Saudi Journal of Biological Sciences 27 (2020) 184–188

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

DNA barcoding of the fire ant genus *Solenopsis* Westwood (Hymenoptera: Formicidae) from the Riyadh region, the Kingdom of Saudi Arabia

Khawaja Ghulam Rasool [a,*], Mureed Husain [a], Shehzad Salman [a], Muhammad Tufail [a,b], Sukirno Sukirno [c], Abdulrahman S. Aldawood [a]

[a] Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia
[b] Ghazi University, Dera Ghazi Khan, Punjab, Pakistan
[c] Entomology Laboratory, Universitas Gadjah Mada, Indonesia

ARTICLE INFO

Article history:
Received 29 April 2019
Revised 18 June 2019
Accepted 30 June 2019
Available online 2 July 2019

Keywords:
Fire ant
DNA barcoding
Cytochrome *C* oxidase I
Biodiversity
Arabian Peninsula

ABSTRACT

The ant genus *Solenopsis* Westwood, 1840 is the largest in Myrmicinae subfamily having almost 200 described species worldwide. They are commonly distributed in the tropics and temperate areas of the world. Some invasive *Solenopsis* species are very dreadful. We have already reported a fire ant species, *Solenopsis saudiensis* Sharaf & Aldawood, 2011, identified using traditional morphometric approaches of species identification. Present study was carried out to develop DNA Barcoding to identify *Solenopsis saudiensis* and to elucidate genetic structure of the various *S. saudiensis* populations across their distribution range in Riyadh, Saudi Arabia. The comparison of DNA barcodes showed no genetic diversity among six populations and a queen from *S. saudiensis* analyzed from the Riyadh region. This genetic resemblance probably reflects their adaptation toward a specific habitat, thus constituting a single and strong gene pool. Our comprehensive field survey did not provide any evidence of *Solenopsis* species except *S. saudiensis* in the Riyadh region. *Solenopsis saudiensis* populations were only found around date palm trees indicating their strong association with date palm groves. Moreover, *S. saudiensis* has 83–86% sequence identity to other *Solenopsis* spp. from other parts of the world. Interestingly, the highest sequence identity of (86%) was with that of *Solenopsis molesta* Say, 1836, the thief ant, from the USA. This study provides a working laboratory procedure and a reference library for the identification of *Solenopsis saudiensis.*

1. Introduction

The scarcity of available information about the genus *Solenopsis* Westwood can perhaps be ascribed to its difficult taxonomy in comparison with other ant genera. Their pale coloration, small size, and cryptic nesting habitats have led to the Arabian *Solenopsis* fauna being overlooked by the collectors. Collingwood and Agosti (1996), were pioneers in reporting Arabian *Solenopsis* fauna from the Arabian Peninsula. So far, in the Arabian Peninsula four *Solenopsis* species have been described including: *S. omana* Collingwood and Agosti, 1996; *S. geminata* (Fabricius, 1804) (Collingwood et al. 1997); *S. zingibara* Collingwood and Agosti, 1996; and *S. sumara* Collingwood and Agosti, 1996.

In Riyadh, Saudi Arabia, the genus *Solenopsis* was originally reported as a new species named *S. saudiensis* Sharaf and Aldawood, 2011 (Sharaf and Aldawood 2011). They also provided an ecological and biological description of the species. In addition, the authors keyed the four known species from Arabian Peninsula together with other four known Egyptian species (Sharaf et al. 2009).

The sole revisionary study of the Arabian *Solenopsis* fauna listed six *Solenopsis* species described based on worker and queen castes from Saudi Arabian southwestern mountains (Sharaf and Aldawood, 2012). In addition, a neotype was designated for the

* Corresponding author.
E-mail addresses: gkhawaja@ksu.edu.sa (K.G. Rasool), mbukhsh@ksu.edu.sa (M. Husain), aldawood@ksu.edu.sa (A.S. Aldawood).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2019.06.018
1319-562X/

Yemeni species *S. sumara* Collingwood and Agosti and the worker caste was re-described and illustrated for the first time. Moreover, the paper presented a taxonomic key to the six known Arabian species.

DNA barcoding intents to render an efficient approach to identify individuals at species level, and consequently, greatly contributes to taxonomic investigations. The DNA barcode is a short DNA sequence derived from a specific part of genomic DNA of the species under investigation that is used to identify the organisms at species level in comparison with already identified sequences submitted to the genomic databases. If the investigated barcode matches any of the bank barcode sequences the species can be identified, otherwise, the new barcode may refer to a newly reported species (Hajibabaei et al. 2007).

DNA barcoding exploits a 650-base pair fragment of COI mitochondrial gene at 5′ end (Hebert et al. 2003), which is the accepted barcode region for animals. DNA barcoding has been reported as useful approach for species identification in many higher animal groups including insects and metazoan (Ward et al. 2005; Smith et al. 2006; Folmer et al. 1994). COI DNA barcoding is an efficient approach for analyzing hypotheses formulated for taxonomic revisions. Moreover, ant taxonomic data is enough to verify DNA barcoding efficacy for ant species identification (Smith et al. 2005).

DNA barcoding has many advantages and can solve many problems of traditional taxonomy: it's a verifiable and reproducible approach, very rapid technique (sample processing takes very few hours, cost effective (approximately 5 USD/sample), work for the smallest animals, and works for any life stage as well as tissues (Rougerie et al. 2009).

DNA barcoding has been used to identify invasive ant species i.e. *Solenopsis geminate* (Kouakou et al. 2017). This approach was used to delimit 24 morphospecies of the genus *Solenopsis*, which is otherwise very hard to identify to species level (Delsinne et al. 2012). This technique was used to identify ant species in hyperdiverse ant genus *Pheidole* in which polymorphism is common which makes identification a challenging task. Phylogenetic analysis revealed that 15 morphospecies out of 19 confirmed with DNA based identified species (Ng'endo et al. 2013). However, first molecular analysis of *S. geminate* confirmed invasion and distribution of this invasive species in Cote d'Ivoire, West Africa (Kouakou et al. 2017). A combination of DNA barcoding with morphological analysis revealed seven ant species belonging to genera *Papyrius* Shattuck 1992, *Iridomyrmex* Mayr 1862, *Cardiocondyla nuda* (Mayr 1866) group, and *Camponotus novaehollandiae* (Mayr 1870) group (Oberprieler et al. 2018). Separation of these seven species was based on COI sequences divergence.

So far, DNA barcoding has been carried out for very few ants species. Fisher and Smith (2008) suggested that DNA barcoding is very useful tool which provides quick and vary precise detail at large scale to systematize alpha-taxonomy that is not possible with traditional morphometric approaches alone.

2. Materials and methods

Solenopsis specimens sampling was carried out from Riyadh region during 2013–2015. In total, we explored 43 areas in the Riyadh region (Table 1). Although, we conducted an extensive survey, yet only six populations of one *Solenopsis* species (*S. saudiensis*), were found in Riyadh city including one population from Al Hayer (23 workers), two populations from Diriyah (43 workers and six queens), two populations from Wadi Hanifa (more than 70 workers), and one population from Al Imam University (13 workers) (Table 2). No other collection site yielded any specimen representing the genus *Solenopsis*. Ants were collected using soil and litter sifting trays since we found that *Solenopsis* sp. nests in the moist soil under date palm trees. After collection, different populations were preserved in ethanol (95%) and for morphological studies of each population, the voucher specimens were point mounted. Specimens were sent to the California Academy of Science, California, USA for photography and high-resolution photographs of *Solenopsis* workers and queen are available on www.AntWeb.org.

Table 1
Fire ant collection sites in the Riyadh region.

1. Afif	18. Hawtet Bani Tamim	35. Sajar35. Sajar
2. Al Ammariyah	19. Hawtet Sudair	36. Salboukh
3. Al Bijadyah	20. Huraymila	37. Tebrik
4. Al Ghat	21. Irqah	38. Thadig
5. Al Hayer	22. Janadiriyah	39. Tumair
6. Al Imam University, Diriyah	23. Kharara	40. Ushairat Sudair
7. King Saud University, Diriyah	24. Layla Alfalaj	41. Wadi Dawasir
8. Al Kharaj	25. Majmaa	42. Wadi Hanifa
9. Al Qarain, Shaqra	26. Malham	43. Zulfi
10. As Sulayyil	27. Marat	44. Zulfi
11. Bijadriyah	28. Mezahmiyah	
12. Dawadimi	29. Naam	
13. Dhurma	30. Na'ajan	
14. Dirab	31. Quwayah	
15. Diqlah	32. Rawdat Khoraim	
16. Ghiyanah	33. Rumah	
17. Hareeq	34. Shaqra	

2.1. DNA extraction of Solenopsis sp.

We tested a number of pre-existing DNA extraction protocols, but none was successful because the specimens were very small. We ultimately settled on a crude DNA extraction procedure. Specimens were removed from alcohol and air-dried in an autoclaved petri dish. Each specimen was transferred into a 200 μL PCR tube having 20 μL of 50 mM NaOH and incubated for 15 min at 95 °C in PCR machine. The incubated samples were normalized in a 20 μL of 200 mM Tris-HCl (pH 8.0), briefly centrifuged to spin down the extracted DNA, and stored at −20 °C for further use.

Mitochondrial cytochrome oxidase 1 (COI) gene barcoding region was amplified by using KOD FX Neo DNA polymerase (Toyobo, Osaka, Japan) and standard primers for DNA barcoding: Folmer primers LCO1490 (Reverse): GGT CAA CAA ATC ATA AAG ATA TTG G and HCO2198 (Forward): TAA ACT TCA GGG TGA CCA AAA AAT CA (Folmer et al., 1994). A 50 μL PCR reactions was prepared by using the following ingredients: 1 μL polymerase enzyme, 1 μL reverse primer (10 μM), 1 μL forward primer (10 μM), 5 μL DNA template, 25 μL 2X buffer, 10 μL dNTP (2 mM), and 7 μL water.

Thermocycler 9700 (Applied Biosystems, California, USA), was used to amplify the DNA template with the given reaction conditions: initial denaturation (94 °C for two minutes), denaturation (98 °C for 10 s), annealing (48 °C for 30 s), and extension (68 °C for 15 s), where steps 2–4 were repeated 35 times.

Gel electrophoresis was carried out to observe the amplified DNA products. In case of a single discrete amplified band, the PCR products were directly purified with FavorPrep™ MicroElute PCR clean up kit (GE Healthcare, Buckinghamshire, UK), whereas, in case of double bands, the required band was excised from the gel and purified with the same clean up kit. The gel purified product was again subjected to gel electrophoresis to observe a discrete single band (Fig. 1). The DNA concentration was measured with NanoDrop (Thermo Fisher Scientific, Wilmington, Delaware, USA).

The purified samples of COI were sent to Beijing Genomic Institute (BGI, Hong Kong, China) for sequencing. Seven DNA barcodes were generated; one for each population of *S. saudiensis* found in

Table 2
Solenopsis saudiensis specimens collected from the Riyadh region with their assigned sample ID.

Sample ID	Collection site	Latitude	Longitude	Elevation
SSHYR01	Al Hayer	24.55775°	46.74115°	587
SSWH01	Wadi Hanifa	24.66497°	46.60575°	633
SSWH02	Wadi Hanifa	24.73507°	46.57518°	674
SSKSU01	King Saud University, Diriyah	24.71383°	46.62557°	660
SSKSU02	King Saud University, Diriyah	24.71383°	46.62557°	660
SSAIU01	Al Imam University	24.81658°	46.71162°	650
SSKSUQ01	King Saud University, Diriyah	24.71383°	46.62557°	660

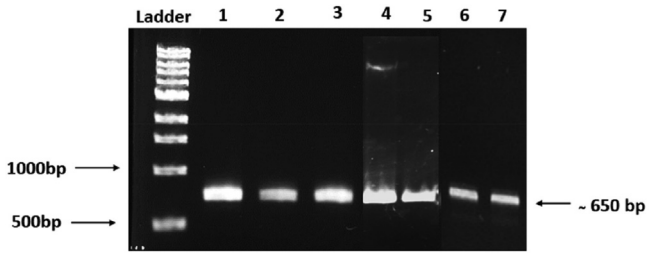

Fig. 1. Sample loaded in lane 1 was obtained from SSAIU01, lanes 2 and 3 contain samples obtained from SSWH01, SSWH02, lanes 4 and 5 contain samples obtained from SSKSU01 and SSKSU02, respectively, lane 6 from SSHYR01, and lane 7 from SSKSUQ01.

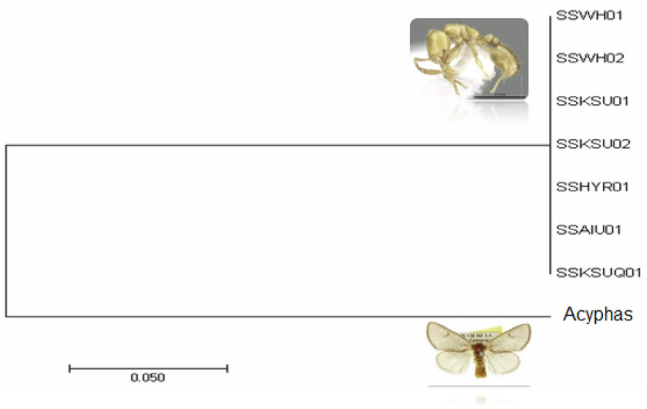

Fig. 2. Neighbor-joining phylogenetic analysis of seven *Solenopsis* specimens mitochondrial COI DNA sequences was performed using *Acyphus* as out group. ClustalW alignment program was used for the distance analysis of available sequences. The aligned sequences were used as input and a neighbor-joining phylogenetic tree was constructed by using MEGA7.0. Information about *Solynopsis* specimens collected for obtaining COI sequences is given in Table 2.

the Riyadh region. These DNA barcodes were deposited to the International Barcode of Life Database (BOLD: http://www.boldsystems.org) and the information is publicly accessible at http://www.boldsystems.org/index.php/Public_SearchTerms (accession number, KR916796–KR916802).

The barcode sequences of Saudi *Solenopsis* ant species and those of other *Solenopsis* ants from 13 countries; whose sequences are available in BOLD, were compared to understand the global locus and phylogenetic relationship of Saudi fire ants to ants from other parts of the globe. A distance analysis of 66 nucleotide sequences representing 14 countries including Saudi Arabia was performed using the ClustalW program (https://www.genome.jp/tools-bin/clustalw) that were used for the construction of neighbor-joining tree, by using MEGA7.0 software (https://www.megasoftware.net).

3. Results and discussion

DNA barcoding is an innovative technology that provides fast and accurate species identification by using COI gene, a short sequence of the mitochondrial DNA. This technique uses a universal set of primers to amplify a small fragment (~650 bp) of the COI gene for the precise identification of different organisms. However, identification through COI sequences was found to be 100% successful in class Hexapoda and 96.4% success was recorded in the kingdom Animalia (Hebert et al. 2003).

Despite an extensive field survey, only six populations of *S. saudiensis* Sharaf & Aldawood were found in the Riyadh region. Additionally, we sequenced one *S. saudiensis* queen (SSKSUQ01) from the King Saud University population that was previously described (Sharaf et al., 2014) (Table 2). Three individuals were analyzed from each population to identify any genetic diversity within the population.

Three COI sequences from each population were analyzed to identify any genetic variability within the population. However, sequence analysis did not reveal any diversity within the population. This finding indicated that the population is completely isolated and there was no blending or gene flow from any other population.

Comparison of COI sequences between populations also showed no genetic variation among six populations including a queen from *S. saudiensis* investigated from the Riyadh region (Fig. 2). This genetic resemblance of all tested populations suggests that they have adapted to the specific habitat, thereby resulted in a single and strong gene pool. In a study of fire ants DNA barcoding from Latin America, found that, gene pool of *Solenopsis quinquecuspis* Forel 1913, was greatly different from two other species including *S. richteri* and *S. invicta*, due to barriers in the gene flow whereas, greater similarities were found between later two species (Ross and Shoemaker, 2005).

The Basic Local Alignment Search Tool (BLAST) comparison of *S. saudiensis*, from Saudi Arabia, COI sequence (query) with that of *Solenopsis molesta* isolate NE68505USA (subject), confirmed that our sequence represents a member of the genus *Solenopsis* and shared 86% sequence identity (Narain et al., 2013). A comparison of *S. saudiensis,* with *Solenopsis* sp. MAS022 voucher BIOUG01261, Costa Rica, revealed 83–86% sequence similarity (Smith et al., 2014).

In the present study we generated DNA barcodes for *Solenopsis saudiensis* Sharaf & Aldawood, collected from the Riyadh region. We generated seven DNA barcodes, one for each population of *S. saudiensis* found in the Riyadh region. These DNA barcodes were deposited to International Barcode of Life Database (BOLD: http://www.boldsystems.org). These barcodes, together with specimen related information, are publicly available on the BOLD website with codes ANTSA001-15 to ANTSA007-15. We were the first to generate and deposit DNA barcodes of fire ants *Solenopsis* species from Riyadh, Saudi Arabia. Furthermore, DNA barcoding of fire ants species accomplished from USA (Shoemaker et al. 2006), Ecuador (Delsinne et al. 2012), Brazil, Peru, and Argentina (Ross and Shoemaker 2005), were in line with our findings.

A phylogenetic analysis using the neighbor joining method with Mega (Version 7.0) (Fig. 2) was performed on seven sequences of *S.*

saudiensis with a Lymantriid moth, *Acyphas pelodes* (accession number KF522526), used as an outgroup. The barcode sequences of Saudi *Solenopsis* ant species and those of other *Solenopsis* ants from 13 countries, whose sequences are available in BOLD, were compared to reveal the global locus and phylogenetic relationship of Saudi fire ants to ants from other parts of the world (Fig. 3).

Our phylogenetic analysis indicates *S. saudiensis* forms a sister cluster with *S. mameti* Donisthorpe 1946 and *S. saevissima* Smith 1855, reported from Mauritius, Comoros, and, Guiana, respectively (Fig. 3). These three species then form a sister group with *Solenopsis* ants from Honduras, Nicaragua and Costa Rica. The mitochondrial genome phylogenetic analysis of *S. saevissima* species group reveals that the ants from Neotropical region are monophyletic and they are evolved from a single origin in Neotropics (Shoemaker et al., 2006). It should not be surprising that the genus *Solenopsis* Westwood includes almost 200 described species worldwide, and many newer species are expected in the future.

Ants of the genus *Solenopsis* have broad distribution in the tropics and warm temperate regions around the globe, among them some species are terribly invasive. In this project, we explored the ant fauna of the genus *Solenopsis*, and are the first to DNA barcode this medically important insect in the Kingdom of Saudi Arabia. We hope this study provides a comprehensive and consolidated structural picture of *Solenopsis* diversity in the Riyadh region. Furthermore, our findings have provided an outline for the structural diversity of *Solenopsis* fauna from other parts of the world. Our current data not only provide a molecular means to identify and validate an ant species and its population biodiversity, but might also be beneficial for programs to quarantine and manage invasive ant species. DNA barcoding tool is not only beneficial for species systematics identification but, it is also helpful for other area of studies like, evolutionary biology, behavior, ecology, and might be helpful for the better understanding of forensic species analysis (Moreau, 2009).

In spite of extensive field survey we found no evidence of any fire ants species in the Riyadh region except *S. saudiensis*, which was only restricted in the city area (Riyadh), strongly suggesting that *S. saudien*is is inhabited in date palm orchards which are regularly irrigated. Our finding of a single gene pool probably reflects the significant natural barriers/factors that impede the dispersal of gene flow of *S. saudiensis* populations. Local restriction and mode of polygyny of *S. saudiensis*, are mainly associated with single limited

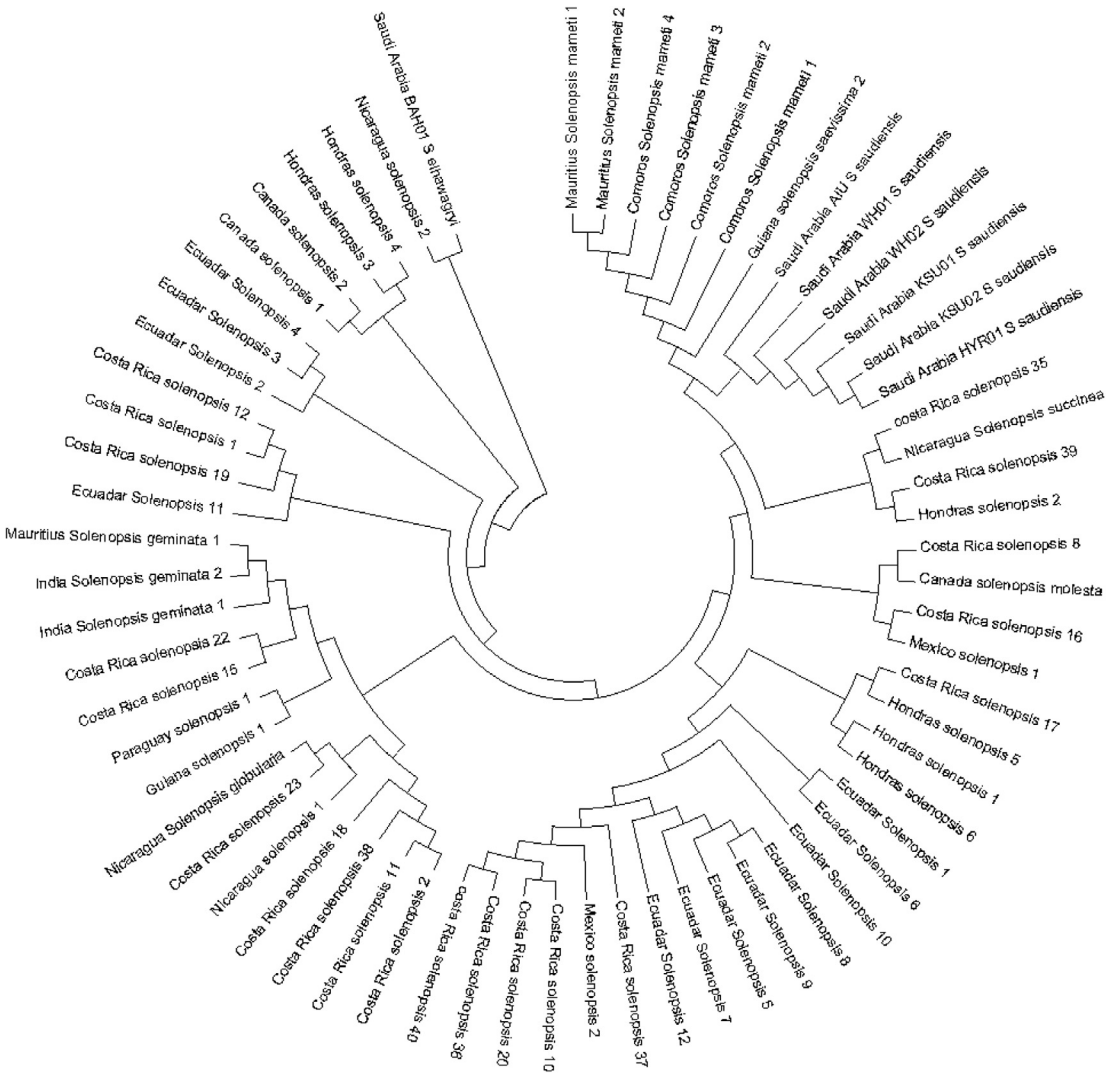

Fig. 3. Phylogenetic analysis comparing Saudi ant barcode sequences and the sequences of other ants from 13 countries. A distance analysis of 66 nucleotide sequences representing 13 countries was performed using the ClustalW program and a neighbor-joining phylogenetic tree was constructed by using MEGA7.0. Information about *Solynopsis* specimens collected for obtaining COI sequences is given in Table 2. Whereas, information about COI sequences of ants from other 13 countries is accessible at International Barcode of Life Database, http://www.boldsystems.org.

gene pool. Our results are in accordance with Ross et al. (1997), where they revealed that isolated populations of *S. invicta* showed restricted gene flow due to the presence of multiple queens and microgeographic patterns. Moreover, *S. saudiensis* had 83–86% sequence identity to other *Solenopsis* spp. from other parts of the world. Interestingly, the highest similarity (86%) was with that of *Solenopsis molesta*, the thief ant, from the USA. This study resulted in a working lab protocol and a reference library beneficial for identifying ants of the genus *Solenopsis* in Saudi Arabia.

4. Conclusions

Only one *Solenopsis* species (*Solenopsis saudiensis*) was found in the Riyadh region, indicating its strong link to regularly irrigated date palm orchards. The DNA barcodes comparison showed no genetic diversity among six populations along with that of a queen in *S. saudiensis,* analyzed from the Riyadh region. This genetic identity of all populations probably reflects their adaptation to a discrete habitat, thereby forming a single and strong gene pool.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Acknowledgements

The present research was supported by the National Plan for Science, Technology and Innovation (MAARIFAH), King Abdulaziz City for Science and Technology, Kingdom of Saudi Arabia, Award Number (11-BIO1974-02). Authors appreciate the support from Dr. Mostafa Rezk Sharaf for the morphometric identification of *Solenopsis* spp.

References

Collingwood, C., Tigarh, B.J., Agosti, D.J., 1997. Introduced ants in the United Arab Emirates. J. Arid Environ. 37, 505–512.
Collingwood, C.A., Agosti, D., 1996. Formicidae (Insecta: Hymenoptera) of Saudi Arabia (Part 2). Fauna Saudi Arabia 15, 300–385.
Delsinne, T., Sonet, G., Nagy, Z.T., Wauters, N., Jacquemin, J., Leponce, M., 2012. High species turnover of the ant genus *Solenopsis* (Hymenoptera: Formicidae) along an altitudinal gradient in the Ecuadorian Andes, indicated by a combined DNA sequencing and morphological approach. Invertebr. Syst. 26, 457–469.
Fisher, B.L., Smith, M.A., 2008. A revision of Malagasy species of *Anochetus* Mayr and *Odontomachus* Latreille (Hymenoptera: Formicidae). PLoS One 3, e1787.
Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.
Hajibabaei, M., Singer, G.A., Hebert, P.D., Hickey, D.A., 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. TIG 23, 167–172.
Hebert, P.D., Cywinska, A., Ball, S.L., Dewaard, J.R., 2003. Biological identifications through DNA barcodes. P. R. Soc. B-Biol. Sci. [Biol] 270, 313–321.
Kouakou, L.M., Yeo, K., Vanderheyden, A., Kone, M., Delsinne, T., Ouattara, K., Dekoninck, W., 2017. First morphological and molecular confirmed report of the invasive tropical fire ant, *Solenopsis geminate* (Fabricius, 1804) (Hymenoptera: Formicidae) from Cote d'Ivoire (West Africa). BioInvas. Rec. 6 (2), 173–179.
Moreau, C.S., 2009. Inferring ant evolution in the age of molecular data (Hymenoptera: Formicidae). Myrmecol. News 12, 201–210.
Narain, R.B., Kamble, S.T., Powers, T.O., Harris, T.S., 2013. DNA barcode of thief ant complex (Hymenoptera: Formicidae). J. Entomol. Sci. 48, 234–242.
Ng'endo, R.N., Osiemo, Z.B., Brandl, R., 2013. DNA barcodes for species identification in the hyperdiverse ant genus Pheidole (Formicidae: Myrmicinae). J. Insect. Sci. 13, 27.
Oberprieler, S., Andersen, A., Moritz, C., 2018. Ants in Australia's monsoonal tropics: CO1 barcoding reveals extensive unrecognised diversity. Diversity 10 (36), d10020036.
Ross, K.G., Shoemaker, D.D., 2005. Species delimitation in native South American fire ants. Mol. Ecol. 14, 3419–3438.
Ross, K.G., Krieger, M.J., Shoemaker, D.D., Vargo, E.L., Keller, L., 1997. Hierarchical analysis of genetic structure in native fire ant populations: results from three classes of molecular markers. Genetics 147, 643–655.
Rougerie, R., Decaëns, T., Deharveng, L., Porco, D., James, S.W., Chang, C.H., Richard, B., Potapov, M., Suhardjono, Y., Hebert, P.D., 2009. DNA barcodes for soil animal taxonomy. Pesq. Agropec. Bras. 44, 789–802.
Sharaf, M.R., Aldawood, A.S., 2012. Ants of the genus *Solenopsis* Westwood 1840 (Hymenoptera: Formicidae) in the Arabian Peninsula with description of a new species *Solenopsis elhawagryi*. PLoS One 7 (1–21), e101856.
Sharaf, M.R., Aldawood, S.A., 2011. First occurrence of *Solenopsis* Westwood 1840 (Hymenoptera: Formicidae), in the kingdom of Saudi Arabia, with description of a new species *S. saudiensis* n. sp. Ann. Soc. Entomol. Fr. 47, 474–479.
Sharaf, M.R., Salman, S., Aldawood, A.S., 2014. First description of the queen of the ant *Solenopsis saudiensis* Sharaf & Aldawood (Hymenoptera: Formicidae) from Saudi Arabia. Zool. Middle East. 61, 50–54.
Sharaf, M.R., Taylor, B., Klingenberg, C., 2009. Ants of the genus *Solenopsis* Westwood, 1840 (Hymenoptera: Formicidae) in Egypt with description of the worker caste of *S. cooperi* Donisthorpe, 1947. Zootaxa 2004, 49–58.
Shoemaker, D.D., Ahrens, M.E., Ross, K.G., 2006. Molecular phylogeny of fire ants of the *Solenopsis saevissima* species-group based on mtDNA sequences. Mol. Phylogenet. Evol. 38, 200–215.
Smith, M.A., Fisher, B.L., Hebert, P.D., 2005. DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: the ants of Madagascar. Philos. Trans. R. Soc. Lond. [Biol.] 360, 1825–1834.
Smith, M.A., Hallwachs, W., Janzen, D.H., 2014. Diversity and phylogenetic community structure of ants along a Costa Rican elevational gradient. Ecography 37, 720–731.
Smith, M.A., Woodley, N.E., Janzen, D.H., Hallwachs, W., Hebert, P.D., 2006. DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). PNAS 103, 3657–3662.
Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., Hebert, P.D., 2005. DNA barcoding Australia's fish species. Philos. Trans. R. Soc. Lond. [Biol.] 360, 1847–1857.