Malaysian Applied Biology (2022) 51(6): 35-45
https://doi.org/10.55230/mabjournal.v51i6.2239

Research Article

Morpho-molecular Characterization and Phylogenetic Relationship of *Tapinoma indicum* Forel from Metropolitan Area

Li Yang Lim and Abdul Hafiz Ab Majid*

Household & Structural Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia
*Corresponding author: abdhafiz@usm.my

ABSTRACT

Tapinoma indicum Forel is one of the most abundant nuisance pests found in Penang Island, Malaysia. However, limited research has been done on *T. indicum,* especially in the molecular genetic field. This paper aims to collect *T. indicum* from three districts in Penang Island, Malaysia including George Town, Gelugor, Balik Pulau, characterize *T. indicum* based on morphological measurements and molecular characterization of *T. indicum* using mitochondrial cytochrome c oxidase subunits 1 (CO1) sequences and 16S ribosomal DNA (16S rDNA) sequences. The morphological measurements resulting in HL = 4.31 ± 0.12 mm, HW = 3.87 ± 0.06 mm, EL = 0.89 ± 0.05 mm, EW = 0.58 ± 0.01 mm, SL = 3.56 ± 0.08 mm, ML= 1.11 ± 0.12 mm, CI = 89.83 ± 1.17, EI = 20.59 ± 0.88 and SI = 82.95 ± 2.34. The CO1 sequences and 16S rDNA sequences of *T. indicum* from each population are deposited and accessible via Genbank (NCBI) database. The phylogenetic trees result in two clades with three haplotypes, but the genetic structure is not well revealed.

Key words: *Tapinoma indicum*, household ants, morphology, phylogeny, CO1, 16S rDNA

Article History
Accepted: 13 November 2022
First version online: 29 December 2022

Cite This Article:
Lim, L.Y. & Ab Majid, A.H. 2022. Morpho-molecular characterization and phylogenetic relationship of *Tapinoma indicum* (Forel) from metropolitan area. Malaysian Applied Biology, 51(6): 35-45. https://doi.org/10.55230/mabjournal.v51i6.2239

INTRODUCTION

Tapinoma indicum Forel (Hymenoptera: Formicidae) is a widely distributed pest in Asian countries that was first described in 1895 (Forel, 1895). It is a nuisance pest that annoys and disrupts human activities due to its nest construction and foraging activities in building structures. *T. indicum* was discovered to be one of the most abundant ant species in residential areas of Penang Island, Malaysia, according to recent research (Ab Majid *et al*., 2016).

Tapinoma indicum is known as the ghost ant because of its small size and because it moves faster than other ants. However, no morphological measurements of *T. indicum* have been reported. Despite its prevalence as a pest, *T. indicum* has received little research attention. The earliest research by Chong & Lee (2006) focused on *T. indicum* foraging preferences and foraging activities. The majority of the research focuses on *T. indicum's* bait preferences (Lee, 2008; Chong & Lee, 2009; Ab Majid *et al*., 2018), whereas the most recent *T. indicum* research by Lim and Ab Majid (2019) involved the development of plant-derived pesticides.

There has been no molecular genetics research on *T. indicum* to date. In contrast, much genetic and molecular information was presented on the other *Tapinoma* genus species, such as *T. melanocephalum* (Fabricius) and *T. sessile* (Say). There are only one set of *T. indicum* mitochondrial cytochrome c oxidase subunit 1 (CO1) sequences published in the National Center for Biotechnology Information (NCBI), and the sequences were not discussed or included in the published paper (Wang *et al*., 2018). Moreover, no 16S

ribosomal DNA (16S rDNA) sequence data for *T. indicum* were reported.

Despite the high abundance of *T. indicum* in Penang Island, Malaysia, the information regarding this nuisance pest is limited. The combination of morphological measurements and genetic data may offer a clear determination of these taxa's taxonomic boundaries. This study aims to provide morphological measurements to distinguish between ant populations, variations between groups, and species with similar morphology. This study also aims to molecular characterize the *T. indicum* collected from the districts of George Town, Gelugor, and Balik Pulau on Penang Island, Malaysia by utilizing CO1 and 16S rDNA gene sequences. Additionally, this study seeks to verify the variation of the mitochondrial gene in *T. indicum* to assess the genetic diversity across the districts of George Town, Gelugor, and Balik Pulau on Penang Island, Malaysia, by utilizing CO1 and 16S rDNA sequences.

MATERIALS AND METHODS

Insect sampling

The household ant, *T. indicum,* was collected from random properties in residential areas around Penang Island from three different districts, including George Town, Gelugor, and Balik Pulau. In each of the different districts, ten mixed random residential houses were selected for the *T. indicum* sampling using the baiting method. A total of five 15-mL of centrifuge tubes were used as baiting traps with peanut butter and honey as attractants (Chong & Lee, 2006). The bottom part of the centrifuge tube was punctured to serve as an entrance, and the attractant was applied on a paper that was clipped to the cape inside the centrifuge tube. Baits were placed in different spots around the houses based on the presence and trails of *T. indicum*. The baits were left for 3 h, from 4.00 p.m. to 7.00 p.m. (Lee, 2008). The collected samples were observed based on morphology appearances for identity confirmation. *T. indicum* workers have several characteristics, including 12-segmented antennae without a distinct club, no spine on the thorax, only one segment in the petiole that is nearly hidden by the front edge of the abdomen, and no hairs around the tip of the abdomen (Mallis & Moreland, 2011). The head and thorax are dark in color, while the legs and abdomen are brown. When crushed, *T. indicum* emits a rotten coconut odor. Identified *T. indicum* from different collection sites was preserved separately in glass vials with a 95% ethanol solution at -20 °C in a dark environment.

Morphological measurements

A total of 30 *T. indicum* workers were measured using a 125x magnification dissection light microscope, one from each collection site. The morphological measurements included head length (HL): maximum length from the mid-point of the anterior clypeal margin to the mid-point of the posterior margin of the head; head width (HW): maximum width of the head excluding eyes; eye length (EL): maximum length of the eye; eye width (EW): maximum width of the eye; scape length (SL): maximum length of scape; and mesosoma length (ML): maximum length of mesosoma from the mid-point of the anterior pronotal declivity to the posterior basal angle of the metapleuron. The cephalic index (CI) was calculated by using HW/HL*100, the eye index (EI) by using EL/HL*100, and the scape index (SI) by using SL/HW*100. The morphological characteristics of each haplotype, including head length (HL), head width (HW), eye length (EL), eye width (EW), scape length (SL), mesosoma length (ML), cephalic index (CI), eye index (EI), and scape index (SI) were analyzed using the one-way ANOVA test.

DNA extraction

To maximize DNA yield, the genomic DNA of *T. indicum* was extracted by using the HiYield Plus™ Genomic DNA Mini Kit (Blood/Tissue/Cultured Cells) (Real Biotech Corp., Taipei, Taiwan) with minimal modifications, including repeating the elution step twice with 50 µL elution. In addition, to minimize DNA extraction interference caused by microbes living in the abdomen, an individual worker from each collection site was isolated after removing the abdomen part. The remaining tissues were vortexed in a lysis buffer containing Proteinase K and incubated for 1 hr at 60 °C. Following the ethanol wash that bound the DNA to the filter column, elution was carried out twice with 50 µL elution buffers. A total of 100 µL DNA sample was collected and validated using the NanoDrop 2000c (Thermo Fisher Scientific, Massachusetts, USA).

DNA amplification

The extracted genomic DNA of each individual was used for 16S ribosomal DNA and mitochondrial cytochrome c oxidase subunits 1 (CO1) gene amplification by PCR method by using primer pairs LR-N-13398 (5′-CGCCTGTTTATCAAAAACAT-3′) with LR-J-12887(5′-CCGGTTTGAACTCAGATCA-3′) (Simon *et al*., 1994) and LCO1490 (5′-GGTCAACAAACATAAAGATATTGG-3′) with HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer *et al*., 1994), respectively. The PCR thermocycler profile was set for 10 min at 94 °C for the initial denaturation stage, followed by 40 cycles of 30 s denaturation at 94 °C, 30 s annealing at 48 °C, and 1 min extension at 72 °C (Simon *et al*., 1994;

Folmer *et al*., 1994). The PCR was completed with a final extension phase of 10 min at 72 °C and a hold at 4 °C. The PCR result was validated through gel electrophoresis using 1% agarose gel and sent to Apical Scientific Sdn. Bhd. for outsourcing sequencings.

Molecular characterization

The CO1 and 16S rDNA gene sequences obtained were subjected to a quality check and multiple alignments using Molecular Evolutionary Genetics Analysis v10 (MEGA-X) (Kumar *et al*., 2018). All nucleotide sequences were deposited to Genbank and acquired accession numbers.

Phylogenetic relationship

The gene sequences were further analyzed by MEGA-X software to construct a phylogenetic tree with the best substitution model based on CO1 and 16S rDNA gene sequences, respectively. The Maximum Likelihood method was used to infer the phylogenetic tree based on CO1 gene sequences, and the Hasegawa-Kishino-Yano model with a discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.5356) (Hasegawa *et al*., 1985). On the other hand, the Maximum Likelihood method and Tamura 3-parameter model were used to infer a phylogenetic tree based on 16S rDNA gene sequences (Tamura, 1992). Both analyses used 1000 bootstrap replicates, and the results were drawn to scale, with branch lengths measured in the number of substitutions per site. All positions with gaps and missing data were eliminated (complete deletion option). Outgroups for phylogenetic trees based on the CO1 gene sequences and 16S rDNA gene sequences used *Tapinoma sessile* sequence with accession number FJ161757.1 and *Tapinoma melanocephalum* sequence with accession number MN397938.1, respectively.

Haplotypes and genetic diversity

Polymorphism, haplotypes, and genetic differentiation F_{ST} values of CO1 and 16S rDNA gene sequences were determined by using DnaSP v6 (Rozas *et al*., 2017).

RESULTS

Sample collection

There were a total of 30 sample collection sites and only *T.indicum* workers were collected. A total of 30 *T. indicum* workers were used for morphological measurements while another 30 *T. indicum* workers were used for molecular characterization. Table 1 displays the latitude and longitude, while Figure 1 displays the location via a map image.

Morphological measurements

The morphology of 30 *T. indicum* workers was observed and measured, as shown in Table 2 and Figure 2. The mean ± standard error of each measurement is as follows: head length = 4.31 ± 0.12 mm, head width = 3.87 ± 0.06 mm, eye length = 0.89 ± 0.05 mm, eye width = 0.58 ± 0.01 mm, scape length = 3.56 ± 0.08 mm, mesosoma length = 1.11 ± 0.12 mm, cephalic index = 89.83 ± 1.17, eye index = 20.59 ± 0.88, and scape index = 82.95 ± 2.34. As shown in Table 3, there was no significant difference in all morphological characteristics including head length (HL), head width (HW), eye length (EL), eye width (EW), scape length (SL), mesosoma length (ML), cephalic index (CI), eye index (EI) and scape index (SI) between the three haplotypes.

Molecular characterization

The CO1 and 16S rDNA gene sequences from each sample have been deposited into the Genbank NCBI database. CO1 has accession numbers ranging from MT522033 to MT522062. 16S rDNA gene sequences have accession numbers ranging from MT539965 to MT 539994. The accession numbers for each of the sequences are indicated in Table 1.

Haplotype and phylogenetic relationship

CO1 gene sequences contain 511 monomorphic and 96 polymorphic sites, with 91 two variants parsimony informative sites and 5 three variants parsimony informative sites. There were three haplotypes found by using DnaSP v6 (Rozas *et al*., 2017), which are U05b and U06b (hp 2), S02b and R10b (hp 3), and the remaining 26 CO1 gene sequences (hp 1). The CO1 gene sequence phylogeny analysis involved 32 nucleotide sequences with 312 base pairs in the final dataset. As shown in Figure 3, the single monophyletic group included three haplotypes that were identified as rooted in the outgroup sequence. Three branches were observed within the monophyletic group which discrete three different haplotypes. The pairwise distance (*p*-distance) between each branch is listed in Table 4 with an overall mean pairwise distance of 0.0355 ± 0.0034.

The 16S rDNA gene sequences contain 481 monomorphic sites and 36 polymorphic sites, with 32 two variants parsimony informative sites and 4 three variants parsimony informative sites. There were three haplotypes found by using DnaSP v6 (Rozas *et al*., 2017), which are U05a and U06a (hp 2), S02a and R10a (hp 3), and the remaining 26 16S rDNA gene sequences (hp 1). The 16S rDNA gene sequences phylogeny analysis involved 31 nucleotide sequences with 511 base pairs in the final dataset. As shown in Figure 4, a single

Table 1. The latitude and longitude of sample collection sites, accession number of CO1 and 16S rDNA sequences, and the haplotypes of each *T.indicum* population.

Collection site	Area	Population	Latitude	Longitude	Accession number CO1	Accession number 16S rDNA	Haplotype
1	Balik Pulau	R01	N 5°22'22.60318"	E 100°13'05.87651"	MT522043	MT539975	Hp1
2	Balik Pulau	R02	N 5°21'07.20000"	E 100°14'18.84000"	MT522044	MT539976	Hp1
3	Balik Pulau	R03	N 5°21'02.09530"	E 100°13'51.56244"	MT522045	MT539977	Hp1
4	Balik Pulau	R04	N 5°20'42.22365"	E 100°13'47.66868"	MT522046	MT539978	Hp1
5	Balik Pulau	R05	N 5°20'33.76815"	E 100°13'41.02418"	MT522047	MT539979	Hp1
6	Balik Pulau	R06	N 5°20'02.22198"	E 100°12'59.38530"	MT522048	MT539980	Hp1
7	Balik Pulau	R07	N 5°20'40.06652"	E 100°13'26.74182"	MT522049	MT539981	Hp1
8	Balik Pulau	R08	N 5°20'05.70951"	E 100°13'39.84012"	MT522050	MT539982	Hp1
9	Balik Pulau	R09	N 5°21'20.06870"	E 100°13'47.52746"	MT522051	MT539983	Hp1
10	Balik Pulau	R10	N 5°22'02.93262"	E 100°12'56.44506"	MT522052	MT539984	Hp3
11	Gelugor	S01	N 5°21'00.64874"	E 100°18'26.67501"	MT522053	MT539985	Hp1
12	Gelugor	S02	N 5°20'59.20185"	E 100°18'02.37219"	MT522054	MT539986	Hp3
13	Gelugor	S03	N 5°20'58.00182"	E 100°17'50.05200"	MT522055	MT539987	Hp1
14	Gelugor	S04	N 5°21'07.84953"	E 100°18'26.11859"	MT522056	MT539988	Hp1
15	Gelugor	S05	N 5°20'41.96047"	E 100°17'41.08443"	MT522057	MT539989	Hp1
16	Gelugor	S06	N 5°21'03.22046"	E 100°18'18.45780"	MT522058	MT539990	Hp1
17	Gelugor	S07	N 5°21'08.38670"	E 100°18'10.54475"	MT522059	MT539991	Hp1
18	Gelugor	S08	N 5°20'57.89796"	E 100°17'39.39706"	MT522060	MT539992	Hp1
19	Gelugor	S09	N 5°21'20.28791"	E 100°17'32.90826"	MT522061	MT539993	Hp1
20	Gelugor	S10	N 5°21'10.27102"	E 100°17'58.28169"	MT522062	MT539994	Hp1
21	George Town	U01	N 5°25'04.52400"	E 100°19'34.66200"	MT522033	MT539965	Hp1
22	George Town	U02	N 5°25'08.11800"	E 100°19'37.49400"	MT522034	MT539966	Hp1
23	George Town	U03	N 5°25'43.45200"	E 100°19'05.87400"	MT522035	MT539967	Hp1
24	George Town	U04	N 5°25'42.61200"	E 100°19'02.55600"	MT522036	MT539968	Hp1
25	George Town	U05	N 5°24'36.31004"	E 100°19'11.42311"	MT522037	MT539969	Hp2
26	George Town	U06	N 5°24'41.33400"	E 100°19'06.75600"	MT522038	MT539970	Hp2
27	George Town	U07	N 5°26'23.01000"	E 100°18'18.76200"	MT522039	MT539971	Hp1
28	George Town	U08	N 5°26'05.97600"	E 100°17'45.96000"	MT522040	MT539972	Hp1
29	George Town	U09	N 5°24'13.35000"	E 100°20'01.58400"	MT522041	MT539973	Hp1
30	George Town	U10	N 5°25'52.78200"	E 100°18'38.76000"	MT522042	MT539974	Hp1

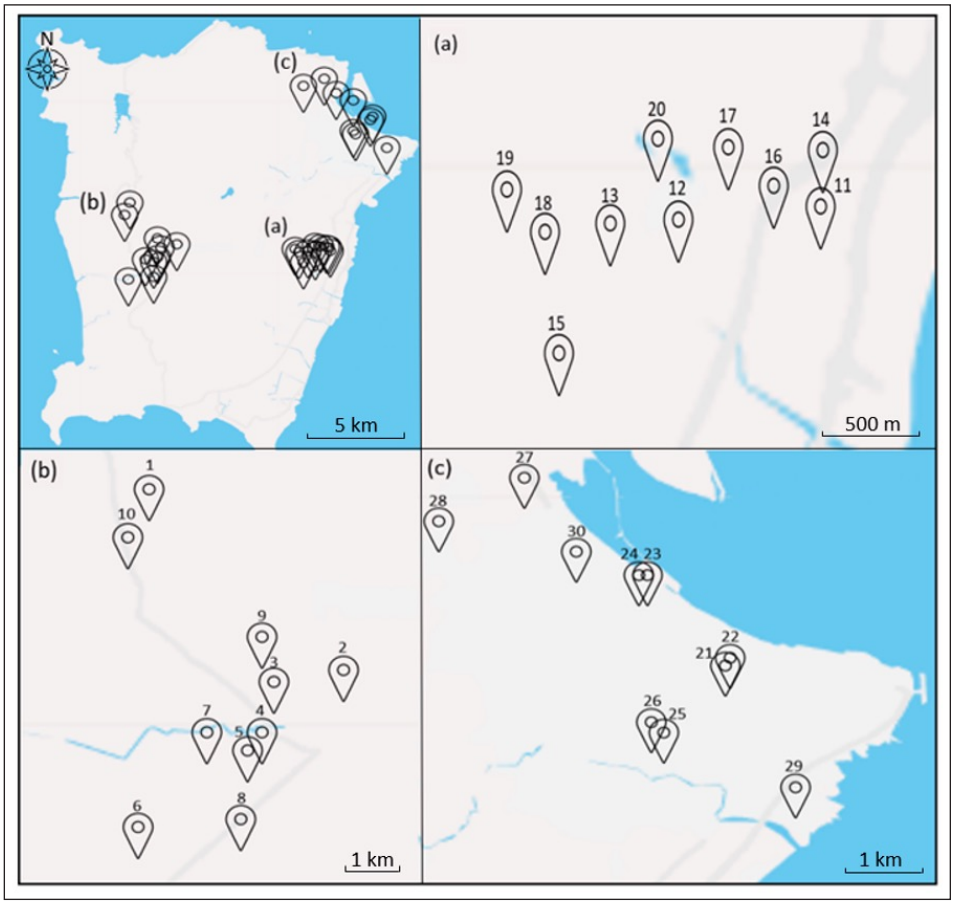

Fig. 1. Sample collection sites on the Penang Island, Malaysia map; (a) Gelugor, (b) Balik Pulau, and (c) George Town. The numerical legends refer to Table 1.

monophyletic group including three haplotypes was observed which was rooted in the outgroup sequence. The pairwise distance (*p*-distance) between each branch is listed in Table 4 with an overall mean pairwise distance of 0.0099 ± 0.0017.

Genetic diversity

The genetic differentiation F_{ST} values between George Town, Gelugor, and Balik Pulau populations based on the CO1 gene are less than zero, indicating no genetic differences between them, as shown in Table 5.

Based on the 16S rDNA gene, both the genetic differentiations F_{ST} values between Balik Pulau and George Town areas and between Gelugor and George Town populations are 0.0828. The genetic differentiation F_{ST} value between Balik Pulau and Gelugor populations is less than zero, as shown in Table 5. There is no genetic difference found between Balik Pulau and Gelugor populations because the genetic differentiation F_{ST} values based on 16S rDNA are less than zero. However, the F_{ST} value 0.0828 indicates that there is low genetic differentiation between George Town and Gelugor populations.

DISCUSSION

Morphological measurement

The morphology appearance of *T. indicum* had been characterized in this study. The head size of *T. indicum* workers was small-sized (mean HL=436.1 µm, HW=386.8 µm) compared to another common pest such as *Pheidole* genus minor workers (mean HL=550 µm, HW=510 µm) (Holley et *al*., 2016). In contrast to other genera notably *Sericomyrmex* (mean CI=101-108) (Ješovnik & Schultz, 2017) and *Tetramorium* (mean CI=108.5) (Schlick-Steiner et *al*., 2006), the heads of *T. indicum* (mean CI=89.2) were slightly longer and rectangular. The *T. indicum* workers were extremely small-sized (mean ML=439 µm) compared to other small-sized ants such as *Sericomyrmex* ant's genus (Mean ML=1710-1040 µm). No unique traits were found on the EI (mean=20.3) and SI (mean=83.7) of *T. indicum* which exhibit similarity to those of other ants such as *Monomorium clavicorne* Andre (EI=17-21, SI=79-86) (Sharaf *et al*., 2018). Furthermore, the morphological measurements of *T. indicum* workers pose similar results to others *Tapinoma* spp., including, *T. melanocephalum* (Guerrero. 2018), *Tapinoma atriceps, and T. atriceps breviscapum* (Escárraga et *al*., 2021), and *T. sessile* (Hamm, 2010).

Table 2. The morphological characteristics of each *T. indicum population,* including head length (HL), head width (HW), eye length (EL), eye width (EW), scape length (SL), mesosoma length (ML), cephalic index (CI), eye index (EI), and scape index (SI).

Population	HL (µm)	HW (µm)	EL (µm)	EW (µm)	SL (µm)	ML (µm)	CI	EI	SI
R01	445.3	392.9	109.2	60.3	364.4	428.4	88.2	24.5	81.8
R02	484.1	417.6	109.2	60.3	392.5	477.4	86.3	22.6	81.1
R03	467.3	403.2	98.3	59.6	334.1	468.7	86.3	21.0	71.5
R04	385.2	358.5	71.2	51.1	346.8	415.9	93.1	18.5	90.0
R05	379.1	366.6	89.9	53.3	337.6	367.2	96.7	23.7	89.1
R06	421.6	381.3	71.7	52.3	336.9	451.2	90.4	17.0	79.9
R07	429.6	393.7	90.2	61.9	393.6	477.0	91.6	21.0	91.6
R08	420.6	373.1	77.1	63.1	365.9	439.7	88.7	18.3	87.0
R09	449.9	391.9	83.8	58.7	335.3	473.1	87.1	18.6	74.5
R10	453.2	407.5	88.7	53.3	370.0	435.7	89.9	19.6	81.6
S01	487.6	404.7	82.5	51.4	396.3	373.7	83.0	16.9	81.3
S02	465.2	395.3	96.9	55.4	382.3	463.5	85.0	20.8	82.2
S03	429.8	377.6	88.3	62.3	320.7	482.7	87.9	20.5	74.6
S04	497.8	404.8	81.1	51.4	353.9	507.8	81.3	16.3	71.1
S05	434.3	373.6	102.4	56.6	391.4	494.2	86.0	23.6	90.1
S06	463.9	388.8	85.6	56.8	326.7	457.2	83.8	18.5	70.4
S07	364.5	359.5	107.8	54.9	368.4	441.1	98.6	29.6	101.1
S08	451.1	378.7	71.7	60.2	369.1	420.2	84.0	15.9	81.8
S09	354.7	424.6	97.7	47.1	388.9	453.6	119.7	27.5	109.6
S10	439.6	389.1	92.7	62.9	405.6	473.5	88.5	21.1	92.3
U01	467.9	372.0	102.5	50.5	337.5	399.4	79.5	21.9	72.1
U02	417.4	364.2	7.2	57.3	341.4	404.5	87.3	1.7	81.8
U03	406.4	410.8	74.9	66.1	370.6	400.9	101.1	18.4	91.2
U04	420.8	359.2	78.3	54.2	364.4	421.6	85.4	18.6	86.6
U05	443.9	386.8	78.3	57.3	351.0	469.8	87.1	17.6	79.1
U06	402.1	397.1	94.1	54.2	373.4	413.2	98.7	23.4	92.9
U07	482.8	387.0	102.5	52.6	349.2	362.2	80.2	21.2	72.3
U08	444.8	386.4	110.9	56.9	348.6	427.7	86.9	24.9	78.4
U09	451.6	367.8	89.3	56.0	339.7	454.1	81.4	19.8	75.2
U10	419.8	388.8	102.9	60.7	414.2	416.1	92.6	24.5	98.7
Mean	436.1	386.8	87.9	56.6	362.3	439.0	89.2	20.3	83.7
SD	35.6	17.6	19.4	4.5	25.0	37.4	7.9	4.8	9.6
SE	6.5	3.2	3.5	0.8	4.6	6.8	1.4	0.9	1.8

Table 3. Morphological characteristic (Mean ± SE) analysis by each haplotype using a one-way ANOVA test.

Characteristic	Hp1 (μm)	Hp2 (μm)	Hp3 (μm)
HL	$435.3 \pm 7.3_{a}$	$423.0 \pm 20.9_{a}$	$459.2 \pm 6.0_{a}$
HW	$385.2 \pm 3.6_{b}$	$391.9 \pm 5.2_{b}$	$401.4 \pm 6.1_{b}$
EL	$87.7 \pm 4.1_{c}$	$86.2 \pm 7.9_{c}$	$92.8 \pm 4.1_{c}$
EW	$56.9 \pm 0.9_{d}$	$55.8 \pm 1.6_{d}$	$54.3 \pm 1.0_{d}$
SL	$361.3 \pm 5.2_{e}$	$362.2 \pm 11.2_{e}$	$376.2 \pm 6.1_{e}$
ML	$438.0 \pm 7.7_{f}$	$441.5 \pm 28.3_{f}$	$449.6 \pm 13.9_{f}$
CI	$89.1 \pm 1.6_{g}$	$92.9 \pm 5.8_{g}$	$87.4 \pm 2.5_{g}$
EI	$20.2 \pm 1.0_{h}$	$20.5 \pm 2.9_{h}$	$20.2 \pm 0.6_{h}$
SI	$83.7 \pm 2.0_{i}$	$86.0 \pm 6.9_{i}$	$81.9 \pm 0.3_{i}$

α. No significant difference was found between the three haplotypes [F (2, 27) = 0.546, p = 0.585]
β. No significant difference was found between the three haplotypes [F (2, 27) = 0.868, p = 0.431]
χ. No significant difference was found between the three haplotypes [F (2, 27) = 0.113, p = 0.893]
δ. No significant difference was found between the three haplotypes [F (2, 27) = 0.317, p = 0.731]
ε. No significant difference was found between the three haplotypes [F (2, 27) = 0.315, p = 0.732]
φ. No significant difference was found between the three haplotypes [F (2, 27) = 0.088, p = 0.916]
γ. No significant difference was found between the three haplotypes [F (2, 27) = 0.262, p = 0.772]
η. No significant difference was found between the three haplotypes [F (2, 27) = 0.020, p = 0.980]
ι. No significant difference was found between the three haplotypes [F (2, 27) = 0.084, p = 0.920]

Table 4. Pairwise distance between each haplotype based on respective phylogeny tree.

Haplotype 1	Haplotype 2	CO1	16S rDNA
Hp1	Hp2	0.2313	0.0655
Hp2	Hp3	0.0235	0.0744
Hp3	Hp1	0.2286	0.0213

Table 5. Genetic differentiations F_{ST} values between each area.

Population 1	Population 2	CO1	16S rDNA
Balik Pulau	Gelugor	-0.1111	-0.1111
Gelugor	George Town	-0.0515	0.0828
George Town	Balik Pulau	-0.0515	0.0828

Haplotypes analysis

Haplotype analysis on 30 CO1 gene sequences resulted in three haplotypes: Hp1, Hp2, and Hp3. Out of 607 monomorphic and polymorphic sites, in between Hp2 and Hp1, 90 parsimony informative sites (14.83%) were found, while 89 parsimony informative sites (informative sites) were found in between Hp3 and Hp1. In addition, 18 parsimony informative sites (0.03%) were found between Hp2 and Hp3. On the other hand, haplotype analysis on 30 16S rDNA sequences showed similar results as CO1 gene sequences. Out of 517 monomorphic and polymorphic sites, Hp2 has 29 parsimony informative sites with Hp1 (0.06%), while Hp3 has 11 parsimony informative sites with Hp1 (0.02%). There were 16 parsimony informative sites detected (0.03%) between Hp2 and Hp3. In both haplotype analyses, no singleton variable site was found. These results indicated that the 16S rDNA gene has less genetic variation than the CO1 gene, which is widely used in DNA barcoding because of its highly conserved feature. There have been studies that suggest using the 16S rDNA gene for barcoding for other animals such as amphibians (Zheng *et al*., 2014) and ticks (Lv *et al*., 2014). Hence, the 16S rDNA gene can be used as an alternative conservative marker to the CO1 gene in the molecular identification of *T. indicum*.

Phylogenetic analysis

The identity of *T. indicum* is confirmed by phylogenetic analysis based on the CO1 gene sequence, as Hp1 sort with the reference sequence in a single branch. At the same time, all the samples are separated from the same genus but different species outgroups. Although Hp2 and Hp3 were in other branches than the reference sequence, the low overall mean pairwise distance of 0.0355 ± 0.0034 reveals the limited genetic divergence between each haplotype. There is no unique characteristic was observed for each haplotype. This is further supported by the results of a one-way ANOVA test conducted on the three haplotypes based on their morphological characteristics. There was no significant difference in morphological characteristics between the three haplotypes. The research on *Tetraponera rufonigra* (Jerdon) ants in Penang Island revealed a similar phylogenetic tree topology with three clades based on CO1 gene sequences, while the sample collection covering the eastern part of Penang

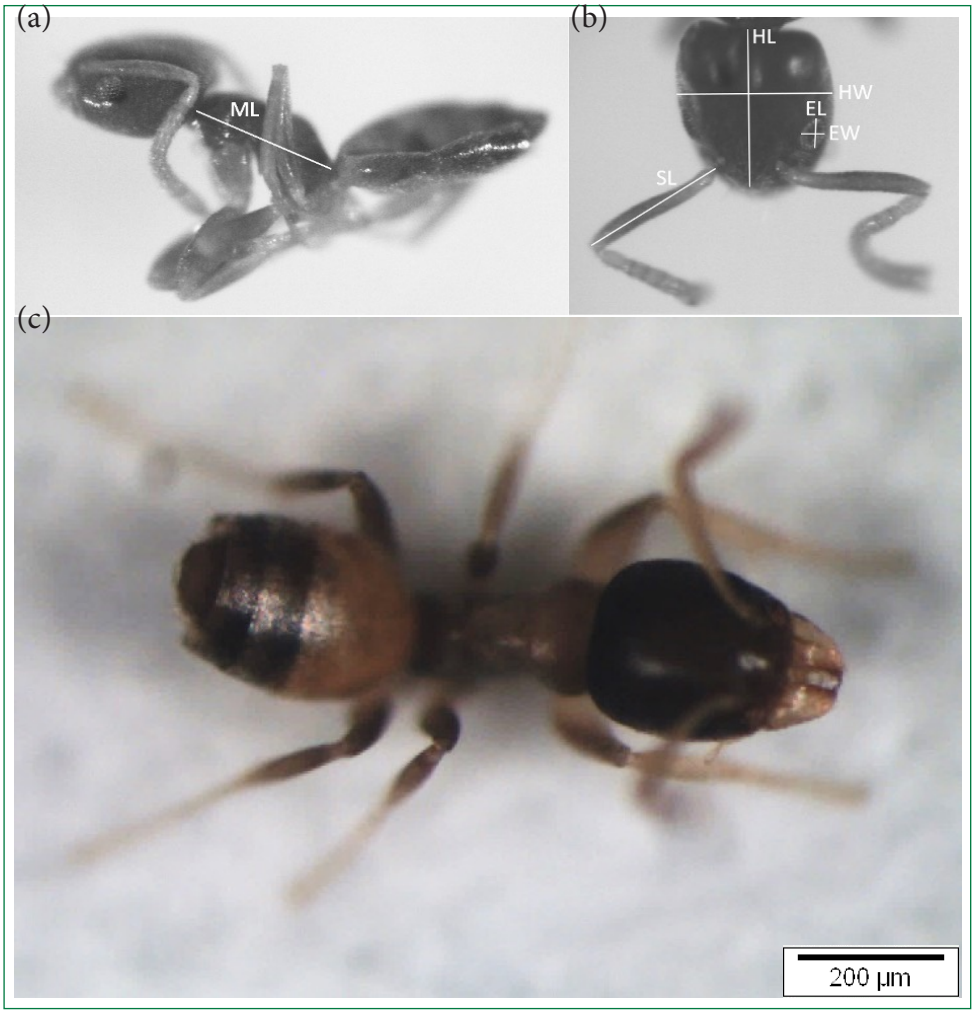

Fig. 2. (a) *Tapinoma indicum* lateral, (b) frontal, and (c) dorsal views. The morphological measurements are labeled as follows: mesosoma length (ML), head length (HL), head width (HW), eye length (EL), eye width (EW), and scape length (SL).

Island includes urban and suburban areas (Sabtu & Ab Majid, 2017). The interpretation of *T. indicum* CO1 phylogenetic analysis is further supported by the similar topology in phylogenetic analysis constructed using 16S rDNA gene sequences (overall mean pairwise distance = 0.0099 ± 0.0017). Hence, the CO1 and 16S rDNA gene sequences of *T. indicum* have been characterized.

Genetic diversity

Genetic differentiation F_{ST} values are widely used to determine the genetic population structure. The F_{ST} value ranges from 0 to 1, with 0 indicating no differentiation and 1 indicating high genetic variances within populations (Meirmans & Hedrick, 2010). Both genetic distances analyses based on CO1 and 16S rDNA gene indicate minimal or no genetic diversity between Balik Pulau, Gelugor, and George Town populations. This may be due to the sample collection sites were not significantly distanced and the conservative feature of CO1 and 16S rDNA gene which causes the genetic distance differentiation Fst value does not offer sufficient polymorphism to demonstrate the genetic diversity. In line with another research on chitons, *Acanthochiton rubrolineatus* Lischke used CO1 and 16S rDNA combined gene sequences, the F_{ST} values are limited when the populations are geographically close to each other, either within northern or southern China, but resulting in significant Fst value when comparing populations between northern and southern China (Xu *et al*., 2020). A similar result by Blekhman *et al*. (2020) on Asian ladybeetle, *Harmonia axyridis* (Pallas) emphasized the F_{ST} value for different levels of population association, showing that the highest F_{ST} value when the group of populations is distinguished into Western and Eastern population groups but the lowest F_{ST} value within both Western and Eastern population groups. Moreover, Ye *et al*. (2018) using the CO1 gene sequence for shell-boring species, *Polydora brevipalpa* Zach's genetic population analysis reported a low F_{ST} value and no genetic structure due to the limited geographic

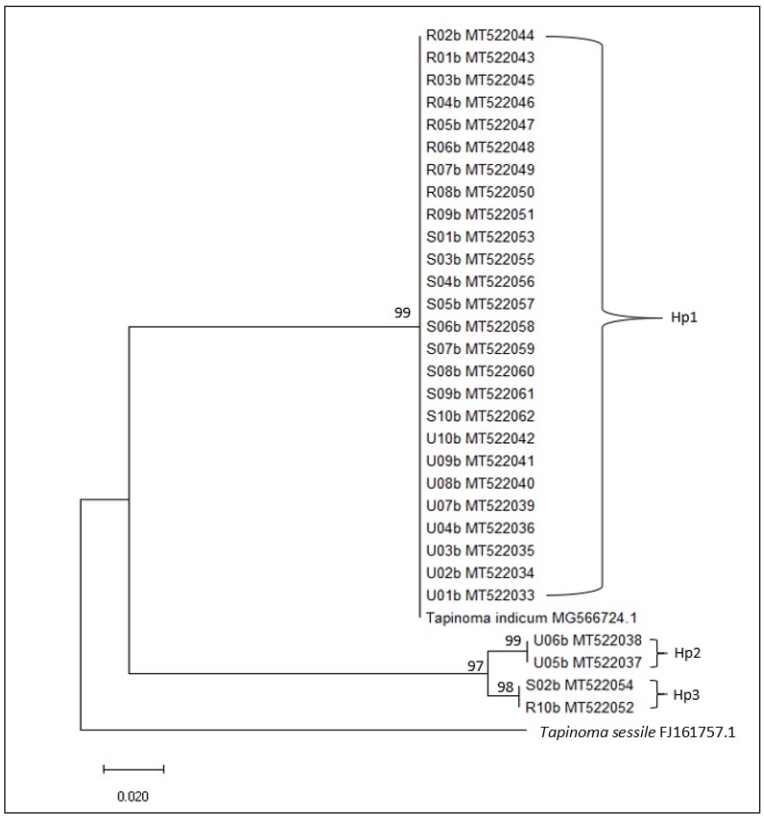

Fig. 3. Maximum likelihood tree inferred from sequences of the CO1 for 30 *T. indicum* individuals collected from Penang Island with outgroup *T. sessile*. Abbreviations are listed in Table 1. Numbers at nodes indicate bootstrap values (%) obtained by 1000 replications.

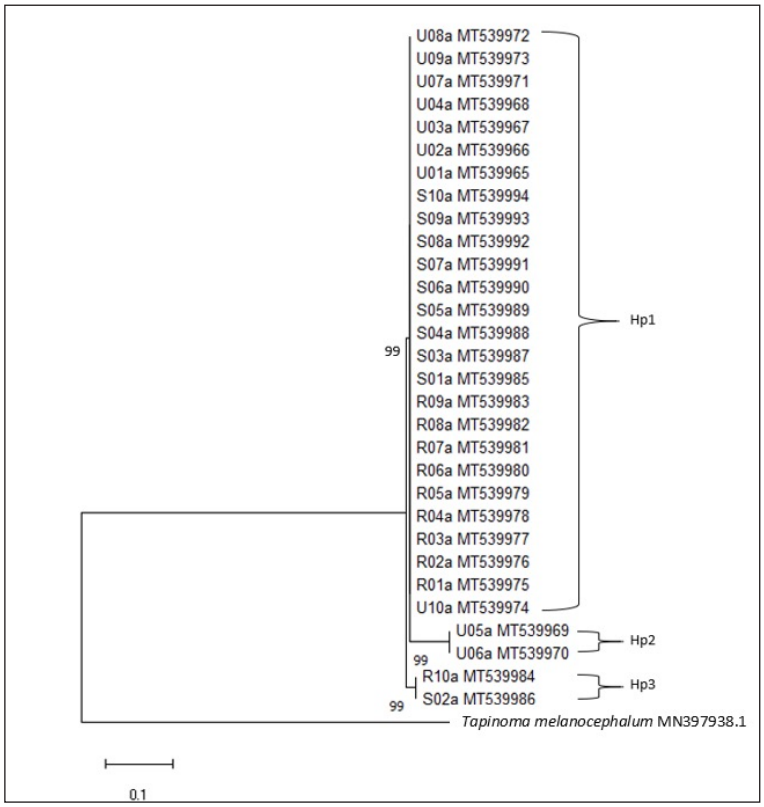

Fig. 4. Maximum likelihood tree inferred from sequences of the 16S rDNA for 30 *T. indicum* individuals collected from Penang Island with outgroup *T. melanocephalum*. Abbreviations are listed in Table 1. Numbers at nodes indicate bootstrap values (%) obtained by 1000 replications.

range of sample populations. This indicates that CO1 and 16S rDNA gene sequences do not adequately reveal the population structure when the sample collection sites are not sufficiently separated geographically. This is because the CO1 and 16S rDNA genes are highly conserved genes and do not provide enough genetic population analysis resolution for the closely related population (Ismail *et al*. 2016). In this case, a higher polymorphic molecular marker is required to determine the genetic diversity of *T. indicum* from the Penang Island areas of George Town, Gelugor, and Balik Pulau.

CONCLUSION

In this study, the morphology and molecular makeup of *T. indicum* workers were characterized. The *T. indicum* workers were extremely small-sized. The heads had a rectangular shape and were slightly longer. The CO1 and 16S rDNA phylogenetic tree found three haplotypes. However, the pairwise distance between each haplotype is minimal. Furthermore, no unique traits can be found in between each haplotype. Besides, the genetic diversity of *T. indicum* in Penang Island, Malaysia could not be identified by using CO1 and 16S rDNA gene sequences. Further study in the genetic population of *T. indicum* may employ other highly polymorphic markers, such as microsatellite markers.

ACKNOWLEDGEMENT

This research was funded by Universiti Sains Malaysia, Research University Grant RUI (Grant No: 1001/PBIOLOGI/8011104).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Ab Majid, A.H., Dieng, H., Ellias, S.S. & Ahmad, H. 2016. Tropical household ants species composition and distribution in rapid urbanization area in Penang, Malaysia. Journal of Entomology and Zoology Studies, 4(1): 496-500.

Ab Majid, A.H., Dieng, H., Ellias, S.S., Sabtu, F.S. & Satho, T. 2018. Olfactory behavior and response of household ants (Hymenoptera) to different types of coffee odor: A coffee-based bait development prospect. Journal of Asia-Pacific Entomology, 21(1): 46-51. https://doi.org/10.1016/j.aspen.2017.11.005

Blekhman, A., Goryacheva, I., Schepetov, D. & Zakharov, I. 2020. Variability of the mitochondrial CO1 gene in native and invasive populations of *Harmonia axyridis* Pall. comparative analysis. PLoS ONE, 15(4): e0231009. https://doi.org/10.1371/journal.pone.0231009

Chong, K.F. & Lee, C.Y. 2006. Food preferences and foraging activity of field populations of a pest ant, *Tapinoma indicum* (Hymenoptera: Formicidae). Sociobiology, 48(3): 875-926.

Chong, K.F. & Lee, C.Y. 2009. Evaluation of liquid baits against field populations of the long-legged ant (Hymenoptera: Formicidae). Journal of Economic Entomology, 102(4): 1586-1590. https://doi.org/10.1603/029.102.0424

Escárraga, M.E., Lattke, J.E., Pie, M.R. & Guerrero, R. J. 2021. Morphological and genetic evidence supports the separation of two *Tapinoma* ants (Formicidae, Dolichoderinae) from the Atlantic Forest biome. ZooKeys, 1033: 35. https://doi.org/10.3897/zookeys.1033.59880

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. Molecular Marine Biology and Biotechnology, 3(5): 294-299.

Forel, A. 1895. Les formicides de l'empire des indes et de ceylan. Part V. Journal of The Bombay Natural History Society, 9: 453-472.

Guerrero, R.J. 2018. Taxonomic identity of the ghost ant, *Tapinoma melanocephalum* (Fabricius, 1793) (Formicidae: Dolichoderinae). Zootaxa, 4410(3): 497-510. https://doi.org/10.11646/Zootaxa.4410.3.4

Hamm, C.A. 2010. Multivariate discrimination and description of a new species of *Tapinoma* from the western United States. Annals of the Entomological Society of America, 103(1): 20-29. https://doi.org/10.1093/aesa/103.1.20

Hasegawa, M., Kishino, H. & Yano, T. 1985. Dating the human-ape split by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution, 22: 160-174. https://doi.org/10.1007/BF02101694

Holley, J.A.C., Moreau, C.S., Laird, J.G. & Suarez, A.V. 2016. Subcaste-specific evolution of head size in the ant genus *Pheidole*. Biological Journal of the Linnean Society, 118(3): 472-485. https://doi.org/10.1111/bij.12769

Ješovnik, A. & Schultz, T.R. 2017. Revision of the fungus-farming ant genus *Sericomyrmex* Mayr (Hymenoptera, Formicidae, Myrmicinae). ZooKeys, 670: 1-109. https://doi.org/10.3897/zookeys.670.11839

Ismail, N.Z., Arsad, H., Samian, M.R., Ab Majid, A.H. & Hamdan, M.R. 2016. Evaluation of genetic

diversity of *Clinacanthus nutans* (Acanthaceaea) using RAPD, ISSR and RAMP markers. Physiology and Molecular Biology of Plants, 22(4): 523-534. https://doi.org/10.1007/s12298-016-0391-x

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, 35: 1547-1549. https://doi.org/10.1093/molbev/msy096

Lee, C. 2008. Sucrose bait base preference of selected urban pest ants (Hymenoptera: Formicidae), in Proceedings of The Sixth International Conference on Urban Pests. Veszpraém, Hungary, OOK-Press Press, pp. 59-63.

Lim, L. & Ab Majid, A.H. 2019. Plant derived pesticides (*Citrus hystrix* DC, *Mentha* x *piperita* L., *Ocimum basilicum* L.) in controlling household ants (*Tapinoma indicum* (F.), *Pheidole megacephala* (F.), *Monomorium pharaonis* (L.) (Hymenoptera: Formicidae). Pertanika Journal of Tropical Agricultural Science, 42: 1321-1342.

Lv, J., Wu, S., Zhang, Y., Chen, Y., Feng, C., Yuan, X., Jia, G., Deng, J., Wang, C., Wang, Q., Mei, L. & Lin, X. 2014. Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the *Ixodida* (Acari: Ixodida). Parasites & Vectors, 7(1): 93. https://doi.org/10.1186/1756-3305-7-93

Mallis, A. & Moreland, D. 2011. Handbook of Pest Control. 10th Ed. Mallis Handboook Company, Ohio. 448-449 pp.

Meirmans, P. & Hedrick, P. 2010. Assessing population structure: FST and related measures. Molecular Ecology Resources, 11(1): 5-18. https://doi.org/10.1111/j.1755-0998.2010.02927.x

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., & Sánchez-Gracia, A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large datasets. Molecular Biology and Evolution, 34: 3299-3302. https://doi.org/10.1093/molbev/msx248

Sabtu, F. & Ab Majid, A.H. 2017. Genetic variation and population structure of the arboreal bicolored ant Tetraponera rufonigra Jerdon from selected urban locations in eastern Penang Island, Malaysia. Journal of Asia-Pacific Entomology, 20(4): 1350-1357. https://doi.org/10.1016/j.aspen.2017.10.003

Schlick-Steiner, B.C., Steiner, F.M. & Zettel, H. 2006. *Tetramorium pacificum* MAYR, 1870, *T. scabrum* MAYR, 1879 sp. rev., *T. manobo* (CALILUNG, 2000) (Hymenoptera: Formicidae)-three good species. Myrmecologische Nachrichten, 8: 181-191.

Sharaf, M., Al Dhafer, H.M., Aldawood, A. S. & Garcia, F.H. 2018. Ants of the *Monomorium monomorium* species-group (Hymenoptera: Formicidae) in the Arabian Peninsula with description of a new species from southwestern Saudi Arabia. PeerJ, 6: e4277. https://doi.org/10.7717/peerj.4277

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of The Entomological Society of America, 87(6): 651-701. https://doi.org/10.1093/aesa/87.6.651

Tamura, K. 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. Molecular Biology and Evolution, 9: 678-687.

Wang, W., Srivathsan, A., Foo, M., Yamane, S. & Meier, R. 2018. Sorting specimen-rich invertebrate samples with cost-effective NGS barcodes: Validating a reverse workflow for specimen processing. Molecular Ecology Resources, 18(3): 490-501. https://doi.org/10.1111/1755-0998.12751

Xu, H., Chu, Z., Zhang, J., Jing, M. & Huang, L. 2020. Genetic diversity and population structure of *Acanthochiton rubrolineatus* (Polyplacophora) based on mitochondrial and nuclear gene markers. Diversity, 12(4): 159. https://doi.org/10.3390/d12040159

Ye, L., Yao, T., Wu, L., Lu, J. & Wang, J. 2018. Morphological and molecular diagnoses of *Polydora brevipalpa* Zachs, 1933 (Annelida: Spionidae) from the shellfish along the coast of China. Journal of Oceanology and Limnology, 37(2): 713-723. https://doi.org/10.1007/s00343-019-7381-0

Zheng, L., He, J., Lin, Y., Cao, W. & Zhang, W. 2014. 16S rRNA is a better choice than COI for DNA barcoding hydrozoans in the coastal waters of China. Acta Oceanologica Sinica, 33(4): 55-76. https://doi.org/10.1007/s13131-014-0415-8