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# The genetic Characterization of *Myrmelachista* Roger Assemblages (Hymenoptera: Formicidae: Formicinae) in the Atlantic Forest of Southeastern Brazil

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**Abstract**

Arboreal ants of the genus *Myrmelachista*, which have ecologically important relationships with different vegetable species, are found exclusively in the Neotropical region. These ant species are difficult to identify, and their taxonomy remains controversial; moreover, little is known regarding their biology. The objective of the present work is to assess the genetic similarities and dissimilarities between and within *Myrmelachista* species, with the goal of expanding knowledge of the relationships among the taxa of this genus. Sample collection in selected regions of the dense ombrophile forest of southeastern Brazil yielded 256 nests, which were found in vegetation or among scattered twigs in the leaf litter; eight species were recorded. A total of 180 specimens were analyzed, producing 123 polymorphic fragments. Data analyses revealed similarity relationships that allowed the examined species to be classified into the following groups: (1) *Myrmelachista* sp. 4, *M. nodigera*, *M. ruszkii* and *M. gallicola*; (2) *M. catharinae* and *M. arthuri*; (3) *M. reticulata*; and (4) *Myrmelachista* sp. 7. The study results also revealed the existence of two morphological variants of *M. catharinae*; *M. arthuri* was more closely related to one of these *M. catharinae* variants than to the other variant. The present work provides important information regarding genetic variation among *Myrmelachista* species that may contribute to interpreting the complex morphology of this genus.

## Introduction

*Myrmelachista* Roger is a genus of the Formicinae subfamily. The geographical distribution of this genus is restricted to the Neotropical region, and 41% of the species in this genus can be found in Brazil (Kempf, 1972; Fernández & Sendoya, 2004). The species in this genus are arboreal (Longino, 2006) and engage in the specialized practice of nesting in trunk cavities and among twigs (Stout, 1979; Brown, 2000; Longino, 2006; Edwards et al., 2009; Nakano et al., 2012, 2013). These ant species may also form complex mutual associations with certain myrmecophytes (Renner & Ricklefs, 1998; Frederickson, 2005; Edwards et al., 2009) or with Coccidae and Pseudococcidae species (Kusnezov, 1951; Stout, 1979; Ketterl et al., 2003; Longino, 2006). Little information is available regarding the biology of *Myrmelachista* species (Brown, 2000); however, it is known that these species generally feed on extrafloral nectaries (Haber et al., 1981) and on animal-derived proteins (Torres, 1984; Amalin et al., 2001; McNett et al., 2010).

At present, 69 *Myrmelachista* species have been described, with a few recognized subspecies (Bolton et al., 2006; Bolton, 2013); the diversity of this genus has most likely been underestimated (Longino, 2006) due to the limited taxonomic knowledge available regarding *Myrmelachista* (Snelling & Hunt, 1975). Previously published reports, such as studies by Wheeler (1934) and Longino (2006), are important sources of taxonomic knowledge regarding *Myrmelachista*; in particular, the report by Longino (2006) is the most recent taxonomic review of the genus, although this review was restricted to *Myrmelachista* species found in Costa Rica. In the most recent molecular data-based phylogenetic proposals for ants (Brady et al., 2006; Moreau

et al., 2006), *Myrmelachista* is a sister group of *Brachymyrmex*, and these groups constitute the most basal and closely related formicine groups.

Longino (2006) emphasized the difficulty of separating *Myrmelachista* species based on morphological characters but suggested that the combined use of worker, queen and male characters could improve the accuracy of species distinctions. Because the collection of alate individuals is more difficult than the collection of workers (Nakano et al., 2013), molecular techniques may be utilized to help establish the systematics of the *Myrmelachista* genus. These techniques have been applied for similar analyses of other insect groups (Reineke et al., 1998). In particular, ISSRs (inter-simple sequence repeats) may serve as a very important tool for these analyses. ISSR-based techniques enable the development of co-dominant microsatellite markers that may be used in population studies (Gupta et al., 1994; Bornet & Branchard, 2001; Wolfe, 2005). These techniques have allowed genetic distinctions to be drawn among Hymenoptera groups (Borba et al., 2005; León & Jones, 2005; Al-Otaibi, 2008) and among other Insecta groups (Souza et al., 2008; Luque et al., 2002; Dusinsky et al., 2006). However, at present, no known published studies in the literature have applied these techniques exclusively for the examination of ants. Thus, the present work sought to identify relationships and levels of genetic similarity (and dissimilarity) between and within *Myrmelachista* species found in the Atlantic Forest of southeastern Brazil, with the goal of providing additional knowledge regarding the relationships among different taxa of this genus.

## Material and methods

### *Samples for molecular analysis*

The collection of *Myrmelachista* nests occurred in three different regions of dense ombrophile forest in southeastern Brazil (Fig 1). A total of 256 nests were collected; these nests were located either in the forest vegetation or among scattered twigs in the leaf litter (for details about biological material collecting see Nakano et al., 2012, 2013). The nests housed eight *Myrmelachista* species: *M. arthuri* Forel, 1903; *M. catharinae* Mayr, 1887; *M. gallicola* Mayr, 1887; *M. nodigera* Mayr, 1887; *M. reticulata* Borgmeier, 1928; *M. ruszkii* Forel, 1903; *Myrmelachista* sp.4; and *Myrmelachista* sp.7.

Whole nests containing living workers were stored at -80°C. To minimize the degradation of genomic DNA, the screening and identification of individuals were performed after specimens were frozen in ice-immersed Petri dishes. Workers from each nest were also collected in the field and stored in 70% ethanol for subsequent morphological identification.

A total of 180 specimens were assessed. For most of the examined species, five individuals per nest were selected from five nests (randomly) for molecular identification (total

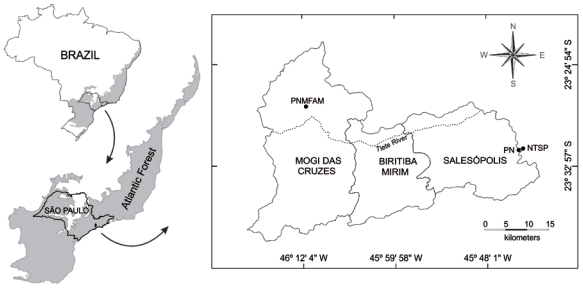


Figure 1. The locations of the Atlantic Forest areas in which *Myrmelachista* species were collected. (PNMFAM: Francisco Affonso de Mello Municipal Natural Park; PN: Ponte Nova Dam; PENT: Tietê Springs State Park).

of 25 individuals and 5 nests per specie), except for *M. reticulata* (5 individuals and 1 nest) and *Myrmelachista* sp.7 (20 individuals and 4 nests). Alate queens (n = 5) were found only in *Myrmelachista* sp.7 nests; these queens were also examined by molecular analyses.

Species (or morphospecies) were identified by comparing the examined specimens with specimens deposited in the reference collection of the Museum of Zoology of the University of São Paulo. Vouchers were deposited in the myrmecofauna collection of the Alto Tietê Myrmecology Laboratory of the University of Mogi das Cruzes and in the Museum of Zoology of the University of São Paulo.

### *Genomic DNA extraction*

Genomic DNA from ants preserved at -80ºC was extracted using the protocol described by Sambrook et al. (1989). DNA extraction was performed independently for each examined specimen. Sample integrity and purity were analyzed by 0.8% agarose gel electrophoresis, and spectrophotometric observations were used to assess DNA quantity and purity.

### *Amplification of ISSRs*

Twenty primers (UBC kit, University of British Columbia) were tested; among these primers, the 10 primers that generated the most distinguishable bands were selected, and optimal annealing temperatures were determined for each of these 10 primers (Table 1). Each amplification reaction was performed in a tube containing a total volume of 20 μL. The reaction mixture included the following reagents: buffer (1.6X), deoxynucleotide triphosphates (dNTPs) (0.2 mM), primer (0.8 μM), $MgCl_2$ (1.5 mM), DNA (8 ng), Taq DNA polymerase (1 U) (GoTaq® Flexi DNA Polymerase, Promega) and autoclaved Milli-Q $H_2O$. The amplifications were performed in a thermal cycler (Peltier Thermal Cycler PTC-200, MJ Research). The following temperature conditions were utilized for the amplification reactions: an initial denaturation at 94°C for 3 min; 40 cycles of denaturation at 92°C for 1 min, annealing (at an optimized temperature for each primer) for 2

min and extension at 72°C for 2 min; and a final extension at 72°C for 7 min.

Table 1. The identifying codes, sequences and optimal annealing temperatures of the ISSR primers utilized in this study.

| Code | 5'-3' Sequence | Annealing temperature (ºC) |
|---|---|---|
| UBC 842 | GAG AGA GAG AGA GAG AYG | 54 |
| UBC 888 | BDB CAC ACA CAC ACA CA | 57 |
| UBC 889 | DBD ACA CAC ACA CAC AC | 58 |
| UBC 890 | VHV GTG TGT GTG TGT GT | 58 |
| UBC 808 | AGA GAG AGA GAG AGA GC | 54 |
| UBC 816 | CAC ACA CAC ACA CAC AT | 54 |
| UBC 836 | AGA GAG AGA GAG AGA GYA | 54 |
| UBC 841 | GAG AGA GAG AGA GAG AYC | 54 |
| UBC 848 | CAC ACA CAC ACA CAC ARG | 54 |
| UBC 811 | GAG AGA GAG AGA GAG AC | 54 |

Y = C or T; R = A or G; B = G, T or C; D = G, A or T; H = A, T or C; V = A, C or G.

Bands were visualized on a 1.8% agarose gel subjected to 2.5 hours of electrophoresis at 120 amperes in a horizontal electrophoresis unit (Gibco). Amplified DNA was visualized by ethidium bromide staining, dissolved in 1X TBE (Tris-borate-EDTA (ethylenediaminetetraacetic acid)) buffer, pH 8.3, and photographed under UV light using a transilluminator equipped with a DC40 digital camera (Kodak).

*Data analysis*

A binary matrix of molecular data was generated by encoding the bands present in the agarose gel (presence = 1; absence = 0). The CP ATLAS 2.0 software program (Lazar Software, 2009) was used to standardize the criteria for determining band detection and band intensity.

To assess intra and interspecific genetic similarity, cluster analysis was performed using the unweighted pair-group method with arithmetic average (UPGMA) method and Nei and Li's coefficient (Nei & Li, 1979). Analyses using this coefficient only examine bands that are present, comparing the number of bands shared among individual specimens. Principal component analysis (PCA) was used to generate graphical representations of interspecies genetic variation and to determine the characters that provide the greatest contribution to genetic differentiation. All statistical analyses were performed using the MVSP 3.1 statistical software package (Kovach, 2007).

## Results

DNA concentrations in samples from the examined species ranged from 3.3 to 55.4 ng/μL, and the $OD_{260}/OD_{280}$ absorbance ratios of these samples ranged from 1.17 to 2.6. A total of 123 different bands were obtained using the 10 selected primers, and a total of 22,140 bands were analyzed. The sizes of the examined DNA fragments ranged from 100 bp to 1000 bp. The number of bands amplified by each primer ranged from seven (for the UBC 848 primer) to 15 (for the UBC 889 primer), with an average of 12 bands generated per primer. The resulting amplification pattern is depicted in Fig 2.

The largest values of intraspecific genetic similarity obtained in the ISSR analyses were found among workers in *M. arthuri* nests (which exhibited similarity values of up to 1.00). The smallest values of intraspecific genetic similarity were found among *M. ruszkii* (with a minimum similarity value of 0.51) and *Myrmelachista* sp.4 (with a minimum similarity value of 0.56) specimens; these two species exhibited the highest rates of intraspecific genetic diversity.

With respect to interspecific genetic similarity, high similarity between *M. arthuri* and *M. catharinae* (with similarity values of up to 0.84) and between *Myrmelachista* sp.4 and *M. nodigera* (with similarity values of up to 0.77) was observed. The pairs of species with the lowest similarity were *Myrmelachista* sp.4 and *M. reticulata* (with a similarity value of only 0.33) and *Myrmelachista* sp.4 and *M. catharinae* (with a similarity value of only 0.37) (Table 2).

Using the UPGMA-based dendogram, seven main groups were defined. Notably, *Myrmelachista* sp.7 (group one) and *M. reticulata* (group two) were the most genetically dissimilar of the examined species; *Myrmelachista* sp.4 (group three) was more closely related to *M. gallicola* (group

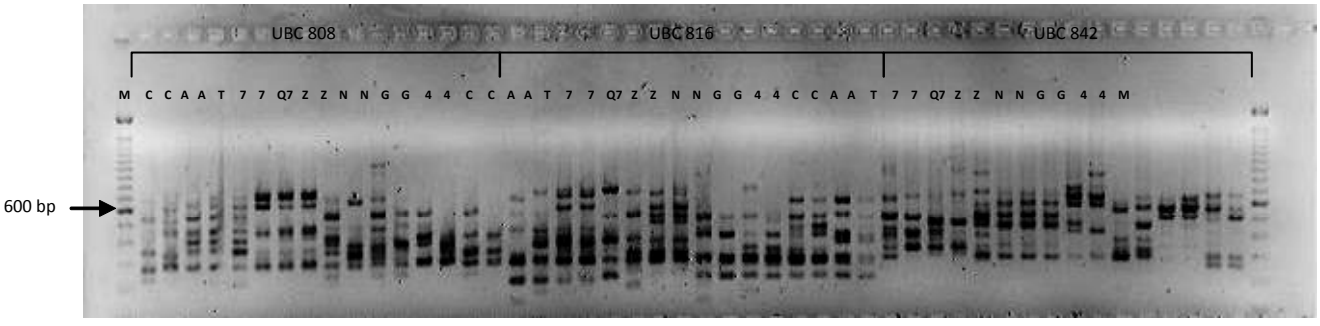


Figure 2. An agarose gel (N%) depicting the ISSR bands obtained from *Myrmelachista* genomic DNA amplified with the UBC 808, UBC 816 and UBC 842 primers. M: 100 bp marker; C: *M. catharinae*; A: *M. arthuri;* T: *M. reticulata;* 7: *Myrmelachista* sp.7; Q7: *Myrmelachista* sp.7 queen; Z: *M. ruszkii;* N: *M. nodigera;* G: *M. gallicola* and 4: *Myrmelachista* sp.4.

Table 2. Variation of intraspecific (underlined) and interspecific molecular similarity for *Myrmelachista* species using ISSR molecular markers and based on the Nei and Li coefficient (Nei & Li, 1979).

| Species | *M. catharinae* | *M. arthuri* | *M. reticulata* | *M.* sp.7 | *M. ruszkii* | *M. nodigera* | *M. gallicola* | *M.* sp.4 |
|---|---|---|---|---|---|---|---|---|
| *M. catharinae* | 0.61 – 099 | | | | | | | |
| *M. arthuri* | 0.52 – 0.84 | 0.81 – 1.00 | | | | | | |
| *M. reticulata* | 0.47 – 0.66 | 0.52 – 0.63 | 0.90 – 0.97 | | | | | |
| *M.* sp.7 | 0.45 – 0.64 | 0.44 – 0.63 | 0.54 – 0.69 | 0.78 – 0.99 | | | | |
| *M. ruszkii* | 0.46 – 0.72 | 0.51 – 0.71 | 0.44 – 0.61 | 0.47 – 0.63 | 0.51 – 0.99 | | | |
| *M. nodigera* | 0.40 – 0.70 | 0.51 – 0.75 | 0.42 – 0.62 | 0.38 – 0.59 | 0.48 – 0.72 | 0.64 – 0.99 | | |
| *M. gallicola* | 0.38 – 0.68 | 0.46 – 0.69 | 0.38 – 0.58 | 0.38 – 0.59 | 0.45 – 0.72 | 0.46 – 0.72 | 0.64 – 0.97 | |
| *M.* sp.4 | 0.37 – 0.64 | 0.45 – 0.71 | 0.33 – 0.57 | 0.37 – 0.62 | 0.42 – 0.66 | 0.44 – 0.77 | 0.43 – 0.74 | 0.56 – 0.97 |

four), *M. nodigera* (group five) and *M. ruszkii* (group six) than to the other examined species; and *M. catharinae* (with two morphological variants) and *M. arthuri*, which are both in group seven, were the most similar of the examined species (Fig 3).

A similar result was derived from PCA, which allowed the examined species to be classified into four groups (Fig 4). The PCA findings confirmed that *Myrmelachista* sp.7 (group one) and *M. reticulata* (group two) were the least similar of the examined species. *M. catharinae* and *M. arthuri* were categorized into group three, whereas *M. nodigera*, *M. ruszkii*, *M. gallicola* and *Myrmelachista* sp.4 were categorized into group four.

## Discussion

*Myrmelachista* species possess between nine and 10 antennal segments. Most nine-segmented *Myrmelachista* species are found in Central America and the Caribbean (with only two known nine-segmented *Myrmelachista* species in South America), whereas 10-segmented *Myrmelachista* species are mostly found in South America (with only three known 10-segmented *Myrmelachista* species found in Mexico and Central America) (Longino, 2006). In the present study, only 10-segmented species were examined; as noted by Snelling and Hunt (1975), these species form an extremely heterogeneous group.

The circumscription of *Myrmelachista* species is a complex task because the morphological differences between individuals of a single species that originate from different colonies can be sufficient to cause these individuals to be erroneously regarded as members of different species (Wheeler, 1934; Snelling & Hunt, 1975). Our results show that the ISSR can be powerful markers identification of *Myrmelachista* and facilitates the identification and morphological interpretation. The ISSR is more advantageous in showing intra and interspecies differences than morphology. According to Nakano (2010), worker ants of the *M. catharinae*, *M. arthuri, M. reticulata* and *Myrmelachista* sp.7 species exhibit extremely similar morphologies. However, the molecular analysis results of this study reveal interspecies differences among these morphologically similar workers. *M. catharinae* and *Myrmelachista* sp.7 queens exhibit morphological differences (Nakano, 2010), and our molecular analysis results confirm the interspecies differences between these queens. Longino (2006) reported that analyses of reproductive ants can facilitate the identification of *Myrmelachista* species; however, these types of ants have rarely been examined, and literature data regarding the reproductive biology of *Myrmelachista* species remain scarce (Nakano et al., 2012, 2013).

Two different *M. catharinae* groups are readily identifiable in the dendogram (Fig 3) of this study, and *M. arthuri* is more closely related to one of these *M. catharinae* groups than to the other *M. catharinae* group. Field observations indicated that *M. catharinae* workers that were more closely related to *M. arthuri* exhibited lighter-colored mesosoma than *M. catharinae* workers that were more distantly related to *M. arthuri*. When disturbed, both *M. arthuri* workers and *M. catharinae* workers with lighter-colored mesosoma exhibit the same aggressive behavior of immediately elevating their gasters. However, in contrast to *M. catharinae* with lighter-colored mesosoma, *M. arthuri* produces large nests and forms trails of intensely foraging workers (MA Nakano, personal observation).

Our results demonstrated that *M. nodigera*, *M. gallicola* and *Myrmelachista* sp.4 are genetically similar species. Previous reports have noted morphologic and morphometric similarities between *M. gallicola* and *M. nodigera* with respect to both workers (Quirán & Martínez, 2006) and queens. Field observations have also revealed that when disturbed, ants of these

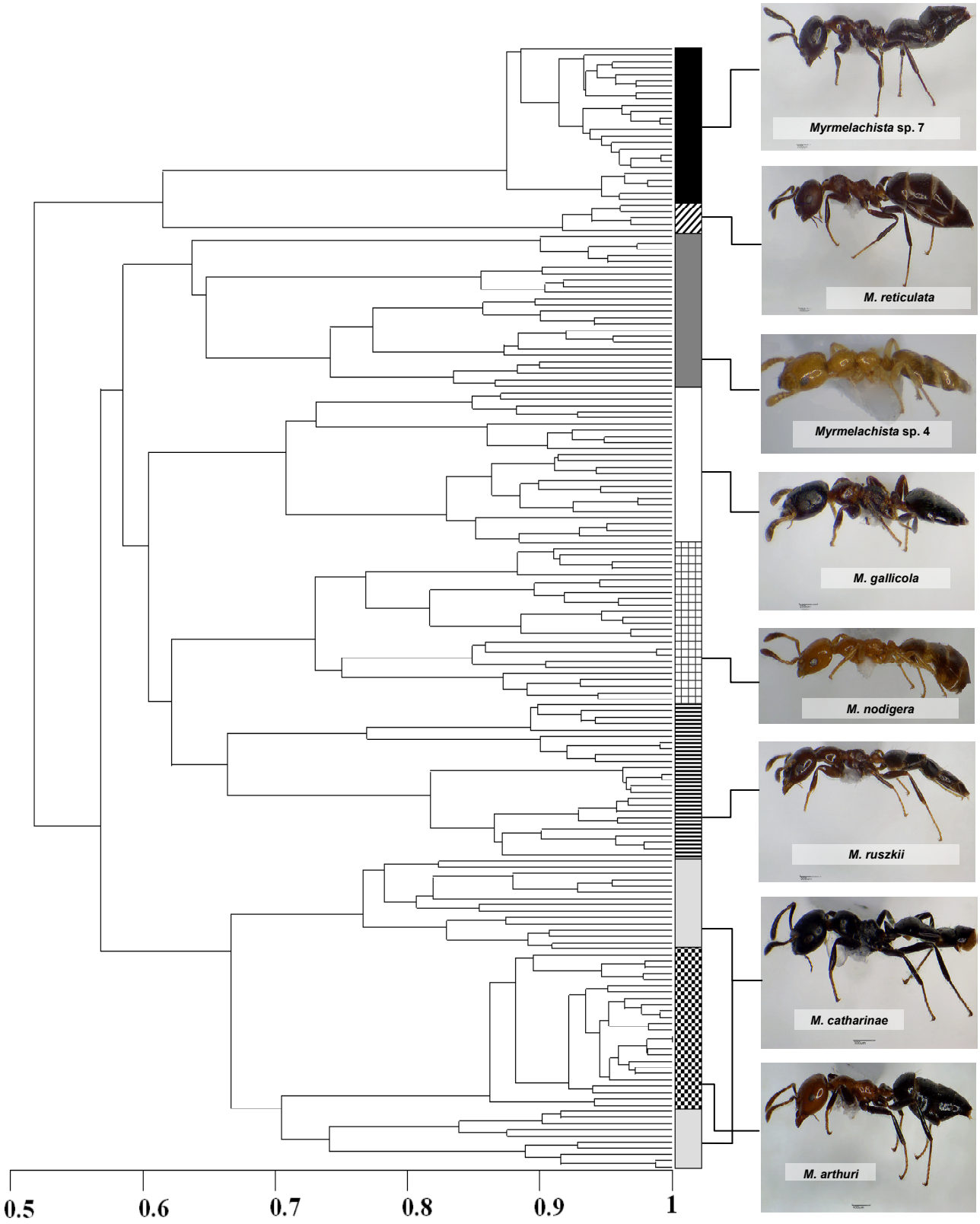


Figure 3. The UPGMA dendrogram generated using Nei and Li's coefficient (Nei & Li, 1979) for eight *Myrmelachista* species. *Myrmelachista* sp.7; *M. reticulata*; *Myrmelachista* sp.4; *M. gallicola*; *M. nodigera*; *M. ruszkii*; *M. catharinae* and *M. arthuri*.

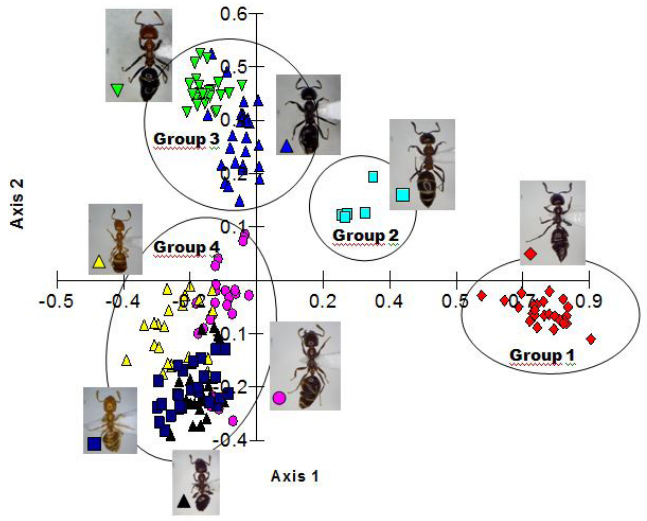


Figure 4. Principal component analysis results for eight *Myrmelachista* species (n = 175) based on 123 bands obtained from ISSR primers. Circles indicate the four different groups into which these species have been classified. PC1 and PC2 account for 16% and 10%, respectively, of the observed variance. ▲ *M. catharinae*; ▼ *M. arthuri*; □ *M. reticulata*; ◆ *Myrmelachista* sp.7; ● *M. ruszkii*; △ *M. nodigera*; ▲ *M. gallicola*; ■ *Myrmelachista* sp.4.

species hastily retreat to nest orifices (Nakano et al., 2013).

The molecular analysis results of this study reveal an interesting scenario that could result in the taxonomic reclassification of the examined groups; that should be studied further with sequencing. For now, the ISSR allowed us to recognize taxonomic unities for *Myrmelachista*. In particular, if less conservative criteria are utilized to interpret intraspecific morphological variations in the examined species (and consequently to delineate the boundaries between species), *M. catharinae* in which workers have lighter-colored mesosoma could be regarded as a new species that is more closely related to *M. arthuri* than to other *M. catharinae*. Similarly, in a more widespread scenario, the superposition of molecular characters among *M. nodigera*, *M. gallicola, M. ruszkii* and *Myrmelachista* sp.4 could lead to the unification of these species under a single specific name that would represent all of these as a single specie with high intraspecific biological and morphological variation.

Prior to the implementation of the suggested nomenclature changes, individuals of the examined species should be sampled in a more widespread manner across a broad swath of the geographic areas in which these species are distributed. Nonetheless, the present work contributes important information regarding the intra and interspecific genetic variations among the examined species. These contributions facilitate the interpretation of morphological variations in *Myrmelachista*, a conspicuous genus that is ecologically important due to the relationship between *Myrmelachista* species and different vegetable species; despite this importance, this biologically diverse and morphologically complex genus remains insufficiently studied.

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**References**

Al-otaibi, S. A. (2008). Genetic variability in mite-resistant honey bee using ISSR molecular markers. Arab Journal of Biotecnology, 11: 241-252.

Amalin, D. M., Pena, J. E., Mcsorley, R., Browning, H. W. & Crane, J. H. (2001). Comparison of different sampling methods and effect of pesticide application on spider populations in lime orchards in south florida. Environmental Entomology, 30: 1021-1027. doi: 10.1603/0046-225X-30.6.1021.

Bolton, B., Alpert, G., Ward, P. S. & Naskrecki, P. (2006). Bolton`s catalogue of ants of the world. Cambridge: Harvard University Press. CD-ROM.

Bolton, B. (2013). An online catalog of the ants of the world. Available from http://antcat.org. (accessed [November, 30, 2013])

Borba, R. S., Garcia, M. S., Kovaleski, A., Oliveira, A. C., Zimmer, P. D., Castelo-Branco, J. S. & Malone, G. (2005). Dissimilaridade genética de linhagens de *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) através de marcadores moleculares ISSR. Neotropical Entomology**,** 34: 565-569.

Bornet, B. & Branchard, M. (2001). Nonanchored Inter Simple Sequence Repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting**.** Plant Molecular Biology Reporter, 19: 209-215.

Brady, S. G., Schultz, T. R., Fisher, B. L. & Ward, P. S. (2006). From de cover: evaluating alternative hypotheses for the early evolution and diversification of ants. Proceedings of the National Academy of Sciences USA**,** 103: 18172-18177. doi: 10.1073/pnas.0605858103.

Brown, W. L. (2000). Diversity of ants. In: Agosti, D., Majer, J. D., Alonso, L. T. & Schultz, T. R. (eds.). Ants: standard methods for measuring and monitoring biodiveristy. Washington: Smithsonian Institution Press 45-46.

Dusinsky, R., Kudela, M., Stloukalova, V. & Jedlicka, L. (2006). Use of inter-simple sequence repeat (ISSR) markers for discrimination between and within species of blackflies (Diptera: Simuliidae). Biologia, Bratislava, 61: 299-304.

Edwards, D. P., Frederickson, M. E., Shepard, G. H. & Yu, D. W. (2009). A plant needs ants like a dog needs fleas: *Myrmelachista schumanni* ants gall many trees species to create housing. Ame-

rican Naturalist, 174: 734-740. doi: 10.1086/606022.

Fernández, F. & Sendoya, S. (2004). Lista de las hormigas Neotropicales (Hymenoptera: Formicidae). Biota Colombiana, 5: 5-27.

Frederickson, M. E. (2005). Ant species confer different partner benefits on two neotropical myrmecophytes. Oecologia, 143: 387-395. doi:10.1098/rspb.2006.0415.

Gupta, M., Chyiy, S., Romero-Severson, J. & Owen, J. I. (1994). Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. Theoretical and Applied Genetics, 89: 998-1006.

Haber, W. A., Frankie, G. W., Baker, H. G., Baker, I. & Koptur, S. (1981). Ants like flower nectar. Biotropica, 13: 211-214.

Kempf, W. (1972). Catálogo abreviado das formigas da Região Neotropical (Hymenoptera: Formicidae). Studia Entomologica, 15: 1-4.

Ketterl, J., Verhaagh, M., Bihn, J. H., Brandão, C. R. F. & Engels, W. (2003). Spectrum of ants associated with *Araucaria angustifolia* trees and their relations to hemipteran trophobionts. Studies on Neotropical Fauna and Environment, 38: 199-206.

Kovach, W. L. (2007). MVSP 3.1: multivariate statistical packkage. http://kovcomp.co.uk/mvsp.html. (accessed date: 13 March, 2010).

Kusnezov, N. (1951). *Myrmelachista* en la Patagônia (Hymenoptera, Formicidae). Acta Zoologica Lilloana, 11: 353-365.

Lazar Software. CP ATLAS 2.0. (2009). http://lazarsoftware.com/index.html. (accessed date: 1 March, 2010).

León, J. H. & Jones, W. A. (2005). Genetic differentiation among geographic populations of *Gonatocerus ashmeadi*, the predominant egg parasitoid of the glassy-winged sharpshooter, *Homalodisca coagulata*. Journal of Insect Science, 5: 1-9.

Longino, J. T. (2006). A taxonomic review of the genus *Myrmelachista* (Hymenoptera: Formicidae) in Costa Rica. Zootaxa, 1141: 1-54.

Luque, C., Legal, L., Saudter, H., Gers, C. & Wink, M. (2002). Brief report ISSR (Inter Simple Sequence Repeats) as genetic markers in Noctuids (Lepidoptera). Hereditas, 136: 251-253.

Mcnett, K., Longino, J., Barriga, P., Vargas, O., Phillips, K. & Sagers, C. L. (2010). Stable isotope investigation of a cryptic ant-plant association: *Myrmelachista flavocotea* (Hymenoptera: Formicidae) and *Ocotea* spp. (Lauraceae). Insectes Sociaux, 57: 67-72. doi: 10.1007/s00040-009-0051-z.

Moreau, C. S., Bell, C. D., Vila, R., Archibald, B. & Piercel, N. (2006). Phylogeny of the ants: diversification in the age of Angiosperms. Science, 312: 101-104.

Nakano, M. A. (2010). Caracterização morfológica, morfométrica e molecular de espécies de *Myrmelachista* Roger, 1865 (Formicidae: Formicinae) do Alto Tietê. Dissertação de Mestrado em Biotecnologia, Universidade de Mogi das Cruzes, SP, 137p.

Nakano, M. A., Feitosa , R. M., Moraes, C.O., Adriano, L. D. C., Hengles, E. P., Longui, E. L. & Morini, M.S.C. (2012). Assembly of *Myrmelachista* Roger (Formicidae: Formicinae) in twigs fallen on the leaf litter of Brazilian Atlantic Forest. Journal of Natural History, 46: 2103-2115. doi:10.1080/00222933.2012.707247.

Nakano, M. A., Miranda, V. F. O., Souza, D. R., Feitosa, R. M. & Morini, M. S. C. (2013). Ocurrence and natural history of *Myrmelachista* Roger (Formicidae: Formicinae) in the Atlantic Forest of southeastern Brazil. Revista Chilena de Historia Natural, 86: 169-179.

Nei, M. & Li, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences USA, 76: 5269-5273.

Quirán, E. M. & Martínez, J. J. (2006). Redescripción de la obrera de *Myrmelachista gallicola* (Hymenoptera: Formicidae) y primera cita para la provincial de La Pampa (Argentina). Revista de la Sociedad Entomológica Argentina, 65: 89-92.

Reineke, A., Karlovsky, P. & Zebitz, P. W. (1998). Preparation and purification of DNA from insects for AFLP analysis. Insect Molecular Biology, 7: 95-99.

Renner, S. S. & Ricklefs, R. E. (1998). Herbicidal activity of domatia-inhabiting ants in patches of *Tococa guianensis* and *Clidemia heterophylla*. Biotropica, 30: 324-327.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). Molecular Cloning: a laboratory manual. 2. ed. Cold Spring: Harbor Laboratory Press.

Snelling, R. R. & Hunt, J. H. (1975). The ants of Chile (Hymenoptera: Formicidae). Revista Chilena de Entomologia, 9: 110-114.

Souza, G. A., Carvalho, M. R. O., Martins, E. R., Guedes, R. N. C. & Oliveira, L. O. (2008). Diversidade genética estimada com marcadores ISSR em populações brasileiras de *Zabrotes subfasciatus*. Pesquisa Agropecuária Brasileira, 43: 843-849.

Stout, J. (1979). An association of an ant, a mealy bug, and an understory tree from a Costa Rican Rain Forest. Biotropica, 11: 309-311.

Torres, J. A. (1984). Niches and coexistence of ant communities in Puerto Rico: Repeated Patterns. Biotropica, 16: 284-295.

Wheeler, W. M. (1934). Neotropical ants collected by Dr. Elisabeth Skwarra and others. Bulletin of the Museum of Comparative Zoology, 77: 157-240.

Wolfe, A. D. (2005). ISSR techniques for evolutionary biology. Methods in Enzymology, 395: 134-144.