personal exceptionalism and rugged individualism (to use a pair of political terms). Of course, not all cultures hold to these values; many Eastern countries, for instance, place a much greater emphasis on the group or collective rather than the individual. If we are to have a more complete understanding of transformative learning, we must remove these overarching assumptions and delve further into the positive or negative nature of perspective changes.

The motivation to change

Mezirow's theory is also based on the underlying assumption that transformative learning, given its emancipatory roots (Habermas, 1971), is strictly voluntary in nature.

There is, however, a very fine line between what is considered purely educational and what ventures into the territory of indoctrination or brainwashing (Mezirow, 2012). In such cases of subtle ethical and pragmatic nuances, it is especially important to understand the intrinsic and extrinsic factors that influence one's motivation to learn (Wlodkowski, 2005) or change perspectives. Despite this clear need, novel theoretical research on the matter is particularly difficult to find and leaves some critical questions unanswered. Are individuals inherently open to transformative experiences or is there some cognitive/psychological threshold that must be overcome in order to reach a particular level of openness? Can this process be any way facilitated (Weimer, 2012)?

The stagnation of experimental methodology

Taylor and Snyder (2012) described modern research in transformative learning as a form of experimental "treadmill" in which authors continuously revisited the similar topics and, as a result, stopped "learning anything new" altogether (Taylor & Cranton, 2013). Most experiments relied entirely on the same three factors: 1) interviews done after learning has already taken place (*i.e.,* retrospectively); 2) an interpretive or antipositivist research paradigm that seeks to understand the subjective nature of knowledge via an individual's viewpoint; and 3) a report of prevailing themes found in the interviewees' responses. It is a simple method – one that has greatly enhanced our basic understanding of transformative learning in the past – though it is also one that has been done to death. Simply put, it is time for transformative learning to undergo a metamorphosis of its own. For instance, rather than conduct retrospective interviews, we could begin to employ more long-term studies that trace the path and progress of transformation through time. Positivist or critical paradigms could also replace our old interpretive perspectives and thematic analyses could yield to any number of different research methods (*e.g.,* participatory action research) (Taylor & Cranton, 2013).

TRANSFORMATIVE LEARNING: NATURAL SELECTION AND EVOLUTION

As mentioned in the introduction, many practitioners of the life sciences are not entirely keen on topics that fall outside of what is strictly empirical or quantifiable and, as such, they oftentimes ignore subjects in more distantly related fields (*e.g.,* adult education). Having gone through and considered the basic principles, influences, and criticisms of transformative learning, however, I now see this is a grave mistake; there is much to be learned from the social sciences, information that could greatly impact not only our own research, but perhaps more importantly, how it is perceived by the general public. In particular, I believe that transformative learning can be used to tackle one of the most controversial issues in the life sciences: the theory of natural selection and evolution.

To a biologist, Darwin's theory of natural selection and evolution (1859) is, quite simply, a given fact; it is the principle that holds all of biology together (Dobzhansky, 1973). Certainly, it has been discussed and revised countless times since its original publication, but its essential meaning has remained intact and has, in fact, grown to encompass a myriad of different perspectives. There is, however, staunch resistance to the theory, particularly from religious groups who claim their particular vision of creation (in whatever form it happens to take) is the one and only truth. For instance, in the United States alone, nearly 40% of adults actively reject evolution (Reiss, 2011). Needless to say, this creates problematic situations for the science educator. It is important to be respectful of differing perspectives; one does not want to come across at hostile or insensitive. Although, from a purely educational standpoint, it is also vital for the instructor to ensure students understand the concept of evolution, as it has tremendous implications on any number of topics in a typical biology course (Cavallo & McCall, 2008). I believe transformative learning theory can be successfully employed to resolve this conflict in a mutually agreeable manner. However, rather than review preexisting methods for teaching natural selection and evolution through a transformative method (*e.g.,* Heddy & Sinatra, 2013), I will choose instead to propose my own ideas that are based, in part, on what I have personally gathered studying the theory's many influences and criticisms. The result is, I hope, the first step towards a new method of teaching this controversial subject.

The potential of narrative inquiry

In their critique of transformative learning, Taylor and Cranton (2013) recognized an overreliance on antiquated procedures (*e.g.,* retrospective interviews) and called for the adoption of more novel approaches. Of the various types listed, one in particular caught my attention: narrative inquiry. As a research methodology, it represents, "the interdisciplinary study of activities involved in generating and analyzing stories of life experience (*e.g.,* life histories, narrative interviews, journals, diaries, memoirs, autobiographies, biographies)" (Schwandt, 2007); moreover, it seeks to elucidate how meaning is constructed (on a personal level) via a narrative format. What I found fascinating about narrative inquiry is the inherent flexibility of its framework.

Though each project has its own idiosyncrasies that may necessitate changes in experimental design, it nevertheless works in a series of four general steps (Clandinin & Connelly, 2000). The researcher – which in this case will refer to the student – first presents a major question or problem that may be elucidated by a more thorough understanding of the experiences presented in the chosen reading. Background research is then conducted to understand the narrative in its original psychological, social, and cultural context. Experience, as previously noted, cannot be viewed as a static entity and must, therefore, be viewed in terms of such internal and external influences (Nohl, 2009). Thirdly, students take the reading as a whole and attempt to broadly reorganize it in terms of chronology or theme, a process known as "restorying" (Creswell, 2007). With a more personalized understanding of the events or experiences in the reading, they can then share it with other students in a form of critical discourse. Based on this general methodology, it is quite clear how transformative learning could fit in the framework of narrative inquiry: a perspective is challenged; the student personally analyzes the development of the argument through a given narrative; communication with fellow participants allow for the validation or rejection of the new perspective. The question now, of course, becomes how it can be used to teach natural selection and evolution.

The voyage of the Beagle

Before the publication of his seminal work, *On the Origin of Species* (1859), Charles Darwin joined a British expedition to survey the flora and fauna of territories across the globe. After nearly five years overseas (1831-1836) – during which he meticulously documented a vast array of strange and foreign ecosystems – Darwin returned to London and published an account of his travels, *The Voyage of the Beagle: Journal of Researches* (1845). Though incredible as a travelogue alone, it also represented an important step in the development of what eventually would become the theory of natural selection and evolution. What Darwin saw in such far-flung locations as Tahiti and the Galapagos called into question all traditional theories of biological diversity and, as such, he struggled for a rational explanation. Thankfully for students of the life sciences, however, he left the evidence of this struggle in his journal; it is this document that I propose as the basis for transformative learning via narrative inquiry.

Proposed course: The narrative of Charles Darwin

At the beginning of our hypothetical course, students would be surveyed to determine their baseline stance on evolution, general understanding of the subject, as well as whatever questions they may have. The subsequent two or three class periods would be spent discussing the cultural and philosophical context of Darwin's work and how it fundamentally clashed with the values of Victorian England. Once context has been established, the instructor would begin to assign select readings from *The Voyage of the Beagle* (1845), each highlighting early notions of natural selection and evolution. Students would then "restory" the narrative of each segment in a way that appeals to them, be it in chronological order or in groups of themes (Creswell, 2007). During each period, a critical discussion would be held regarding the readings and how students interpreted it; afterwards they would also keep a record of how their understanding of evolution had changed from the previous meeting. At the end of the semester, a final discussion and survey would be held to analyze the overall course or development of both evolutionary theory and the students' perspective of it. If conducted properly, I truly believe such a narrative method could facilitate transformative learning, particularly if used in conjunction with other immersive learning techniques such as Reacting To The Past (RTTP; Carnes, 2014). The course contains all the key characteristics of Mezirow's (2006) theory and, perhaps of greater interest to educators, represents the application of a novel idea to an area that doesn't normally utilize such methodologies.

CONCLUSIONS

I began my investigation of transformative learning with much reluctance; at first glance, the theory did not seem to have the intellectual merit, the conceptual backbone to stand on its own. It appeared nebulous, diffuse, and only applicable to a small number of cases in the social sciences. However, I now conclude having undergone a change of perspective. I now realize that transformative learning, despite its various limitations and criticisms, is a powerful tool for the adult educator. This critical synthesis of philosophical and pedagogical thinking – from Kuhn, to Freire, to Habermas – has birthed a methodology that instills real learning in students of all background and, moreover, can be used in any number of situations. I sincerely hope I can carry these ideas of transformative learning into my own educational career and, perhaps, make contributions of my own in the future.

CHAPTER 5

USING FIRE ANTS AS A MODEL TO EXPLORE SPECIES CONCEPTS AND DELIMITATION[4]

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[4] Chialvo P. To be submitted to *The American Biology Teacher*

Abstract

Speciation represents both a foundational aspect of evolutionary biology, as well as a conceptual bridge between many areas of life sciences. As such, it is critical that students have a thorough understanding of related subjects such as species concepts and delimitation. However, given the sheer amount of historical debate regarding these topics, it can be difficult to cover them in a balanced manner. In this paper, we present an inquiry-based activity that utilizes genetic data collected from fire ants to explore the conceptual and practical difficulties of species concepts and delimitation.

INTRODUCTION

Despite being one of the most fundamental units of biology, the exact definition of a species nevertheless remains unresolved. Attempting to describe the "undiscovered and undiscoverable essence of the term" has proven to be a long-standing challenge for both theorists and researchers alike (Darwin, 1859). Dozens of competing species concepts have been proposed since the time of Darwin, each with particular criteria or defining characteristics (Singh, 2012). Given the controversy and confusion surrounding species concepts, many biology textbooks (and as a result, many biology instructors) largely emphasize one viewpoint, the Biological Species Concept (BSC; de Quieroz, 2005; Mayr, 1942, 1982), which gained massive popularity in the 1940s – 90s due in part to its relatively simple criterion for species status (*e.g.*, actual or potential reproductive isolation). However, no single concept can adequately encompass all known biodiversity. The issue lies primarily in the process of speciation itself; speciation, as a biological phenomenon, is a spectrum of events in which various evolutionary factors can have profound effects on the current genotype and phenotype (Fig. 4.1; de Queiroz, 2007). Thus, species boundaries can change radically depending on not only when the lineage is observed, but also what lines of evidence are utilized for delimitation.

Such issues are particularly evident in historically difficult taxa such as the fire ants (Formicidae: *Solenopsis*), which have undergone numerous taxonomic revisions (Wheeler, 1915; Creighton, 1930, 1950; Trager, 1991) and consequently have earned a degree of notoriety amongst myrmecologists (Pitts, 2005). Rather than rely solely on morphology, more recent studies (Chialvo *et al.*, in review) have employed a large set of variable genetic markers (microsatellites) to test several common criteria for species delimitation, including the presence of genotypic clusters, reproductive isolation or cohesion, and monophyly of populations. In this paper, we present an inquiry-based activity that utilizes real world data collected by Chialvo *et al*. (in review) to explore the conceptual and practical difficulties of both species concepts and delimitation. The activity is designed for undergraduate evolutionary biology courses and, as such, students are expected to have a general understanding of evolution and speciation. Conversely, no prior knowledge about fire ants (or insects) is required.

OBJECTIVES

The activity is firmly grounded in recommendations made by the AAAS in their "Vision and Change" report (AAAS, 2011). In particular, we focus on developing core competencies in the ability to apply the process of science, quantitative reasoning, effective communication, and the relationship between science and society. Furthermore, we present a highly abstract concept (*i.e.*, species) in the context of a scientific study.

DESCRIPTION OF LESSON

Preassessment and Set-up (20 minutes)

Prior to class, assign a reading that broadly highlights the controversies surrounding species concepts and delimitation (*e.g*., de Queiroz, 2007); avoid articles that simply emphasize or otherwise describe at length competing concepts. To ensure students read the article (and thus are informed for the subsequent activity), begin the lesson with a short quiz (2-3 questions) concerning the author's major points or arguments. Once students complete the quiz, review the answers as a class and discuss any remaining questions. It is important that students have a firm grasp on the differences between species conceptualization and delimitation and, moreover, that they understand the importance of those distinctions. Such background provides the essential context for the main activity. Next, introduce the Chialvo *et al.* study as a concrete example of the more abstract issues raised in the class discussion. Ask students whether they or someone they know have encountered fire ants in the past. Given the prevalence of invasive species in the United States (Shoemaker *et al.*, 1996; Ascunce *et al.*, 2011) – as well as the engrained animosity towards them by the general public – it is highly likely that someone has a story. The purpose of this slight detour is to further ground the activity in events and subjects that students relate to and find interesting (Osborne & Collins, 2001; Çimer, 2012). Finally, present the taxonomic history of the native fire ants (Fig. 2.1) and the issues encountered by previous researchers (*e.g.*, lack of characters, extensive variation)

Main activity (30 minutes)

Students break up into groups of 3-5 (or larger, depending on the size of the class) and receive one of the four activity worksheets (Appendix B). Each of the worksheets in turn consists of four components: 1) a species concept; 2) a description of the species concept from the primary literature; 3) a related dataset from Chialvo *et al.*; and 4) a series of questions to consider. The biological species concept (BSC) worksheet, for instance, contains one of the original definitions (Mayr, 1942), a table of genetic differentiation measurements (F_{ST} and G''_{ST}; Weir & Cockerham, 1984; Meirmans & Hedrick, 2011), and questions regarding the BSC and gene flow. Ask students to rewrite the species concept in their own words and use this new definition to examine the associated dataset and, subsequently, determine how many species they believe are represented. Circulate around the room and visit each group to answer any questions that may arise. If the discussion beings to lag in some groups, encourage members to consider the questions provided at the bottom of the worksheet.

Debriefing (25 minutes)

After students finish the main activity, call on a specific group to explain their rephrased species concept, describe what data they analyzed, and list how many species they felt are present. Challenge students to explain the reasoning behind their responses. Was there ambiguity in the data (*i.e.,* no clear cut answers)? How did members come to a consensus? Repeat with one group for each of the four species concepts. Next, use a classroom response system such as Top Hat (Top Hat Monocle, Denver, CO) to determine how the number of species reported differed both among groups of the same concept and between groups of different concepts. Use these results to revisit the controversies surrounding species concepts and delimitation presented in the reading.

Post-lesson questions

As a post-lesson writing assignment, ask students to consider the following questions: how could such disagreement concerning species boundaries affect other areas of biology (*e.g.*, biodiversity, conservation, pest management)? Why is it more difficult to delimit species that have recently radiated than those which have been separated for a relatively long time?

OUTCOMES

We piloted this activity in an undergraduate evolutionary biology course at the University of Alabama. To determine whether the lesson met the desired learning outcomes (specifically, that students would be able to explain and appreciate the issues surrounding species concepts and delimitation), we gave each student a short questionnaire (Appendix B). The results of this survey can be found below (Fig. 4.2). Notably, 13 of the 15 students reported a higher level of comprehension in species concepts and/or species delimitation. Moreover, the vast majority stated they enjoyed the activity and that it fit the course in terms of time and difficulty.

CONCLUSIONS

Speciation represents an important conceptual bridge between evolutionary biology and other disciplines within life sciences (de Queiroz, 1998). However, given the sheer amount of historical disagreement regarding species concepts and delimitation, it may seem more practical to simply ignore the controversy and instead focus on a single definition of species. In our view, such an omission is quite misleading. Using inquiry-based activities such as the one presented in this paper, it is possible to present a broader, more accurate perspective on speciation while simultaneously developing core competencies that are essential for modern biology students.

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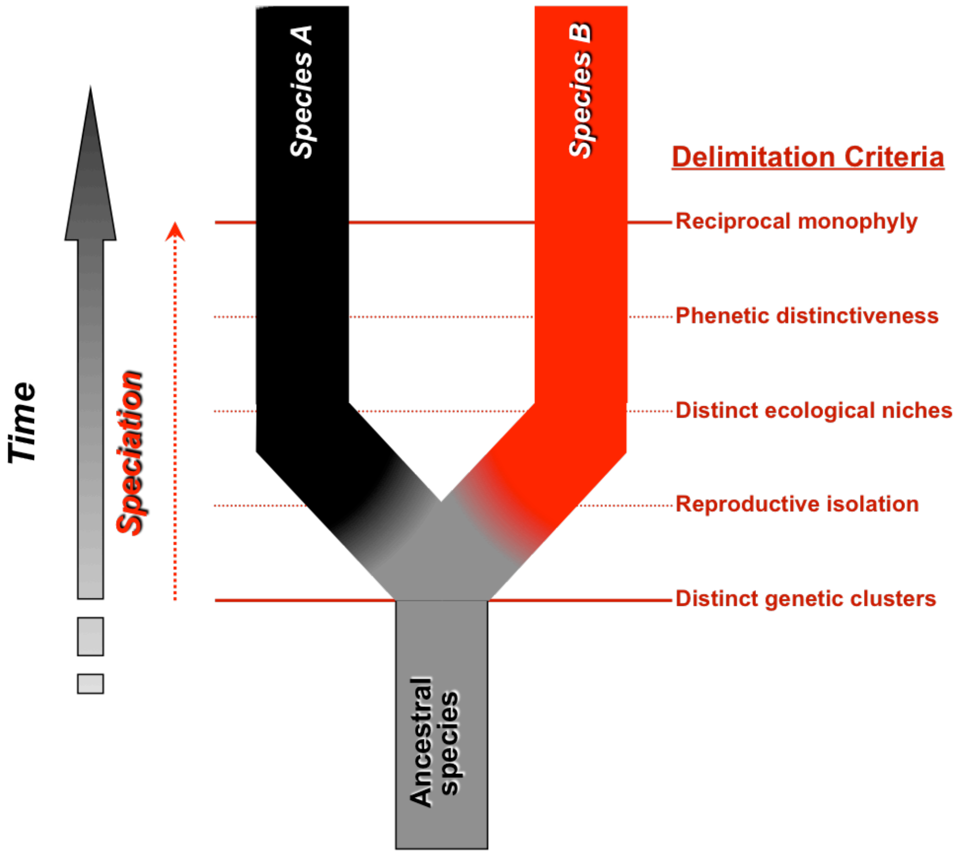

Figure 4.1: Simplified illustration of the speciation process, which demonstrates how different species criteria can emerge at different points in time (modified from de Queiroz, 2007).

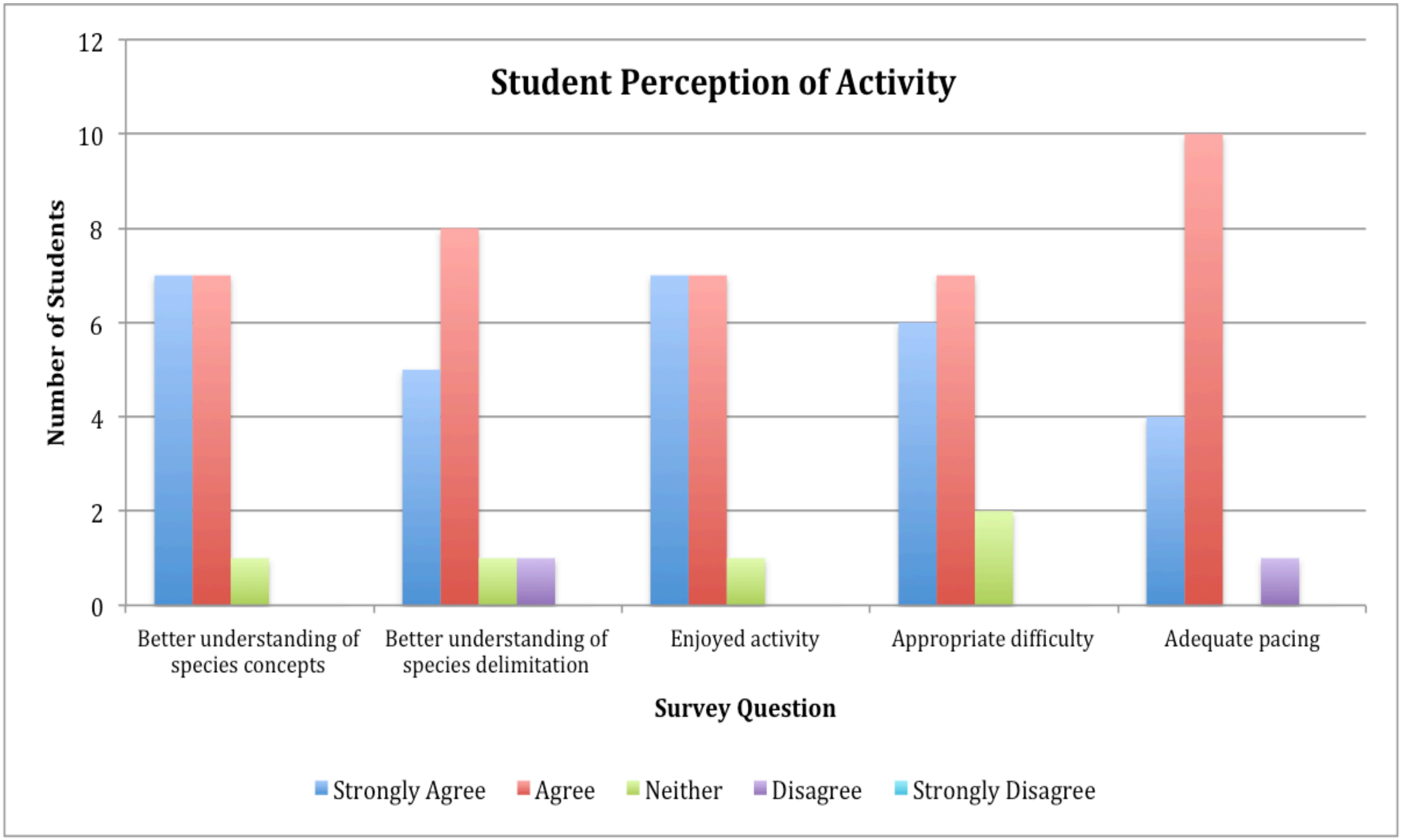

Figure 4.2: Students responses to the activity questionnaire (Appendix B).

CHAPTER 6

CONCLUSIONS

Fire ants (particularly the South American species) have become a model for the study of complex reproductive and social behaviors and their underlying evolutionary, ecological, and physiological mechanisms (Tschinkel 2006; Hölldobler & Wilson, 2009; Tschinkel & Wilson, 2014). Its members exhibit many unique features, including extensive hybridization within introduced ranges and major behavioral polymorphisms associated with known genetic elements (Shoemaker, *et al*. 1996; Ross & Keller, 1998; Gotzek & Ross, 2007). Unfortunately, our knowledge of the native North American fire ants is severely lacking in comparison. Walter Tschinkel (2007), the man who quite literally wrote the book on fire ants, admitted, “nearly nothing is known about the biology of other [non-*invicta*] fire ant species.”

Of the native species, only *S. geminata* has garnered much attention, due to its complex invasion history (Gotzek *et al*. 2015), status as a pest species (Holway *et al*., 2002), and potential for hybridization with other natives (Hung & Vinson, 1977; Axen *et al*., 2014). This distinct gap in our knowledge can be attributed in part to the taxonomic instability of the other native North American fire ants. Nominal taxa have shifted repeatedly between species and subspecies ranks, as well as between valid and synonym status, over a relatively short time period, reflecting continued disagreement among researchers. The group’s morphology can be highly variable (Trager, 1991), and such variation is confounded by extensive size polymorphism in the worker caste of most species (Tschinkel, 2013). Moreover, due to the relatively recent divergence of its members (~0.4 MYA, Gotzek unpublished), it can be especially difficult to distinguish between population genetic structure and independently evolving metapopulation lineages (*i.e.*, species; de Queiroz, 2007).

Despite these recurring issues, my dissertation stands as the latest chapter in the taxonomic history of *Solenopsis*. With the addition of *S. x. maniosa* and *S. arieli*, we now have a more complete understanding of species boundaries and can use this framework as a basis for future studies in native fire ant ecology and evolution. Though some natural and life history information is available (Mallis, 1938; Francke *et al.*, 1986; Hooper & Rust, 1997) there is still a plethora of other questions to consider. For instance, do native species (other than *S. xyloni* and *S. geminata*) hybridize with one another? What is the prevalence of polygyny within these species? At a more specific level, does *S. arieli* truly forage during the day, as hinted at by Snelling & George (1979), and if so what allows it to be (presumably) more heat/desiccation tolerant than other fire ants (Potts *et al*., 1984; Cokendolpher & Francke, 1985; Braulick *et al*., 1986)?

I have no doubt that this group will be revisited in the future and I am truly excited to see what discoveries we uncover in the process. *Solenopsis* is a fascinating taxon and deserves further exploration. With all that said, I must agree with Creighton's (1950) conclusions regarding *Solenopsis*: "The student of North American ants may count himself fortunate that so few species of this difficult genus occur in our latitudes."

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APPENDIX A

SAMPLES USED IN DELIMITATION STUDY

Genus	*Species*	Original Code	Project Code	Collection Date	State/Province	Collection Site	Latitude	Longitude
Solenopsis	*sp.*	DAG185	DAG185	8/8/11	Arizona	Yavapai Co.: Cottonwood, Cottonwood Riverfront Park	34.74875397	-112.013467
Solenopsis	*sp.*	DAG186	DAG186	8/8/11	Arizona	Yavapai Co.: Cottonwood, Cottonwood Riverfront Park	34.74886101	-112.013502
Solenopsis	*xyloni (black)*	DAG187	DAG187	8/8/11	Arizona	Yavapai Co.: Cottonwood, Cottonwood Riverfront Park	34.74753801	-112.013209
Solenopsis	*amblychila*	HA337	HA337		Arizona	Douglas	31.345	-109.5414
Solenopsis	*aurea*	JPAZ6	SD11	08/06/99	Arizona	Cochise Co.: Southwestern Research Station	31.884919	-109.206019
Solenopsis	*amblychila*	RAJ #254	SD37	7/15/93	Arizona	Cochise: Chiricahua Mtns, 0.5 mi E Portal	31.9166667	-109.133333
Solenopsis	*amblychila*	RAJ #1160	SD38	7/22/97	Arizona	Cochise: Chiricahua Mtns, 1.0mi NW Portal	31.9166667	-109.166667
Solenopsis	*amblychila*	RAJ #69	SD39	1/17/93	Arizona	Maricopa: Chandler, nr Price & Elliot Rds, Urban backyard, Small tumulus nest	33.35	-111.899999
Solenopsis	*amblychila*	RAJ #467	SD40	6/9/94	Arizona	Pima: Oro Valley at Rancho Vistoso, Arizona upland association, In foothills palo verde	32.4666667	-110.983333
Solenopsis	*amblychila*	RAJ #2074	SD41	8/22/00	Arizona	Santa Cruz: 1.4 mi W Ruby on FS Rd 39	31.4666667	-111.25
Solenopsis	*amblychila*	RAJ #608	SD42	12/3/94	Arizona	Santa Cruz: Pajarito Mtns, Sycamore Canyon, rolling oak woodland, nest under rock	31.4333333	-111.2
Solenopsis	*amblychila*	RAJ #3869	SD43	4/13/06	Arizona	Santa Cruz: Yanks Canyon, Nest under stone, Rolling oak woodland	31.4166667	-111.166666
Solenopsis	*amblychila*	RAJ #3880	SD44	4/16/06	Arizona	Santa Cruz: Sycamore Canyon, Nest under stone, Rolling oak woodland	31.4333333	-111.183333
Solenopsis	*amblychila*	RAJ #1927	SD51	4/6/00	Arizona	Yavapai: 3.0 mi NW Peeples Valley, chapparal woodland, coarse granitic soil, nest under rock	34.3	-112.766666
Solenopsis	*amblychila*	RAJ #1497	SD52	8/18/98	Arizona	Yavapai: USFS Rd 21 at 5.0 mi W Cty Hwy 5, pinyon-juniper-scrub oak, nest under rock	34.8166667	-112.716666

Solenopsis	*aurea*	JPAZ1	SD6		Arizona	Cochise Co.: Southwestern Research Station	31.884919	-109.206019
Solenopsis	*sp.*	RAJ #1000	SD67	6/2/97	Arizona	Maricopa: Tempe, nr Baseline & McClintock, Urban backyard, Foundress on ground	33.3833333	-111.9
Solenopsis	*aurea*	JPAZ2	SD7		Arizona	Cochise Co.: Southwestern Research Station	31.884919	-109.206019
Solenopsis	*amblychila*	PW13574	PW13574		Baja (MEX)	28km E Ensenada	31.8833333	-116.3
Solenopsis	*amblychila*	AVS1316	AVS1316		California		33.6	-117.55
Solenopsis	*amblychila*	PW12985	PW12985		California	San Diego Co.: Chula Vista (E end)	32.6666667	-116.983333
Solenopsis	*amblychila*	PW14322	PW14322		California	Orange Co.: Starr Ranch	33.6	-117.55
Solenopsis	*amblychila*	TVD #4515	SD45	3/28/10	MEX	Sonora: 27.7 km (by air) SSE of Sásabe, desert grassland, forager	31.2366667	-111.481666
Solenopsis	*amblychila*	TVD #4851	SD46	5/11/11	MEX	Sonora: Mpio. Guaymas, Rancho Ojo de Agua, Stream canyon, Goodding willow riparian forest	28.4666667	-110.316666
Solenopsis	*amblychila*	TVD #4438	SD48	3/28/10	MEX	Sonora: Rancho Agua Caliente, cottonwood riparian gallery forest	30.95	-110.85
Solenopsis	*amblychila*	TVD #4451	SD49	3/19/10	MEX	Sonora: Sierra El Tigre, Cañada el Tejano, Rocky canyon, canyon riparian forest	30.4	-109.283333
Solenopsis	*sp.*	TVD #4962	SD68	2/2/12	MEX	Sonora: Mpio. Hermosillo, 39.7 km (by air) E Hermosillo, Rocky limestone hill, foothills thornscrub	29.0666667	-110.55
Solenopsis	*sp.*	DAG234	DAG234	8/15/11	New Mexico	Socorro Co.; Rt.1 just N of Luis Lopez, after Rt.1 crosses I-25	33.99160098	-106.894153
Solenopsis	*sp.*	DAG219	DAG219	8/13/11	New Mexico	Chaves Co.; Rt.380 & Rt.256 jct.; LE Ranch Devils Tank Pens	33.41950701	-104.003906
Solenopsis	*sp.*	DAG220	DAG220	8/13/11	New Mexico	Chaves Co.; Rt.380 & Rt.256 jct.; LE Ranch Devils Tank Pens	33.41950198	-104.003984
Solenopsis	*aurea*	PW3786	PW3786		Texas	The Basin, Chisos Mtns.	29.277225	-103.302365
Solenopsis	*aurea*	AVS2036	AVS2036		Arizona		31.8833333	-109.233333
Solenopsis	*aurea*	AW1576	AW1576		Arizona	Cochise Co.	31.884919	-109.206019
Solenopsis	*aurea*	HA350	HA350		Arizona	Cochise Co.: SW. Research Station	31.884919	-109.206019
Solenopsis	*aurea*	PW14482	PW14482		Arizona	Gila Co.: First Water Canyon, Sierra Ancha	33.7333333	-110.966666

Solenopsis	*aurea*	PW14748	PW14748		Arizona	Cochise Co.: Miller Canyon, Huachuca Mtns.	31.4166667	-110.283333
Solenopsis	*aurea*	PW14936	PW14936		Arizona	Cochise Co.: Chiricahua Mtns	31.8833333	-109.233333
Solenopsis	*aurea*	PW15495	PW15495		Arizona	Cochise Co.: 3km 292d WNW Portal	31.9316667	-109.178333
Solenopsis	*aurea*	PW5632	PW5632		Arizona	Cochise Co.: 47km E Douglas	31.342952	-109.052998
Solenopsis	*aurea*	FMNH 0000-118-557	SD165	3/27/73	Arizona	Wild Horse Ranch	33.594271	-112.185156
Solenopsis	*aurea*	RAJ #3913	SD54	10/14/06	Arizona	Cochise: Chiricahua Mtns,0.5 km SW SWRS, Rolling oak-juniper woodlands, nest under stone	31.8833333	-109.216666
Solenopsis	*aurea*	RAJ #1895	SD61	4/1/00	Arizona	Pinal: Queen Creek Canyon	33.3333333	-111.05
Solenopsis	*aurea*	RAJ #3879	SD65	4/16/06	Arizona	Santa Cruz: Yanks Canyon, Nest under stone, Rolling oak woodland	31.4333333	-111.183333
Solenopsis	*aurea*	FMNH 0000-118-976	SD148	4/6/73	Baja (MEX)	Isla San Esteban	28.6975	-112.5775
Solenopsis	*aurea*	aurea-7	aurea-7		MEX	La Colorado, Las Mercedes, Sonara	28.65344361	-110.016178
Solenopsis	*amblychila*	RAJ #4596	SD47	11/10/10	MEX	Sonora: Presa San Miguel, 4.7 km SSE of Benjamín Hill, Sonoran desertscrub, heavily grazed area	30.1166667	-111.1
Solenopsis	*amblychila*	RAJ #3395	SD50	4/28/04	MEX	Sonora: Sierra Mazatán, Encinal – thornscrub habitat, 2"" tumulus nest	29.1	-110.15
Solenopsis	*aurea*	RAJ #2791	SD59		MEX	Mexico: Hwy 16 at 44 mi E Yecora, oak woodland with scattered juniper, nest under rock	28.4333333	-108.5
Solenopsis	*aurea*	RAJ #2797	SD60		MEX	Mexico: Hwy 16 at 44 mi E Yecora, oak woodland with scattered juniper, nest under rock	28.4333333	-108.5
Solenopsis	*sp.*	TVD #4967	SD69	2/15/12	MEX	Sonora: Mpio. Ímuris, Río Magdalena between San Ignacio & Terrenate, Riparian forest	30.7166667	-110.916666
Solenopsis	*sp.*	DAG205	DAG205	8/9/11	New Mexico	Hidalgo Co,: Gin Road, Route 80	31.834186	-109.043312
Solenopsis	*sp.*	RAJ #4921	SD71	4/26/12	Arizona	Yuma: I-8 at Avenue 10 E South side, Urban roadside habitat, Nest under and adjacent to stone	32.6666667	-114.466666
Solenopsis	*aurea*	IN-2	IN-2		California	Riverside Co.: Indio	33.720556	-116.21556

Solenopsis	*aurea*	IN-B	IN-B		California	Riverside Co.: Indio	33.720556	-116.21556
Solenopsis	*aurea*	M-1	M-1		California	Riverside Co.	33.57347	-116.075426
Solenopsis	*aurea*	MEC-1	MEC-1		California	Riverside Co.: Mecca	33.571667	-116.077222
Solenopsis	*xyloni*	PW11584	PW11584		California	San Diego Co.: Borrego Springs	33.2472	-116.3719
Solenopsis	*aurea*	USDA-3	USDA-3		California	Riverside Co.: Coachella	33.6794	-116.1744
Solenopsis	*aurea*	USDA-4	USDA-4		California	Riverside Co.: Salton Sea	33.3	-115.8
Solenopsis	*sp.*	AW2309	AW2309		MEX	Puebla: Lands of S. Miguel Tzinacapan, Mpio. Cuetzalan	20.030556	-97.540556
Solenopsis	*aurea*	RAJ #2376	SD66	4/11/01	Arizona	Yavapai: Bumble Bee Rd at 5.0 mi NW I-17, mesic stream bottom, tumulus nest	34.1833333	-112.166666
Solenopsis	*xyloni*	GMNH_X1	GMNH_X1		Georgia	Athens-Clarke Co.: Whitehall Forest, Lot 82-74	33.945343	-83.377993
Solenopsis	*xyloni*	GMNH_X2	GMNH_X2		Georgia	Athens-Clarke Co.: Whitehall Forest, Lot 82-74	33.945343	-83.377993
Solenopsis	*xyloni*	FMNH 0000-062-855	SD139	12/8/73	MEX	San Luis Potosi	22.838278	-101.133395
Solenopsis	*xyloni*	DAG209	DAG209	8/12/11	New Mexico	Otero Co.: Alamogordo, in grass by gas station off Rt.70	32.93662003	-105.963559
Solenopsis	*xyloni*	DAG210	DAG210	8/12/11	New Mexico	Otero Co.: Tularosa, at historic marker off Rt.70/54	33.05902499	-106.014219
Solenopsis	*xyloni*	DAG211	DAG211	8/12/11	New Mexico	Chaves Co., Roswell, W 3rd St.	33.39539403	-104.547497
Solenopsis	*xyloni*	DAG212	DAG212	8/12/11	New Mexico	Chaves Co., Roswell, W 3rd St.	33.39538498	-104.548391
Solenopsis	*sp.*	DAG213	DAG213	8/12/11	New Mexico	Chaves Co.; Roswell, Kentucky Ave, Administration & Educational Services Complex	33.39583601	-104.527665
Solenopsis	*xyloni*	DAG214	DAG214	8/12/11	New Mexico	Chaves Co., Bottomless Lakes State Park, Lea Lake campground	33.31741098	-104.329454
Solenopsis	*xyloni*	DAG215	DAG215	8/12/11	New Mexico	Chaves Co., Bottomless Lakes State Park, Lea Lake campground	33.31737796	-104.329393
Solenopsis	*xyloni*	DAG218	DAG218	8/13/11	New Mexico	Chaves Co., Rt.380 & Rt.256 jct., LE Ranch Devils Tank Pens	33.41952302	-104.003801
Solenopsis	*xyloni*	DAG222	DAG222	8/14/11	New Mexico	Guadalupe Co., Newkirk, exit 300 off I-40	35.06787599	-104.266873
Solenopsis	*xyloni*	DAG223	DAG223	8/14/11	New Mexico	Guadalupe Co., Newkirk, exit 300 off I-40	35.06766502	-104.266828

Solenopsis	*xyloni*	DAG224	DAG224	8/14/11	New Mexico	Lincoln Co., Carrizozo S end at historical marker on Rt.54	33.63590302	-105.883641
Solenopsis	*xyloni*	DAG225	DAG225	8/14/11	New Mexico	Lincoln Co., Carrizozo S end at historical marker on Rt.54	33.63573597	-105.883973
Solenopsis	*xyloni*	DAG226	DAG226	8/14/11	New Mexico	Lincoln Co., ~3.3 miles S of Carrizozo on Rt.54	33.60654096	-105.928258
Solenopsis	*xyloni*	DAG227	DAG227	8/14/11	New Mexico	Lincoln Co., ~7 miles S of Carrizozo on Rt.54	33.57180501	-105.980162
Solenopsis	*xyloni*	DAG228	DAG228	8/14/11	New Mexico	Lincoln Co., County Rt.A004 (Ibar X Ranch) across railroad tracks off Rt.54	33.54174499	-106.012526
Solenopsis	*xyloni*	DAG229	DAG229	8/14/11	New Mexico	Lincoln Co., Carrizozo, Rt.380 & Water Canyon Rd. jct	33.64175904	-105.860321
Solenopsis	*xyloni*	DAG231	DAG231	8/15/11	New Mexico	Lincoln Co., Valley of Fire Recreation Area	33.684739	-105.919786
Solenopsis	*xyloni*	DAG232	DAG232	8/15/11	New Mexico	Lincoln Co., Valley of Fire Recreation Area	33.68454102	-105.919815
Solenopsis	*xyloni*	DAG233	DAG233	8/15/11	New Mexico	Socorro Co., Rt.1 btwn San Antonio & Luis Lopez	33.98327499	-106.887445
Solenopsis	*xyloni*	DAG235	DAG235	8/15/11	New Mexico	Socorro Co., Rt.1 across from Socorro landfill	33.99908602	-106.895754
Solenopsis	*xyloni*	DAG236	DAG236	8/15/11	New Mexico	Socorro Co., Socorro, grassy area in front of WalMart parking lot	34.06459998	-106.891299
Solenopsis	*xyloni*	DAG237	DAG237	8/15/11	New Mexico	Socorro Co., Socorro, grassy area in front of WalMart parking lot	34.06473602	-106.891317
Solenopsis	*xyloni*	DAG244	DAG244	8/16/11	New Mexico	Socorro Co., exit 175 (Bernardo) off I-25 on Rt.60	34.41925004	-106.83588
Solenopsis	*xyloni*	DAG245	DAG245	8/16/11	New Mexico	Socorro Co., exit 175 (Bernardo)	34.41926203	-106.835728
Solenopsis	*xyloni*	OX	OX		Oklahoma	Benyan Co.: Caddo	34.126667	-96.265556
Solenopsis	*xyloni*	Orange 32	SD19	4/21/13	Oklahoma	Altus	34.6709667	-99.3344833
Solenopsis	*xyloni*	Orange 34	SD21	4/21/13	Oklahoma	Altus	34.6709667	-99.3344833
Solenopsis	*xyloni*	Orange 18	SD14	4/16/13	Texas	Monahans	31.59535	-102.890983
Solenopsis	*xyloni (bicolor)*	DAG080	DAG080	7/24/11	California	Fresno Co.: Coalinga, West Hills Community College	36.150262	-120.355038
Solenopsis	*xyloni (bicolor)*	DAG081	DAG081	7/24/11	California	Fresno Co.: Coalinga, West Hills Community College	36.150262	-120.355038

Solenopsis	*xyloni (bicolor)*	DAG082	DAG082	7/24/11	California	Fresno Co.: Coalinga, West Hills Community College	36.150262	-120.355038
Solenopsis	*xyloni (bicolor)*	DAG084	DAG084	7/24/11	California	Fresno Co.: Coalinga, West Hills Community College	36.150262	-120.355038
Solenopsis	*xyloni (bicolor)*	DAG092	DAG092	7/25/11	California	Fresno Co.: Coalinga, Keck Park	36.139309	-120.368948
Solenopsis	*xyloni*	DAG093	DAG093	7/25/11	California	Fresno Co.: Coalinga, Keck Park	36.139309	-120.368948
Solenopsis	*xyloni*	DAG094	DAG094	7/25/11	California	Fresno Co.: Coalinga, Keck Park	36.139309	-120.368948
Solenopsis	*xyloni*	DAG095	DAG095	7/25/11	California	Fresno Co.: Coalinga, Keck Park	36.139309	-120.368948
Solenopsis	*xyloni*	DAG096	DAG096	7/25/11	California	Fresno Co.: Coalinga, Keck Park	36.139309	-120.368948
Solenopsis	*xyloni*	DAG097	DAG097	7/25/11	California	Fresno Co.: Coalinga, Keck Park	36.139309	-120.368948
Solenopsis	*xyloni*	PW12950	PW12950		California	Santa Barbara Co.: S end Sedgewick Ranch	34.693062	-120.040663
Solenopsis	*xyloni*	PW12956	PW12956		California	Santa Barbara Co.: S end Sedgewick Ranch	34.693062	-120.040663
Solenopsis	*xyloni*	PW13337	PW13337		California	SL Obispo Co.: 19km SSE Calif. Valley, Carrizo Plain Natural Area	35.373485	-119.018755
Solenopsis	*xyloni*	PW13989	PW13989		California	Contra Costa Co.: Black Diamond Mines Regional Preserve	37.971892	-121.861387
Solenopsis	*xyloni*	PW14802	PW14802		California	Kern Co.: Cudahy Camp, Red Rock Canyon State Park	35.38374	-117.974681
Solenopsis	*xyloni*	PW5319	PW5319		California	Yolo Co.: Davis	38.55453	-121.737176
Solenopsis	*xyloni*	PW7043	PW7043		California	Yolo Co.: 10km W Winters	38.535784	-122.093728
Solenopsis	*xyloni*	PW7108	PW7108		California	Napa Co.: 6km SSW Knoxville	38.800106	-122.359573
Solenopsis	*xyloni*	PW7251	PW7251		California	Davis, Yolo Co.	35.38374	-117.974681
Solenopsis	*xyloni*	PW9433	PW9433		California	Sutter Co.: 7km NNW Sutter	39.239876	-121.795801
Solenopsis	xyloni	DAG195	DAG195	8/8/11	Arizona	Gila Co.; Globe, Willow St. and Rt.60	33.40211104	-110.792536
Solenopsis	*xyloni*	DAG199	DAG199	8/9/11	Arizona	Graham Co.; Safford, Rt.191, in wash behind Church of Latter Day Saints	32.79314197	-109.70978
Solenopsis	*xyloni*	PW15020	PW15020		Arizona	Yavapai Co.: Beaver Creek Campground	33.3718	-108.120224
Solenopsis	*aurea*	JPAZ5	SD10	08/05/99	Arizona	Cochise Co.: Southwestern Research Station	31.884919	-109.206019
Solenopsis	*xyloni*	RAJ #36-1	SD78	11/26/93	Arizona	Pima: 2 mi S Corona de Sol, AZ	31.9333333	-110.766666

Solenopsis	*xyloni*	RAJ #37-1	SD79	8/17/91	Arizona	Pima: 2 mi S Corona de Sol, AZ upland association	31.9333333	-110.766666
Solenopsis	*xyloni*	JPAZ3	SD8		Arizona	Cochise Co.: Southwestern Research Station	31.884919	-109.206019
Solenopsis	*xyloni*	JPAZ4	SD9		Arizona	Cochise Co.: Southwestern Research Station	31.884919	-109.206019
Solenopsis	*xyloni*	PW13245	PW13245		Baja (MEX)	Isla Smith [=Isla Coronado], S end	29.041893	113.498952
Solenopsis	*xyloni*	FMNH 0000-118-618	SD136	4/2/73	Baja (MEX)	Isla Salsipuedes	28.727103	-112.956011
Solenopsis	*xyloni*	RAJ #2249	SD25	3/21/01	Baja (MEX)	San Carlos Canyon, 9.0 mi NE Hwy 1, mesic canyon bottom	31.8	-116.501666
Solenopsis	*xyloni*	RAJ #BC 95-25	SD26	1/25/95	Baja (MEX)	6.9 mi N El Huerfanito	30.2166667	-114.675
Solenopsis	*xyloni*	RAJ #2307	SD27	3/25/01	Baja (MEX)	12.9 mi W Meling Ranch, coastal scrub	31	-115.915
Solenopsis	*xyloni*	RAJ #BC 121	SD28	2/10/93	Baja (MEX)	38.5 mi NW Catavina (1 mi N El Progreso)	29.9833333	-115.216666
Solenopsis	*xyloni*	RAJ #BC 148	SD29	2/19/94	Baja (MEX)	6.5 mi SE Las Arrastras, rocky hillside, nest under rock	29.4666667	-114.299999
Solenopsis	*xyloni*	RAJ #BC 1311	SD30	3/11/98	Baja (MEX)	Sierra San Borja, 9.9 mi N San Borja, nest under rock	28.8583333	-113.791666
Solenopsis	*xyloni*	RAJ #BC 1180	SD31	3/8/92	Baja (MEX)	10.5 mi E El Arco	29.1166667	-113.333332
Solenopsis	*xyloni*	RAJ #BCS 118	SD32	2/16/93	Baja (MEX)	Hwy 1 at 8.3mi W San Ignacio, nest under stone	27.2916667	-113.074998
Solenopsis	*xyloni*	RAJ #BCS 95-19	SD33	1/26/95	Baja (MEX)	0.4 mi S Sierra San Francisco, nest under rock	27.5916667	-113.016666
Solenopsis	*xyloni*	RAJ #BC 109	SD35	2/9/93	Baja (MEX)	NE end Bahia Falsa	30.45	-115.991666
Solenopsis	*xyloni*	RAJ #BCS 159	SD36	2/18/93	Baja (MEX)	Hwy 1 at 10mi N Mulege	26.9916667	-112.066666
Solenopsis	*xyloni*	PW16074	PW16074		California	Los Angeles Co.: Phantom Trail, Malibu Creek State Park	34.105104	-118.73153
Solenopsis	*xyloni*	PW9379	PW9379		California	Los Angeles Co.: Azusa	34.133703	-117.90852
Solenopsis	*xyloni*	RAJ #9-1	SD84	6/28/94	California	San Diego: I-8 at 0.5mi E Cameron Station	32.7166667	-116.466666
Solenopsis	*xyloni*	FMNH 0000-	SD152	10/18/74	MEX	Los Mochis	25.7835	-108.9937

		118-540						
Solenopsis	sp.	TVD #4974	SD70	2/15/12	MEX	Sonora: Mpio. Moctezuma, 3.2 km SSE (by air) Moctezuma, Rocky slope, foothills thornscrub	29.7833333	-109.666666
Solenopsis	*xyloni*	RAJ #3383	SD89	4/27/04	MEX	Sonora: La Pila, Mesic canyon bottom, Mesquite with sandy soil, Deciduous thorn scrub, Nest under stone	29.3	-109.716666
Solenopsis	*xyloni*	RAJ #92-4	SD90	10/9/92	MEX	Sonora: Punta Cirio	29.8333333	-112.65
Solenopsis	*xyloni*	RAJ #3360	SD91		MEX	Sonora: Rancho Agua Caliente, Cienega above ranch, Nest under stone	30.65	-109.466666
Solenopsis	*xyloni*	ANM1a	ANM1a		New Mexico	Animas	31.952951	-108.806107
Solenopsis	*xyloni*	ANM3b	ANM3b		New Mexico	Animas	31.952951	-108.806107
Solenopsis	*xyloni*	DAG208	DAG208	8/11/11	New Mexico	Otero Co.; White Sands National Monument, Rt.70; turn-off to White Sand N.P.	32.77728299	-106.173637
Solenopsis	*xyloni*	PW13423	PW13423		New Mexico	Catron Co.: 1km NNE Visitor Center, Gila Cliff Dwellings Natl. Mon.	33.229013	-108.270899
Solenopsis	*xyloni*	PW13448	PW13448		New Mexico	Sierra Co.: Hillsboro	32.921807	-107.567017
Solenopsis	*xyloni*	Orange 24	SD16		Texas	West Point	33.4058333	-102.2541667
Solenopsis	*xyloni*	Orange 29	SD18		Texas	Crosbyton	33.9547222	-101.471111
Solenopsis	*xyloni*	Orange 3	SD2		Texas	Alpine/US-67	30.3833333	-103.866111
Solenopsis	*xyloni*	Orange 4	SD3		Texas	Alpine/US-67	30.3833333	-103.866111
Solenopsis	*xyloni*	Orange 12	SD4		Texas	Pecos	31.5419444	-103.656944
Solenopsis	*xyloni*	Orange 14	SD5		Texas	Pecos	31.5419444	-103.656944
Solenopsis	*xyloni*	DAG177	DAG177	8/5/11	Arizona	Mohave Co.; exit 27 (Black Rock Rd.) off I-15	36.98458197	-113.645606
Solenopsis	*xyloni*	DAG178	DAG178	8/5/11	Arizona	Mohave Co.; exit 8 (Littlefield) off I-16	36.89097103	-113.928472
Solenopsis	*xyloni*	DAG179	DAG179	8/6/11	Arizona	Mohave Co.; Rt.68, mile post 4, La Puerta Rd. turn-off	35.18608396	-114.502201
Solenopsis	*xyloni*	DAG180	DAG180	8/6/11	Arizona	Mohave Co.; Rt.68, mile post 4, La Puerta Rd. turn-off	35.186019	-114.502088

Solenopsis	*xyloni*	DAG181	DAG181	8/6/11	Arizona	Mohave Co.; Kingman, exit 51 (Stockton Hill Rd.) off I-40	35.21638303	-114.034453
Solenopsis	*xyloni*	DAG184	DAG184	8/7/11	Arizona	Yavapai Co.; Rt.89	34.20047399	-112.807463
Solenopsis	*xyloni*	DAG197	DAG197	8/9/11	Arizona	Graham Co.; Rt.70 ~5 m. W of	33.18144604	-110.154007
Solenopsis	*xyloni*	DAG198	DAG198	8/9/11	Arizona	Graham Co.; Pima, along Rt.70	32.89905301	-109.839202
Solenopsis	*xyloni*	DAG202	DAG202	8/9/11	Arizona	Cochise Co.; Bowie	32.33025597	-109.506862
Solenopsis	*xyloni*	DAG203	DAG203	8/9/11	Arizona	Cochise Co.; Bowie	32.33026796	-109.507149
Solenopsis	*xyloni*	DAG204	DAG204	8/9/11	Arizona	Cochise Co.; Southwestern Research Station, next to gift shop	31.884919	-109.206019
Solenopsis	*xyloni*	PW14973	PW14973		Arizona	Coconino Co.: Grand Canyon Park, Colorado River at 91 Mile Creek	36.1064	-112.1471
Solenopsis	*xyloni*	RAJ #370	SD77	11/8/93	Arizona	Maricopa: 4 mi SE Vulture Mine, creosote flats	33.8	-112.785
Solenopsis	*xyloni*	RAJ #41	SD81	7/23/90	Arizona	Pinal: 0.5 km N San Tan Mtn, creosote flats	33.2	-111.716667
Solenopsis	*xyloni*	RAJ #40	SD83	2/18/91	Arizona	Pinal: McCartney Rd at 2 mi E Jct I-10, Atriplex association	32.9333333	-111.666667
Solenopsis	*aurea*	CN_9	CN_9		California	Imperial Co.: El Centro	32.8	-115.5667
Solenopsis	*xyloni*	DAG104	DAG104	7/27/11	California	Riverside Co.; Joshua Tree National Park; Pine City Trail	34.023711	-116.077701
Solenopsis	*xyloni*	DAG105	DAG105	7/28/11	California	Riverside Co.; Joshua Tree National Park; Jumbo Rocks campground	33.991001	-116.062181
Solenopsis	*xyloni*	DAG108	DAG108	7/28/11	California	Riverside Co.; Joshua Tree National Park; Jumbo Rocks campground	33.991001	-116.062181
Solenopsis	*xyloni*	DAG109	DAG109	7/29/11	California	Riverside Co.; Joshua Tree National Park; road to Belle campground	34.00238	-116.022009
Solenopsis	*xyloni*	DAG111	DAG111	7/29/11	California	Riverside Co.; Joshua Tree National Park; road to Split Rock	34.007849	-116.053829
Solenopsis	*xyloni*	DAG113	DAG113	7/29/11	California	Riverside Co.; Joshua Tree National Park; Skull Rock trail	33.996321	-116.060366
Solenopsis	*xyloni*	DAG114	DAG114	7/29/11	California	San Bernardino Co.; Mojave National Reserve; Hole-in-the-Wall campground	35.050542	-115.393506
Solenopsis	*xyloni*	DAG115	DAG115	7/29/11	California	San Bernardino Co.; Mojave National Reserve; Hole-in-the-Wall campground	35.050542	-115.393506

Solenopsis	*xyloni*	DAG116	DAG116	7/29/11	California	San Bernardino Co.; Mojave National Reserve; Hole-in-the-Wall campground	35.050542	-115.393506
Solenopsis	*xyloni*	DAG117	DAG117	7/29/11	California	San Bernardino Co.; Mojave National Reserve; Hole-in-the-Wall campground	35.050542	-115.393506
Solenopsis	*xyloni*	DAG118	DAG118	7/29/11	California	San Bernardino Co.; Mojave National Reserve; Hole-in-the-Wall campground	35.05050902	-115.393794
Solenopsis	*xyloni*	DAG119	DAG119	7/30/11	California	San Bernardino Co.; Mojave National Reserve; Essex Road; ~10 miles S Essex Rd & Black Canyon Rd jct.	34.808848	-115.333127
Solenopsis	*xyloni*	DAG120	DAG120	7/30/11	California	San Bernardino Co.; Mojave National Reserve; Essex Road; ~10 miles S Essex Rd & Black Canyon Rd jct.	34.808848	-115.333127
Solenopsis	*xyloni*	DAG122	DAG122	7/30/11	California	San Bernardino Co.; Mojave Nat. Reserve; Essex Road; ~10 miles S Essex Rd & Black Canyon Rd	34.808848	-115.333127
Solenopsis	*xyloni*	DAG123	DAG123	7/30/11	California	San Bernardino Co.; Mojave Nat. Reserve; Essex Road; ~10 miles S Essex Rd & Black Canyon Rd jct.	34.808848	-115.333127
Solenopsis	*xyloni*	DAG124	DAG124	7/30/11	California	San Bernardino Co.; Mojave Nat. Reserve; Essex Road; ~10 miles S Essex Rd & Black Canyon Rd jct.	34.808848	-115.333127
Solenopsis	*xyloni*	DAG125	DAG125	7/30/11	California	San Bernardino Co.; Mojave Nat. Reserve; Essex Road; ~5 miles S Essex Rd & Black Canyon Rd jct.	34.844697	-115.381721
Solenopsis	*xyloni*	DAG126	DAG126	7/30/11	California	San Bernardino Co.; Mojave Nat. Reserve; Essex Rd & Black Canyon Rd jct.	34.908428	-115.423343
Solenopsis	*xyloni*	DAG127	DAG127	7/30/11	California	San Bernardino Co.; Mojave Nat. Reserve; Essex Rd & Black Canyon Rd jct.	34.908447	-115.423817
Solenopsis	*xyloni*	DAG128	DAG128	7/30/11	California	San Bernardino Co.; Mojave Nat. Reserve; Essex Rd & Black Canyon	34.908445	-115.4397

Solenopsis	*xyloni*	DAG129	DAG129	7/30/11	California	San Bernardino Co.; Mojave Nat. Reserve; Essex Rd & Black Canyon Rd jct.	34.908133	-115.423808
Solenopsis	*xyloni*	DAG131	DAG131	7/30/11	California	San Bernardino Co.; Mojave Nat. Reserve; Essex Road; ~5 miles N Essex Rd & Black Canyon Rd jct.	34.976086	-115.394452
Solenopsis	*xyloni*	DAG133	DAG133	7/30/11	California	San Bernardino Co.; Mojave Nat. Reserve; intersection Kelbaker Rd & dirt road to Kelso Dunes	34.90058703	-115.64922
Solenopsis	*xyloni*	DAG134	DAG134	7/30/11	California	San Bernardino Co.; Mojave Nat. Reserve; intersection Kelbaker Rd & dirt road to Kelso Dunes	34.90053498	-115.649212
Solenopsis	*xyloni*	DAG135	DAG135	7/30/11	California	San Bernardino Co.; Mojave National Reserve; 5 miles N jct. Nat. Rd & dirt road to Kelso Dunes	34.97096996	-115.64502
Solenopsis	*xyloni*	DAG136	DAG136	7/30/11	California	San Bernardino Co.; Mojave Nat. Reserve; 5 miles N jct. Kelbaker Rd & dirt road to Kelso Dunes	34.97081699	-115.644988
Solenopsis	*xyloni*	DAG137	DAG137	7/30/11	California	San Bernardino Co.; Mojave Nat. Reserve; 5 miles N Kelso Depot on Kelso Cima Rd	35.08483099	-115.569122
Solenopsis	*xyloni*	DAG138	DAG138	7/30/11	California	San Bernardino Co.; Mojave Nat. Reserve; 5 miles N Kelso Depot on Kelso Cima Rd	35.08490902	-115.569158
Solenopsis	*xyloni*	DAG139	DAG139	7/30/11	California	San Bernardino Co.; Mojave Nat. Reserve; 5 miles N Kelso Depot on Kelso Cima Rd	35.08503098	-115.569248
Solenopsis	*xyloni*	DAG140	DAG140	7/30/11	California	San Bernardino Co.; Mojave Nat. Reserve; Mojave Rd & Kelso Cima Rd jct.	35.17581303	-115.507498
Solenopsis	*xyloni*	DAG141	DAG141	7/31/11	California	San Bernardino Co.; I-15 & Excelsion Mine Rd. intersection	35.44752901	-115.677237
Solenopsis	*xyloni*	DAG142	DAG142	7/31/11	California	San Bernardino Co.; I-15 & Excelsion Mine Rd. intersection	35.44757604	-115.677313
Solenopsis	*xyloni*	DAG143	DAG143	8/1/11	California	San Bernardino Co.; Rt. 127 ~6 miles N of Baker	35.36077104	-116.103196

Solenopsis	*xyloni*	DAG144	DAG144	8/1/11	California	San Bernardino Co.; Rt. 127 ~26 miles N of Baker	35.61352104	-116.261959
Solenopsis	*xyloni*	DAG145	DAG145	8/1/11	California	San Bernardino Co.; Rt. 127 ~26 miles N of Baker	35.61363	-116.2622
Solenopsis	*xyloni*	DAG146	DAG146	8/1/11	California	Inyo Co.; Death Valley National Park; Texas Spring campground	36.45942698	-116.855597
Solenopsis	*xyloni*	DAG148	DAG148	8/2/11	California	Inyo Co.; Death Valley National Park; Texas Spring campground, wash along road, under bushes	36.45810197	-116.858724
Solenopsis	*xyloni*	DAG150	DAG150	8/2/11	California	Inyo Co.; Death Valley National Park; Texas Spring campground, wash along road, under bushes	36.45920704	-116.858402
Solenopsis	*xyloni*	DAG151	DAG151	8/2/11	California	Inyo Co.; Death Valley National Park; Texas Spring campground, wash along road, under bushes	36.45929497	-116.858114
Solenopsis	*xyloni*	DAG152	DAG152	8/2/11	California	Inyo Co.; Death Valley National Park; Texas Spring campground, wash along road, under bushes	36.45960602	-116.857859
Solenopsis	*xyloni*	DAG153	DAG153	8/2/11	California	Inyo Co.; ~11 miles W of CA-190 & CA-127 intersect. Between Death Valley Jct and Death Valley N.P.	36.34214303	-116.603615
Solenopsis	*xyloni*	DAG154	DAG154	8/2/11	California	Inyo Co.; Death Valley Jct. at Rt.190 & Rt.127	36.30407997	-116.414934
Solenopsis	*xyloni*	DAG155	DAG155	8/2/11	California	Inyo Co.; Death Valley Jct. at Rt.190 & Rt.128	36.30411199	-116.414926
Solenopsis	*xyloni*	DAG156	DAG156	8/2/11	California	Inyo Co.; Rt.127 in front of Shoshone Trailer Park	35.98106699	-116.270829
Solenopsis	*xyloni*	DAG157	DAG157	8/2/11	California	Inyo Co.; Rt.127 in front of Shoshone Trailer Park	35.98141098	-116.270807
Solenopsis	*xyloni*	DAG158	DAG158	8/2/11	California	Inyo Co.; Shoshone	35.97587599	-116.270347
Solenopsis	*xyloni*	DAG159	DAG159	8/2/11	California	Inyo Co.; Rt.178 ~13.5 miles NE of Shoshone	36.12078602	-116.17901
Solenopsis	*xyloni*	EN_1	EN_1		California	Imperial Co.	32.847777	-115.571685
Solenopsis	*xyloni*	PW4911	PW4911		California	49 Palms, Joshua Tree National Monument	34.121	-116.112198
Solenopsis	*xyloni*	PW4934	PW4934		California	San Bernadino Co.: 50km N Baker	35.694814	-116.307166

Solenopsis	*xyloni*	PW9351	PW9351		California	San Diego Co.: 15km SW Borrego Springs	33.116111	-116.44637
Solenopsis	*aurea*	WS_5	WS_5		California	Imperial Co.: Westmorland	33.0372	-115.6214
Solenopsis	*xyloni*	PW13471	PW13471		MEX	2km SW Punta Narragansett, Isla Tiburón	29.386768	-112.33194
Solenopsis	*xyloni*	SON# 6	SD87	5/28/94	MEX	Sonora: 3 mi W Punta Cirio, Foraging on senita cactus	29.5	-112.583333
Solenopsis	*xyloni*	SON #10	SD88	10/10/92	MEX	Sonora: 5 mi N Seri Desemboque, coastal desert, nest in sand	29.5666667	-112.383333
Solenopsis	*xyloni*	DAG160	DAG160	8/3/11	Nevada	Clark Co.; Las Vegas Blvd. ~8 miles S of Las Vegas	35.896492	-115.215642
Solenopsis	*xyloni*	DAG162	DAG162	8/3/11	Nevada	Clark Co.; Las Vegas Blvd. ~9.5 miles S of Las Vegas	35.88211996	-115.226441
Solenopsis	*xyloni*	DAG163	DAG163	8/3/11	Nevada	Clark Co.; Las Vegas Blvd. ~9.5 miles S of Las Vegas	35.88223496	-115.226343
Solenopsis	*xyloni*	DAG165	DAG165	8/4/11	Nevada	Clark Co.; exit 90 off I-15 twd Moapa, at Muddy River wash	36.66206997	-114.576649
Solenopsis	*xyloni*	DAG166	DAG166	8/4/11	Nevada	Clark Co.; exit 90 off I-15 twd Moapa, at Muddy River wash	36.66216996	-114.57684
Solenopsis	*xyloni*	DAG167	DAG167	8/4/11	Nevada	Clark Co.; exit 90 off I-15 twd Moapa, at Muddy River wash	36.66202001	-114.57668
Solenopsis	*xyloni*	DAG168	DAG168	8/4/11	Nevada	Clark Co.; exit 100 off I-15	36.73411301	-114.436018
Solenopsis	*xyloni*	DAG169	DAG169	8/4/11	Nevada	Clark Co.; Mesquite, exit 120 off I-15	36.80279701	-114.105402
Solenopsis	*xyloni*	AW1608	AW1608		New Mexico		33.05902499	-106.014219
Solenopsis	*xyloni*	DAG170	DAG170	8/4/11	Utah	Washington Co.; Hurricane, Community Center park, corner of Main St. & 100S	37.17539901	-113.288275
Solenopsis	*xyloni*	DAG171	DAG171	8/4/11	Utah	Washington Co.; Hurricane, Community Center park, corner of Main St. & 100S	37.17519298	-113.288473
Solenopsis	*xyloni*	DAG172	DAG172	8/4/11	Utah	Washington Co.; Hurricane, Community Center park, corner of Main St. & 100S	37.176015	-113.288362
Solenopsis	*xyloni*	DAG174	DAG174	8/5/11	Utah	Washington Co.; SR-9 & 3400W intersect dirt road next to gas station	37.16955003	-113.367263

Solenopsis	*xyloni*	DAG175	DAG175	8/5/11	Utah	Washington Co.; St. George, exit 4 off I-15, Pioneer Rd & parking lot	37.06339398	-113.584307

APPENDIX B

ACTIVITY MATERIALS

Instructions: Please answer the following questions regarding today's activity, as well as the topics it covered. All responses are anonymous and confidential.

1) <u>Prior</u> to today, how would you have described your understanding of the following topics?

	Excellent	Good	Average	Poor	No Understanding
Species concepts					
Process of speciation					
Importance of species delimitation					

2) <u>After</u> the activity, how would you describe your understanding of the following topics?

	Excellent	Good	Average	Poor	No Understanding
Species concepts					
Process of speciation					
Importance of species delimitation					

3) To what extent do you agree with the following statements? (Circle your response)

a) Today's activity helped me more thoroughly understand species concepts.

Strongly Agree Agree Neither Agree/Disagree Disagree Strongly Disagree

b) Today's activity helped me more thoroughly understand species delimitation.

Strongly Agree Agree Neither Agree/Disagree Disagree Strongly Disagree

c) *I enjoyed participating in the activity.*

Strongly Agree Agree Neither Agree/Disagree Disagree Strongly Disagree

d) I believe the activity was at an appropriate level (in terms of difficulty) for the course.

Strongly Agree Agree Neither Agree/Disagree Disagree Strongly Disagree

e) I believe the activity was adequately paced and/or time-appropriate for the course.

Strongly Agree Agree Neither Agree/Disagree Disagree Strongly Disagree

Optional: Use the space below (or back of the page) for any comments or concerns you have regarding the activity.

Taxonomic Species Concept

Description

"… I look at the term species as one arbitrarily given for the sake of convenience to a set of individuals closely resembling each other and that it does not essentially differ from the term variety… Hence, in determining whether a form should be ranked as a species or a variety, the opinion of naturalists having sound judgment and wide experience seems the only guide to follow." (Darwin, 1859)

Evidence

Researcher & Evidence	**Groups Recognized**
Wheeler (1915): morphology	*S. geminata* *S. geminata xyloni* *S. geminata maniosa* *S. aurea* *S. aurea amblychila*

Researcher & Evidence	**Groups Recognized**
Creighton (1930): morphology	*S. geminata* *S. xyloni* *S. xyloni aurea* *S. xyloni amblychila* *S. xyloni maniosa*

Researcher & Evidence	**Groups Recognized**
Trager (1991): morphology	*S. geminata* *S. amblychila* *S. aurea* *S. xyloni*

Researcher & Evidence	**Groups Recognized**
Chialvo (2017): morphology and genetics	*S. geminata* *S. amblychila* *S. aurea* *S. xyloni* *S. xyloni maniosa* *S. arieli*

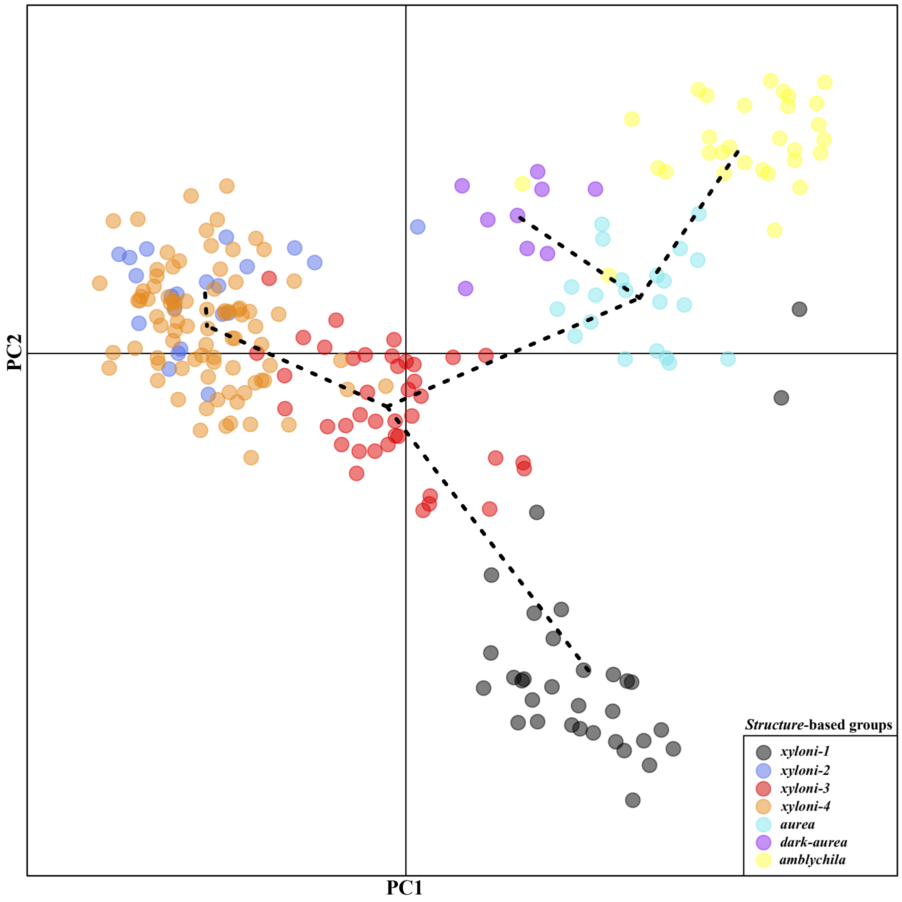
Issues to consider: Who should you trust? Is morphology a more reliable set of characters to use than genetic markers? Do you feel it is better to describe all diversity/varieties, or lump it into larger groups?

Genotypic Clustering Concept

Description

"When we observe a group of individuals within an area, we intuitively recognize species by means of morphology if there are no or few intermediates between two morphological clusters, and because independent characters that distinguish these clusters are correlated with each other. Adding genetics to this definition, we see two species rather than one if there are two identifiable genotypic clusters. These clusters are recognized by a deficit of intermediates, both at single and multiple loci. Mendelian variation is discrete; therefore we expect quantized differences between individuals. We use the patterns of the discrete genetic differences, rather than the discreteness itself, to reveal genotypic clusters." (Mallet, 1995)

Evidence



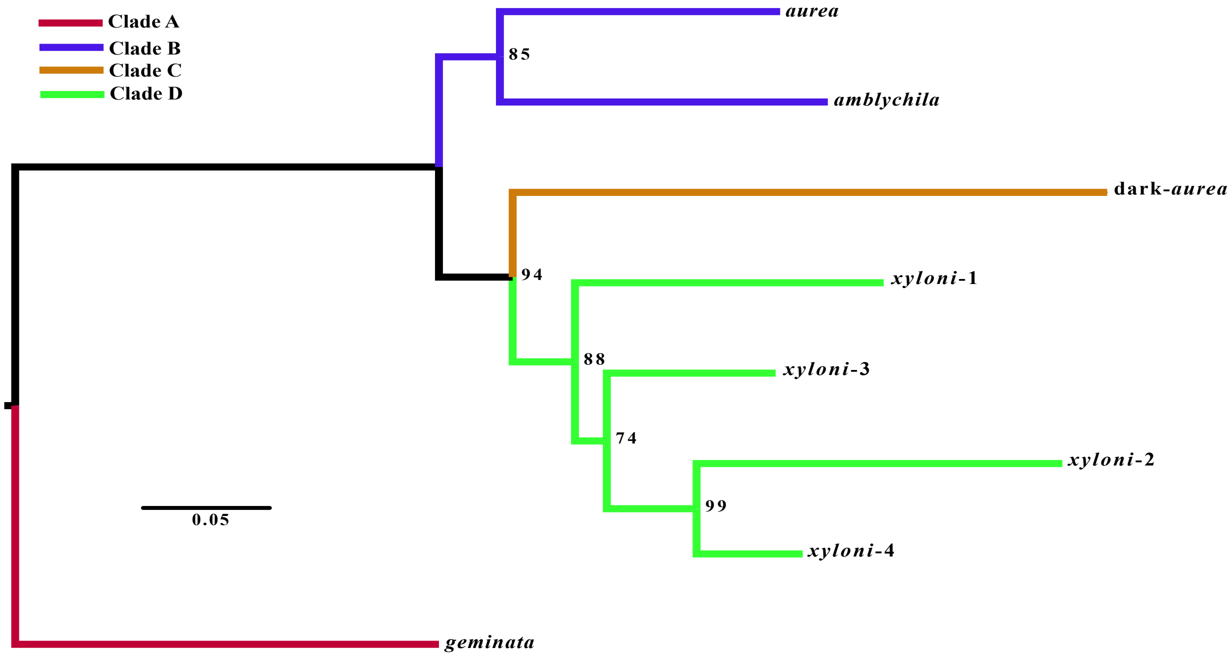
Issues to consider: What degree of separation is considered significant? What does the overlap suggest?

Phylogenetic Species Concept

Description

"Accordingly, a species can be defined as an irreducible cluster of organisms, within which there is a parental pattern of ancestry and descent, and which is diagnosably distinct from other such clusters. Species are thus basal, differentiated taxa." (Cracraft, 1987)

Evidence



Issues to consider: What does the paraphyly of *aurea* suggest?

Biological Species Concept

Description

“Species are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups.” (Mayr, 1942)

Evidence

Table 1. Two different measurements of genetic differentiation (F_{ST} below diagonal, G''_{ST} above diagonal) between populations. Scale: 0 (no differentiation) to 1 (total differentiation).

	xyloni-1	*xyloni*-2	*xyloni*-3	*xyloni*-4	*aurea*	dark-*aurea*	*amblychila*	*geminata*
xyloni-1	---	0.449	0.234	0.332	0.345	0.462	0.439	0.648
xyloni-2	0.217	---	0.348	0.220	0.427	0.539	0.557	0.757
xyloni-3	0.106	0.163	---	0.179	0.278	0.393	0.374	0.646
xyloni-4	0.150	0.100	0.079	---	0.337	0.435	0.419	0.683
aurea	0.151	0.192	0.118	0.144	---	0.417	0.258	0.615
dark-*aurea*	0.227	0.284	0.184	0.201	0.188	---	0.515	0.718
amblychila	0.201	0.264	0.167	0.191	0.110	0.247	---	0.690
geminata	0.260	0.312	0.253	0.275	0.219	0.294	0.270	---

Issues to consider: What degree of level of differentiation is considered “enough” to justify species status? Do these results suggest ongoing gene flow between the populations? If yes, can they be considered species according to this concept? If no, what could cause these results? (Think about timescale).