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Identification of Ants (Hymenoptera: Formicidae) in Urban Manila, Philippines Through DNA Barcoding

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A B S T R A C T
Urbanization is a driving force behind habitat destruction and has a dramatic impact on ant richness and composition. This ecological and taxonomic study of urban ants generally focused on identifying species richness in different urban habitat types using DNA barcoding techniques. 17 species of ants collected from Manila, Philippines were sequenced and barcoded for a 600 – 700 range bp region of the mitochondrial cytochrome c oxidase subunit 1 gene (CO1). The AT content in the DNA of 16 ant species was estimated at 68.5%, which is following invertebrates. The distance within species was calculated using the Kimura 2-parameter (K2P) and it was 0.244, 0.168, and 0.331% for mean, minimum, and maximum, respectively. The phylogeny tree constructed using the N-J method also revealed two clusters. The first cluster consisted of three sub-clades representing four subfamilies: Myrmicinae, Ponerinae, while Pseudomyrmicinae, and Dolichoderinae are within the same sub-clade but with different sub-group. The second cluster is formed by the sub-clades of the Formicinae subfamily, which is not in contradiction with the cladistics analysis of morphological data for ants and is consistent with the traditional phylogeny. The results demonstrate that DNA barcoding is an additional tool for providing pertinent information for the systematist for quick and reliable identification of urban ants.

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INTRODUCTION

It has been emphasized that interest in urban ecology research has been greater than before (McIntyre et al., 2000; Picket et al., 2001 Savard et al. 2000) as urban and suburban areas are increasing in number and population density globally (Pauchard et al. 2006 and UN World Urbanization Prospects 2008). The worldwide census has revealed that the year 2003 was the start when more people lived in urban areas than in rural ones (Cohn 2005). With these present inclinations, the emphasis on the role of urban ecological health and conservation has been steadily increasing (Cohn 2005; Miller and Hobbs 2002; Dunn et al., 2006). Townsend and Hildrew (1994) suggested that urbanization results in disturbance, which they defined as any event that removes biomass. Ant habitat of all kinds is modified by a myriad of natural disturbances, furthermore, many terrestrial ecosystems, especially in the tropical regions, have been altered by a series of human activities including urbanization (Philpott et al., 2010). Ants can be very sensitive to habitat transformation and disturbance, and for this reason, have been extensively used as indicator species (Hoffman and Andersen 2003). Disturbance effects of urbanization on ant communities include alteration of interspecific interactions, changes in trophic interactions with ant-plants and honeydew-producing hemipterans, modification of ant-provided ecosystem services such as seed dispersal, predation, and soil modification, changes in species composition, and most importantly, loss of diversity (Philpott et al., 2010). That is why central to this study is the identification of the remaining ant fauna in the local urbanized area using molecular DNA barcoding techniques.

DNA barcoding is a useful method for the molecular identification of organisms to overcome taxonomic impediments (Ng'endo et al., 2012) such as requiring an expert in the field (Monaghan et al., 2005) and is a cost-effective system with which non-specialists can assign unidentified specimens to known species (Schindel and Miller 2005). This technique uses PCR to amplify a fragment of the cytochrome oxidase I (COI) gene, which is then sequenced and compared to a database of known organisms (Keele et al., 2014). The modern concept of DNA barcoding as a proposed standard method for identifying species, as well as potentially allocating unknown sequences at higher taxa such as orders and phyla, could be traced from the 2003 study of Paul Hebert and colleagues who demonstrated the utility of the 648 base pair region of the COI using a primer developed by O. Folmer and co-workers from Rutgers University in 1994.

Manila, officially called the National Capital Region or NCR is subdivided into 17 local government units (LGUs) composed of 16 cities and a lone municipality, wherein each of these 17 LGUs is headed by an elected official or its mayor. Among all regions in the Philippines, NCR is the only region with no province. According to the 2015 census, NCR has an approximate population of 12, 877, 253 Filipinos distributed over a land area of 619.7 square kilometers.

Early western entomologists and myrmecologists who were able to settle or had access to ant specimens of Manila were able to record a diverse population of ant fauna. Among them was Fornicidae expert Gustav Mayr, an Austrian entomologist, and professor in Budapest and Vienna (Obit. Gustav Mayr 1908). Swiss myrmecologist, Auguste-Henri Forel was interested in both the cephalic anatomy of humans and ants, while American entomologists documented several ant genera found in Manila during their Philippine occupation. It should also be noted that most collections in the national museum were contributed by collectors. Dr. P.L. Stangl, of the U.S. army, and Father W.A. Stanton, of Manila. Another American zoologist, William Wheeler recorded numerous trifling assemblies of ants that have been received at various times during the past five years by the American Museum of Natural History (Wheeler, 1910). Emery recorded these ant genera from manila: *Diacamma*, *Odontoponera*, *Brachyponera, Odontomachus, Sima, Monomorium*, *Solenopsis, Pheidologeton, Pheidole Crematogaster, Tetramorium, Dolichoderus, Tapinoma*, *Technomyrmex, Plagiolepis, Oecophylla, Camponotus*, and *Polyrhachis*. Later in 1925, Wheeler and Chapman co-authored an article entitled: "The ants of the Philippine Islands Part I, Dorylinae and Ponerinae" which was published in the Philippine Journal of Science.

The objective of this study was to identify ants collected in the urban and disturbed area of Manila, Philippines up to the species level if possible using DNA barcoding. These ants were previously identified using traditional morphological techniques in an initial study. Results obtained by various approaches can aid in overcoming methodological issues in taxonomic identification (Mengual et al., 2006; Smith et al., 2008). Another advantage of integrating molecular and morphological approaches (Dayrat 2005; Cardoso et al., 2009) is that it promotes taxonomic stability (Padial et al., 2010).

Materials and methods

THEORETICAL REVIEW

The study was carried out in Manila or officially known as the National Capital Region of the Philippines. As the study aims to collect ant communities in an urban area, a roadmap or route map that primarily displays roads, transport links, and political boundaries rather than natural geographical information was used as a guide to determine collection sites.

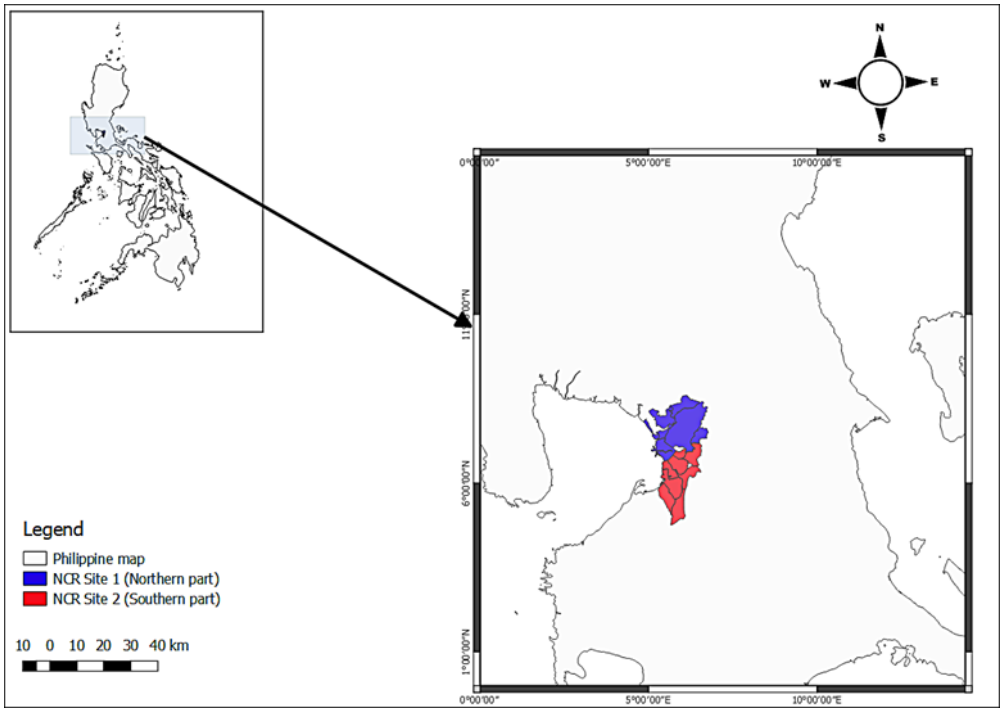

Figure 1. Map showing the study site location in National Capital Region, Luzon Philippines. (Figure generated by the senior author using QGIS 2.14.2)

Table 1. The 16 specific sampling sites, urban area, size, and GPS coordinates.

Site1	Specific sampling site	Urban area	Size	GPS Coordinates
1.1	Mac Arthur Highway, Valenzuela	Park; Median Lane	1.5 ha; 50 x 1m	14° 41' 35" N, 120° 58' 19" E
1.2	Bgy. 168 Deparo, Caloocan	Vacant Lot; Easement	400 sq. m; 15 sq. m.	14° 44' 20" N, 121° 1' 3" E
1.3	North Bay Blvd., Navotas	Median Lane	50 x 5 m	14° 41' 47" N, 121° 0' 44" E
1.4	Arroceros Park, Manila	Urban Forest Patch	2.2 ha	14° 35' 50" N, 121° 0' 4" E
1.5	Mindanao Av. Ext., Quezon City	Median Lane	50 x 5 m	14° 41' 29" N, 121° 1' 25" E
1.6	La Mesa Eco Park, Quezon City	Urban Forest Park	33 ha	14° 42' 46" N, 121° 3' 39" E
1.7	Visayas Ave., Quezon City	Vacant Lot	200 sq. m.	14° 41' 28" N, 121° 2' 24" E
1.8	Bgy. Malanday, Marikina	Vacant Lot	250 sq. m.	14° 40' 20" N, 121° 3' 24" E
Site 2	Specific sampling site	Urban area	Size	GPS Coordinates
2.1	Buendia Ave., Pasay	Median Lane	50 x 5 m	14° 36' 23" N, 120° 59' 7" E
2.2	Gil Puyat Ave., Makati	Median Lane	50 x 5 m	14° 33' 6" N, 121° 1' 48" E
2.3	Pamplona Tres, Las Pinas	Vacant Lot	185 sq. m.	14° 26' 47" N, 120° 58' 24" E
2.4	Manila Mem. Park. Paranaque	Urban Cemetery	96 ha	14° 27' 42" N, 121° 1' 28" E
2.5	Boni Ave., Mandaluyong	Median Lane	50 x 5 m	14° 34' 12" N, 121° 1' 12" E
2.6	Pinagbuhatan, Pasig	Median Lane	50 x 5 m	14° 33' 0" N, 121° 4' 12" E
2.7	Madrigal Business. Park, Muntinlupa	Business Park	27.34 ha	14° 26' 38" N, 121° 0' 36" E
2.8	Arca Circle, Western Bicutan	Open Rotunda Area	150 sq. m.	14° 30' 23" N, 121° 3' 24" E

Table 2. The Ant Specimens Collected In The Study According To Subfamily And Its Corresponding Frequency

Manila was divided into two parts to ensure each part would have representative collections during random sampling. Each collection part was further equally divided into eight for a total of 16 sub-samples. Each sub-sample had 10 collection or sampling points. A total of 160 sampling points were used to collect ants in the study. Figure 1 shows the route map used in the study while table 1 shows the 16 sub-sampling areas with the specific urban areas of collection where traffic was available for more than 12 hours a day and

their respective GPS coordinates. Permission letters to collect specimens were sent to each Local Government Unit (LGUs) before the study was initiated.

Ant sampling

Ant collections were in March – November 2015. These months represented both dry and rainy seasons. The collection of ants followed the techniques of Bestlemeyer *et al*. (2000) and Bestlemeyer and Casanova (2010). Each sampling point had a distance of 20 meters apart. Baiting for a standard time of 60 minutes for each sampling point using different food sources such as peanut butter, tuna, dead insects, and seeds to attract different ants with a variety of diets was used. To capture foraging ants during the night, pit-fall trapping was utilized, and a plastic cup four inches tall with a lid diameter of two inches was set on ground level with a diluted automotive coolant solution used as a killing and preserving agent. Ethylene glycol, a major component of automotive coolant known to be non-toxic to the environment was used to ensure that the solution will not evaporate when the temperature goes up. A drop of detergent solution was added to break water surface tension to minimize escape. Leaf litter sifting was used in areas of urban forest parks and forest patches. The siftate was then placed in a Winkler sack (Agosti and Alonso 2000) for the extraction of ants. General hand-collection of ants for a standard time of five minutes each for each sampling point was done to collect ants proximal to the sampling point.

Ant Identification Using Traditional Morphological Characteristics

Ant specimens were identified using published keys (General and Alpert 2012; Wilson 1964; Fernandez 2010; Hosoishi and Ogata 2009; Sorger and Zettel 2011; Yamane 2009; La Polla *et al*. 2010; and Shattuck, 1999). For confirmation of identification, dorsal, profile, and full-face images for individual specimens were sent to Dr. Edward Wilson of Harvard University – Museum of Comparative Zoology in Cambridge Massachusetts.

DNA Extraction, Amplification, And Sequencing

Field collections were preserved in 95% ethanol and placed in a freezer until the time for the DNA extraction process. The specimens are to genus and if possible up to species level and distributed into their respective subfamilies (Table 2). Mitochondrial DNA was isolated from the worker caste representing the 17 genera using the Qiagen DNeasy tissue extraction kit following a modified version of the manufacturer's protocols. Whole individuals were used to ensure that more tissues will be extracted for nucleic acids. All specimen samples were handled on a clean working surface and all instruments were acid or flame sterilized between each sample. Utmost care was performed when loading plates with samples to avoid cross-contamination between wells.

Polymerase chain reaction (PCR) was conducted under the following reaction volumes: 13.3 µl DNA template, 2 µl 10× PCR buffer, 1.6 µl of dNTPs in 10mM concentration, 1 µl of each primer in 10 mM concentration, 0.2 µl of Taq DNA polymerase for a total reaction of 20 µl. Reactions conditions (Moreau

2014) included: initial denaturation at 94°C for 1 minute; 40 cycles at 94°C for 30 seconds; annealing temperature of 45°C for 1 minute; elongation temperature of 72°C for 2 minutes and final extension temperature of 72°C for 3 minutes. Full-length sequences were amplified using universal DNA primer pair LCO1490–GGTCAACAAATCAAAAGATATTGG and HCO2198-TAAACTTTCAGGGTG ACCAAAAAATCA (Folmer et al. 1994).

Before proceeding to cycle sequencing, 2% agarose gel electrophoresis for 30 minutes was administered to visualize the PCR product and ensured that the DNA and target PCR product size was successfully amplified. Gel electrophoresis also permits confirmation of a single amplified PCR. Amplicons were sent to 1st Base Laboratories in Malaysia for sequencing and PCR product clean-up to remove residual primers and unincorporated nucleotides.

DATA ANALYSIS

The pairwise analysis of the available 18 sequences using the Kimura 2-parameter (K2P) method in MEGA6. The number of base substitutions per site was analyzed between all sequences. Codon positions included were 1st + 2nd + 3rd + non-coding. All positions containing gaps and missing data were eliminated from the data set.

Subfamily Myrmicinae	Frequency
Solenopsis geminata	1007
Tetramorium lanuginosum	130
Pheidole fervens	803
Monomorium floricola	493
Carebara diversa	111
Crematogaster sp.	3
Cardiocondyla sp.	1
Trichomyrmex destructor	823
Total	3371
Subfamily Formcinae	**Frequency**
Paratrechina longicornis	576
Oecophylla smaragdina	100
Camponotus sp.	3
Anoplolepis gracilipes	38
Nylanderia sp.	23
Total	740
Subfamily Dolichoderinae	**Frequency**
Dolichoderus thoracicus	23
Tapinoma melanocephalum	602
Technomyrmex sp.	4
Total	629
Subfamily Ponerinae	**Frequency**
Hypoponera sp.	3
Anochetus sp.	3
Odontomachus simillimus	2
Odontoponera denticulata	14
Brachyponera sp.	2
Total	24
Subfamily Pseudomyrmicinae	**Frequency**
Tetraponera sp.	1

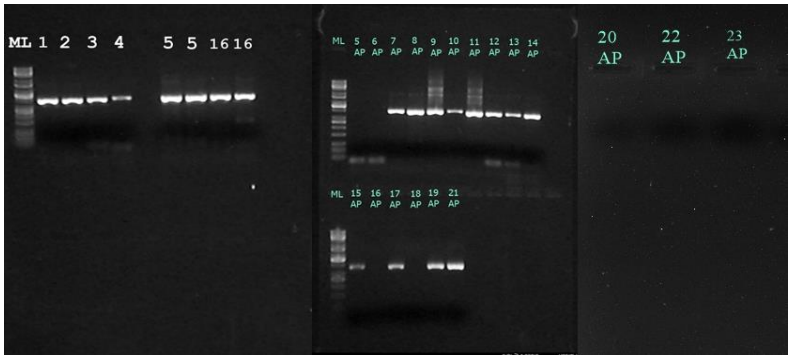

Figure 3. PCR Amplification Of CO1 Region (ML, Molecular Ladder; 1, *Solenopsis Geminata*; 2, *Paratrechina Longicornis*; 3, *Tetramorium Lanuginosum*; 4, *Pheidole Fervens*; 5, *Hypoponera* Sp.; 7, *Monomorium Floricola*; 8, *Tetraponera* Sp.; 9, *Odontomachus simillimus*; 10, *Dolichoderus thoracicus*; 12, *Tapinoma melanocephalum*; 13, *Camponotus* sp.; 14, *Oecophylla smaragdina*; 15, *Anoplolepis gracilipes*; 16, *Nylanderia* sp.; 17, *Odontoponera denticulata*; 11&19, *Carebara diversa*; 21, *Trichomyrrmex* sp.)

Sequenced products were assembled using DNA Baser application. Assembled contigs were checked and verified through National Center for Biotechnologies' (NCBI) Basic Local Alignment Search Tool (BLAST). Assembled contigs were then aligned using MEGA 6-ClustalW software together with sequences of the same genera extracted from GenBank. Sequence divergences were calculated and a Neighbor-joining (NJ) tree of distances was created to provide a graphic representation of the among-species divergences (Saitou and Nei 1987). A sequence of ant genera not included in the collection that acted as an out-group was also included in the alignment.

RESULTS AND DISCUSSION

All 17 CO1 sequences were submitted to the NCBI – GenBank under submission number 2170456. PCR products from different ant species were easily produced and aligned as no insertions, or deletions were observed. The visualized PCR product contained only discrete single bands (Fig. 2), thus indicating that the sequences obtained were mitochondrial DNA and not nuclear pseudogenes (Bensasson et al. 2001). A total of 17 ant species for identification were used in the study. The number of base substitutions per site from between sequences is shown in Figure 3. Analyses were conducted using the Kimura 2-parameter model (Kimura 1980). The rate variation among sites was modeled with a gamma distribution. The analysis involved 17 nucleotide sequences. Codon positions included were 1^{st} + 2^{nd} + 3^{rd} + Noncoding. All positions containing gaps and missing were eliminated. There were a total of 623 positions in the final data set. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013). Comparative analysis of A, T, G, and C content was found to have a relatively high AT content of 68.5% (38.3 + 30.2) compared to 31.3 CG content (18.9 + 12.7). This difference could be attributed to the AT content of the 1^{st} codon (AT_1), which ranged from 27 – 34.6% (Table 3). The

phylogeny tree constructed using the N-J method also revealed two clusters. The first cluster consisted of three sub-clades representing four subfamilies: Myrmicinae, and Ponerinae, while Pseudomyrmicinae and Dolichoderinae are within the same sub-clade but different sub-group. The second cluster is formed by the sub-clades of the Formicinae subfamily.

The sequences obtained in the present study [*Solenopsis geminata* (712 bp); *Tetramorium lanuginosum* (697 bp); *Pheidole fervens* (712 bp); *Hypoponera* sp. (715 bp); *Monomorium floricola* (697 bp); *Tetraponera* sp. (704 bp); *Odontomachus simillimus* (689 bp); *Dolichoderus thoracicus* (701 bp); *Carebara diversa* (686 bp); Tapinoma melanocephalum (711 bp); Camponotus sp. (685 bp); Oecophylla smaragdina (702 bp); Anoplolepis gracilipes (710 bp); Nylanderia sp. (674 bp); Odontoponera denticulate (710 bp); Trichomyrmex sp. (712 bp)] were compared to homologous sequences available in Genbank and it indicated that closely allied species, which grouped closely in the N-J tree, showed high bootstrap (89 – 99 range) values and thus represents phylogenetic confidence for the tree topology (Figure 4).

This study clearly shows that the availability of DNA barcoding as a system for fast and accurate species identification that enables ecological systems more accessible by using short DNA sequences instead of the whole genome for diversity assessment will greatly facilitate and complement taxonomic studies. The authors very much agree when Ebach and Holdrege (2005) said that: "DNA barcoding generates information, not knowledge". The Consortium for the Barcode of Life (CBOL) believes that this information can make systematists and consumers of taxonomic information more knowledgeable. Therein lies its potential value. The combination of DNA sequencing data with traditional taxonomy will serve as a model that can be applied across disciplines. It will further increase the rate of species identification, which will eventually help to deal with the current biodiversity crisis (Smith et al. 2005). In a situation where species identification is difficult, the potential utility of DNA barcoding is immense. The results reveal that CO1 barcoding will permit the unambiguous identification of ant species in Manila, Philippines, thus there is a need to look for an integrated approach for quick identification of insect biodiversity (Ojha et al. 2014).

Ant species	T(U)	C	A	G	Total	T-1	C-1	A-1	G-1	Pos #1	T-2	C-2	A-2	G-2	Pos #2	T-3	C-3	A-3	G-3	Pos #3
1 AP (*Solenopsis geminata*)	35.8	23.0	28.7	12.5	634.0	23	21.7	35.8	19.8	212.0	44	24.2	17.1	15.2	211.0	41	23.2	33.2	2.4	211.0
2 AP (*Paratrechina longicornis*)	38.5	18.9	30.0	12.6	634.0	25	19.8	33.5	21.7	212.0	44	24.6	18.5	13.3	211.0	47	12.3	37.9	2.8	211.0
3 AP (*Tetramorium lanuginosum*)	38.8	16.6	32.8	11.8	634.0	28	16.0	36.8	18.9	212.0	44	24.2	17.1	15.2	211.0	45	9.5	44.5	1.4	211.0
4 AP (*Pheidole fervens*)	38.6	19.1	30.2	12.0	632.0	28	17.9	34.4	19.8	212.0	44	24.3	17.1	14.8	210.0	44	15.2	39.0	1.4	210.0
5 AP (*Hypoponera sp.*)	38.8	16.2	33.0	12.0	634.0	29	15.1	35.4	20.8	212.0	44	23.2	18.0	14.7	211.0	44	10.4	45.5	.5	211.0
7 AP (*Monomorium floricola*)	39.4	17.2	31.9	11.5	634.0	27	17.9	35.4	19.3	212.0	44	25.1	16.1	14.7	211.0	47	8.5	44.1	.5	211.0
8 AP (*Tetraponera sp.*)	37.2	20.2	28.2	14.4	634.0	24	19.3	34.0	22.6	212.0	44	24.2	16.1	15.6	211.0	44	17.1	34.6	4.7	211.0
9 AP (*Odontomachus simillimus*)	43.1	12.3	32.9	11.7	633.0	35	12.3	33.0	19.8	212.0	43	24.2	19.4	13.7	211.0	52	.5	46.2	1.4	210.0
10 AP (*Dolichoderus thoracicus*)	39.0	17.8	28.7	14.5	634.0	26	18.4	33.5	22.2	212.0	44	24.2	17.5	14.7	211.0	47	10.9	35.1	6.6	211.0
12 AP (*Tapinoma melanocphalum*)	36.1	20.2	29.7	14.0	634.0	27	17.0	33.0	22.6	212.0	43	24.6	17.1	15.2	211.0	38	19.0	38.9	4.3	211.0
13 AP (*Camponotus sp.*)	39.6	19.3	30.2	10.9	632.0	27	18.9	36.3	17.9	212.0	43	25.7	16.7	14.3	210.0	49	13.3	37.6	.5	210.0
14 AP (*Oecophylla smaragdina*)	40.9	13.7	33.0	12.5	634.0	31	14.2	34.4	20.8	212.0	44	24.2	18.0	14.2	211.0	48	2.8	46.4	2.4	211.0
15 AP (*Anoplolepis gracilipes*)	39.3	19.1	28.9	12.8	634.0	26	20.3	32.5	21.2	212.0	44	25.1	18.0	13.3	211.0	48	11.8	36.0	3.8	211.0
16 AP (*Nylanderia sp.*)	37.7	19.5	29.2	13.6	631.0	26	19.0	33.6	21.3	211.0	43	25.2	18.1	13.3	210.0	44	14.3	35.7	6.2	210.0
17 AP (*Odontoponera denticulata*)	34.4	22.2	30.1	13.2	634.0	25	19.3	34.9	20.3	212.0	42	24.2	19.4	14.7	211.0	36	23.2	36.0	4.7	211.0
19 AP (*Carebara diversa*)	37.7	22.4	27.0	12.9	634.0	25	20.8	34.9	19.8	212.0	44	24.2	17.1	15.2	211.0	45	22.3	28.9	3.8	211.0
21 AP (*Trichomyrmex sp*)	35.8	23.0	28.7	12.5	634.0	23	21.7	35.8	19.8	212.0	44	24.2	17.1	15.2	211.0	41	23.2	33.2	2.4	211.0
Average	**38.3**	**18.9**	**30.2**	**12.7**	**633.5**	**27**	**18.2**	**34.6**	**20.5**	**211.9**	**43**	**24.4**	**17.6**	**14.5**	**210.8**	**45**	**14.0**	**38.4**	**2.9**	**210.8**

Table 4. Nucleotide Frequencies Of The 17 Ant Species Found In Manila, Philippines.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. 21 AP Trichomyrmex destructor																	
2. 17 AP Odontoponera denticulata	0.225																
3. 16 AP Nylanderia bourbonica	0.260	0.272															
4. 15 AP Anoplolepis gracilipes	0.254	0.266	0.189														
5. 14 AP Oecophylla smaragdina	0.228	0.250	0.217	0.220													
6. 13 AP Camponotus sp.	0.293	0.324	0.289	0.244	0.233												
7. 12 AP Tapinoma melanocephalum	0.239	0.265	0.273	0.265	0.255	0.295											
8. 11 AP Carebara sp.	0.272	0.289	0.297	0.298	0.285	0.317	0.287										
9. 10 AP Dolichoderus thoracicus	0.250	0.248	0.248	0.223	0.227	0.275	0.257	0.291									
10. 9 AP Odontomachus simillimus	0.245	0.221	0.240	0.214	0.168	0.244	0.252	0.293	0.218								
11. 8 AP Tetraponera sp.	0.260	0.280	0.302	0.264	0.291	0.300	0.281	0.326	0.252	0.288							
12. 7 AP Monomorium floricola	0.200	0.254	0.215	0.231	0.216	0.231	0.257	0.254	0.245	0.209	0.251						
13. 5 AP Hypoponera sp.	0.242	0.263	0.270	0.275	0.233	0.258	0.268	0.331	0.254	0.184	0.294	0.255					
14. 4 AP Pheidole fervens	0.236	0.268	0.228	0.248	0.223	0.232	0.263	0.264	0.251	0.247	0.274	0.200	0.234				
15. 3 AP Tetramorium lanuginosum	0.221	0.257	0.224	0.217	0.187	0.235	0.246	0.274	0.230	0.201	0.247	0.178	0.240	0.205			
16. 2 AP Paratrechina longicornis	0.236	0.261	0.207	0.194	0.183	0.240	0.253	0.303	0.201	0.224	0.309	0.245	0.235	0.226	0.197		
17. 1 AP Solenopsis geminata	0.204	0.253	0.232	0.262	0.241	0.257	0.263	0.259	0.230	0.259	0.275	0.206	0.273	0.223	0.208	0.251	

Figure 5. Estimates Of Evolutionary Divergence Between Sequences, The Numbers Between Sequences, And The Numbers Of Base Substitutions Per Site Are Shown.

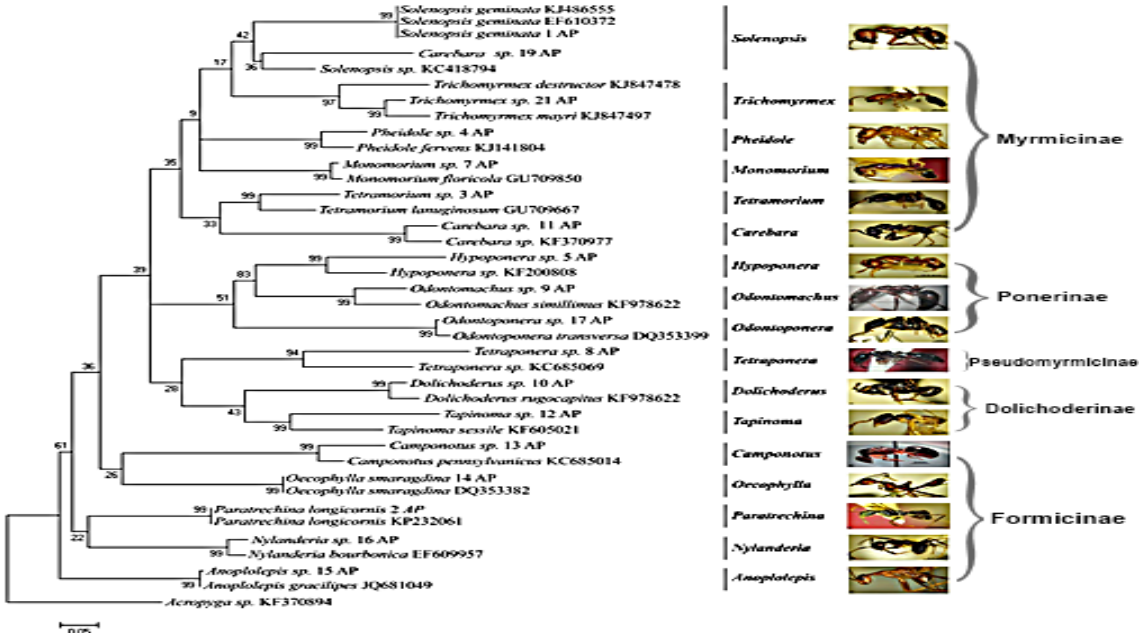

Figure 6. Cluster analysis-based dendrogram depicting genetic relationships among 17 different ant species from collections in Manilal 62 Genbank ant sequences for reference, generated by Bootstrap Test Phylogeny using N-J (Neighbor-joining) method of MEGA 6 Software

FURTHER STUDY

This research still has limitations so that further research is still needed related to the topic Identification of Ants (Hymenoptera: Formicidae) in Urban Manila, Philippines Through DNA Barcoding.

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