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# Genetic analysis and molecular phylogeny of leafhopper *Batracomorphus signatus* (Hemiptera: Cicadellidae) from Egypt

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**Abstract**

Classic identification of leafhoppers is based only on the morphology of male genitalia. However, genetic analysis and molecular phylogeny are effective tools to identify different leafhopper species in any stage of their life cycle and to study the evolution of any species to estimate its phylogenetic relationships at different taxonomic levels. The mitochondrial cytochrome oxidase I gene (mtCOI) region has been the source of DNA sequence data frequently used to infer evolutionary relationships among insects at various taxonomic levels. The current work explores the molecular evolution of the leafhopper *Batracomorphus signatus* Lindberg (Hemiptera: Cicadellidae) and its applicability in reconstructing phylogenetic connections within and among the leafhopper species by using the COX gene and 28SrDNA (NCBI accession No. LC775122.1 and LC670604.1, respectively.

## Introduction

Cicadellidae is one of the largest families of the order Hemiptera. It comprises plant-feeding insects known as leafhoppers. This family was classified into 25 subfamilies (Krishnankutty *et al.,* 2016). The subfamily Iassinae Walker, 1870 includes about 2000 species classified into 184 genera and 12 tribes. This subfamily is one of the largest subfamilies of the Cicadellidae and is distributed all around the world (Dai *et al*., 2015; Krishnankutty *et al*., 2016; Domahovski *et al*., 2020 and Dietrich *et al*. 2020). Cicadellidae species are phytophagous, or specifically, sap-feeders. They suck plant sap with their piercing-sucking mouthparts, particularly from xylem. Cicadellid species can directly harm plants through feeding and oviposition, or indirectly by spreading harmful plant infections like bacteria and viruses (Carter, 1973; Harris, 1979; Larivière *et al.,* 2010 and Albre and Gibernau, 2019). The morphological diagnoses of the Iassinae subfamily and each of the included tribes were revised by Sindhu *et al.* (2016).

The leafhopper *Batracomorphus signatus* Lindberg is a member of the Iassinii tribe (Hemiptera: Cicadellidae: Iassinae) that specializes in feeding on grass. This species is widely distributed across various geographic regions except the New World. The identification of all stages of these insects has been achieved using molecular techniques, specifically DNA barcoding. This approach allows non-experts to

objectively identify species, even in cases where specimens are small or damaged, complementing traditional identification methods. By combining DNA information with morphological features, the accuracy and reliability of leafhopper identification can be improved. Additionally, sequencing and annotating the entire mitochondrial genome of *B. signatus* has provided valuable insights into the properties of mitochondria and the evolutionary history of this species. The partial coding sequence of the COI gene has proven to be a powerful tool for accurately identifying organisms (Hebert and Gregory, 2005).

The current work explores the molecular evolution of the leafhopper *Batracomorphus signatus* Lindberg (Hemiptera: Cicadellidae) and its applicability in reconstructing phylogenetic connections within and among the leafhopper species by using the COX gene and 28SrDNA (NCBI accession No. LC775122.1 and LC670604.1, respectively.

## Materials and methods

### 1. Collection and identification of samples:

Between 2018 and 2022, *Batrachomorphus* specimens were collected from several Egyptian Governorates and conserved in 70% ethanol in preparation for additional research. Collected adult specimens have been identified morphologically. Morphological terminology follows Dietrich (2005). Each specimen abdomen was separated, and the genitalia structure was examined to confirm the identification. The specimens that had been collected were kept at -20°C until the DNA was extracted. Photos were taken using an Olympus Stereomicroscope with Olympus camera EP 50 (5 MP).

### 2. Molecular studies:

#### 2.1. DNA Extraction:

Five insects were crushed using a pestle and mill while submerged in liquid nitrogen. DNA was extracted using the Gene JET Genomic DNA Purification Kit (Thermo Scientific, USA). Using a pestle and mortar and liquid nitrogen, each specimen was ground into a fine powder before being placed in a 1.5 ml microcentrifuge tube. Then, 1 ml of extraction buffer (100 mM Tris-Cl, 50 mM EDTA, 50 mM NaCl, 20% SDS) was added to the powdered sample and thoroughly mixed. The homogenate was then kept in a microcentrifuge tube in a hot water bath at 65°C for 30 minutes. After that, the tubes were taken out of the water bath and the supernatant was transferred to a clean microcentrifuge tube. Next, equal amounts of isoamyl alcohol (25:24:1), phenol, and chloroform were added, and everything was thoroughly combined. After that, the samples were centrifuged at 10,000 rpm for 15 minutes. After carefully pouring the supernatant into microcentrifuge tube, 100% cooled ethanol was added in double the volume, and the tube was chilled at 4°C for a full night. The DNA was pelletized for 10 minutes at 10,000 rpm using centrifugation. The DNA pellet was then cleaned using cold 70% ethanol. The recovered DNA pellet was allowed to air dry before being dissolved in 50 µl of 1× TE buffer that contained 1 mM EDTA and 10 mM Tris (pH 8).

#### 2.2. Amplification and Sequencing of 28srDNA and COX gene:

Each DNA sample was treated with 5 µl of RNase and incubated at 37°C for 30 min to purify the DNA. For many specimens, the 28srDNA gene was amplified using the primers 28SF1 and 28R (Table 1). The DreamTaq kit (Thermo Scientific, USA) was used to produce the PCR. Using a Big Dye v3.1 Terminator kit and the following PCR protocol, sequences were fractionated on an ABI 3730XL (Life Technologies,

Carlsbad, CA, US): 95°C for 3 min, 35 cycles of 95°C for 0.5 min, 60°C for 30 s, 72°C for 1 min, and a final extension step of 72°C for 10 min (Applied Biosystems, Foster City, CA, US). The mitochondrial cytochrome oxidase (COX) gene was amplified using HCO (5'TTTTCTACHAAYCATAAAGAT ATTGC3') and LCO (5TATAAACYTCDGGATGNCCAA AAAA3), covering a length of about 550 kb. 50 µl reaction volumes were used for the PCRs, which contained 25 µl of the Type-DreamTaq kit (Thermo-Scientific, USA), 0.5 µl of each primer, and 4 µl of DNA template. The reaction was thermo cycled for three minutes at 95°C, 35 cycles at 95°C for one minute, 55°C for thirty seconds, and 72°C for one minute, resulting in a final extension step of 72°C for ten minutes. Using 2% agarose gel electrophoresis, the PCR products were quantitatively evaluated following amplification.

**2.3. Sequence analysis and Phylogenetic analysis:**

Using an ABI 3730XL automatic DNA sequencer (Macrogen, Korea), the 28srDNAamplicon was purified using a gel extraction kit (Thermo Fisher, USA) and then immediately sequenced in both directions using the Big Dye Ò Terminator method. Bio Edit software was used to edit and trim chromatograms. After being edited, sequences were submitted to GenBank and aligned using Clustal X of Clustal W packages (Thompson *et al.*, 1994). In the tree-based study, neighbor-joining (NJ) trees were built in MEGA11 for the pairwise genetic distance (PWG) method. To evaluate the accuracy of the inferred phylograms, 1000 heuristic replicates were used to assess the bootstrap support (Tamura *et al.,* 2021).

**Table (1): Specific primer of 28S-rDNA and COX markers used in this study.**

| Gene | Primer | Orientation | Primer sequence (5=3) | References |
|---|---|---|---|---|
| 28S | 28S | F | 5´-AGAGAGAGTTCAAGAGTACGTG-3´ | Hancock *et al.*, 1988 and Campbell *et al.*, |
| | | R | 5´-TTGGTCCGTGTTTCAAGACGGG-3´ | |
| COX | HCO | F | 5'- | Linares *et al.*, 1991 |
| | LCO | R | 5'- | |

**Results and discussion**

**1. Taxonomy:**

*Batracomorphus* Lewis

*Batracomorphus* Lewis, 1834: 51.

Type species: *Batracomorphus irroratus* Lewis, 1834: 52.

**Diagnosis:**

**Coloration:** Yellow to pale green, rarely with brown markings.

**Head**: Head slightly equal to or wider than pronotum. Vertex short, transversely striate with uniform length, rarely slightly longer medially. Anterior margin broadly rounded. Face short, wide, with broad maxillary plates, lateral margins sinuate. Lora widely separated from margin. Frontoclypeus broad, approximately circular in outline, Clypellus distinct, short, broad, sides parallel. Antennae near ventral margin of eyes. Ocelli distinct, near anterior margin of face, Antennal ledges prominent. Antennal pits deep.

**Thorax:** Pronotum longer than vertex, rarely parallel-sided, with lateral margins long, strongly carinate; posterior margin shallowly concave; transversely striate. Scutellum long. Forewings long, exceeding abdomen, with wide appendix.

***Batracomorphus* (*Batracomorphus*) *signatus* Lindberg, 1923**

*Batracomorphus signata* Lindberg, 1923:69

*Batracomorphus glaber* Haupt, 1927:15

*Batracomorphus flavovirens* Lindberg, 1948:134

*Batracomorphus signatus* Metcalf, 1966:132

**Remarks.**

This species was recorded in Egypt by Linnavuori, 1964; Metcalf, 1966; Nast, J., 1972; Herakly, 1970 and 1980 and El-Hady *et al.*, 2020.

Distribution: *Batracomorphus* occurs in all geographical regions, except the New World.

Ethiopian region, Cyprus, Egypt, Syria, Israel, Libya (Metcalf Cat.) In Egypt, Assiut, some, 30. -31. VII. 1961; Cairo, some, IX. 1962; Dakhla, many, 20. - 21. IX. 1962; Fayoum, some, 18. IX. 1962; Kharga, some, 19. 22. IX. 1962. on Acacia spp. Eremian (Linnavuori, 1964). Alexandria and Qena (El-Hady *et al*., 2020)

**Diagnosis:**

**Measurement:** Body length, male 4.0–4.5 mm (Figure 1A), and female 4.3–5.2 mm. Crown length 0.3-0.5 mm, width 1.3mm. Pronotum length 0.6-0.9 mm, width 1-1.2 mm. Scutellum length 0.27-0.30 mm, width 0.8-1.0 mm (Figure 1E). Forewing length 3.54-3.60 mm.

**Structure**: Body yellow to light green (Figure 1A). Pronotum transversely striate; scutellum with two clear light-yellow triangles; forewing with dark green venation (Figure 1A).

**Male genitalia** (Figure 1F): Pygofer with short spine-like setae scattered posteriorly; valve small, triangular and fused to pygofer; subgenital plate wide, triangular with rounded apex, with long hair-like setae. Style elongate, with a small dorsal hook; lateral lobe well-developed. Connective Y-shaped with anterior arms fused and articulated to aedeagus. Aedeagus simple, stout, curved dorsally. Anal tube long and membranous. Gonopore is usually present.

**Material examined:**

Meadi, 8.XI.1914 (1); Minia, 5. X.1925; Heliopolis (1), 17.IV.1930; Kafr Hakim (2); Abu Rawash, 6.XII. 1932 (2); Kerdasa 11. XII. 1932 (1); Kerdasa 11. I. 1933 (1), [The Reference Egyptian Museum of Insects, Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC)].

**Collection:** Dishna, 30.I.2018 (18); Dishna, 30.II.2018 (52); Dishna, 30.III.2018 (45); Dishna, 30.IV.2018 (112); Dishna, 30.V.2018 (88);, 30.VI.2018 (305); Dishna, 30.VII.2018 (94); Dishna, 30.VIII.2018 (103); Al Qlamina, 14. V11.2018 (15); Al Qlamina,15.VIII.2018(25); Al Qlamina,15.VIII.2018(80); Al Qlamina,15.VIII.2018(65); Al Qlamina,15.VIII.2018(58); Qus, 30.VI.2018 (11); Qus, 30.VII.2018 (9); Qus, 1.IX.2018 (25), Borg el Arab, 15.XI.2018 (16); Borg el Arab, 15.V.2018 (19); Borg el Arab, 15.V.2018 (10); Saft, 5.IV.2019 (7); Saft, 6.II.2020 (3); Saft, 12.VI.2022 (2). Voucher specimens were deposited in The Reference Egyptian Museum of Insects, PPRI, and ARC.

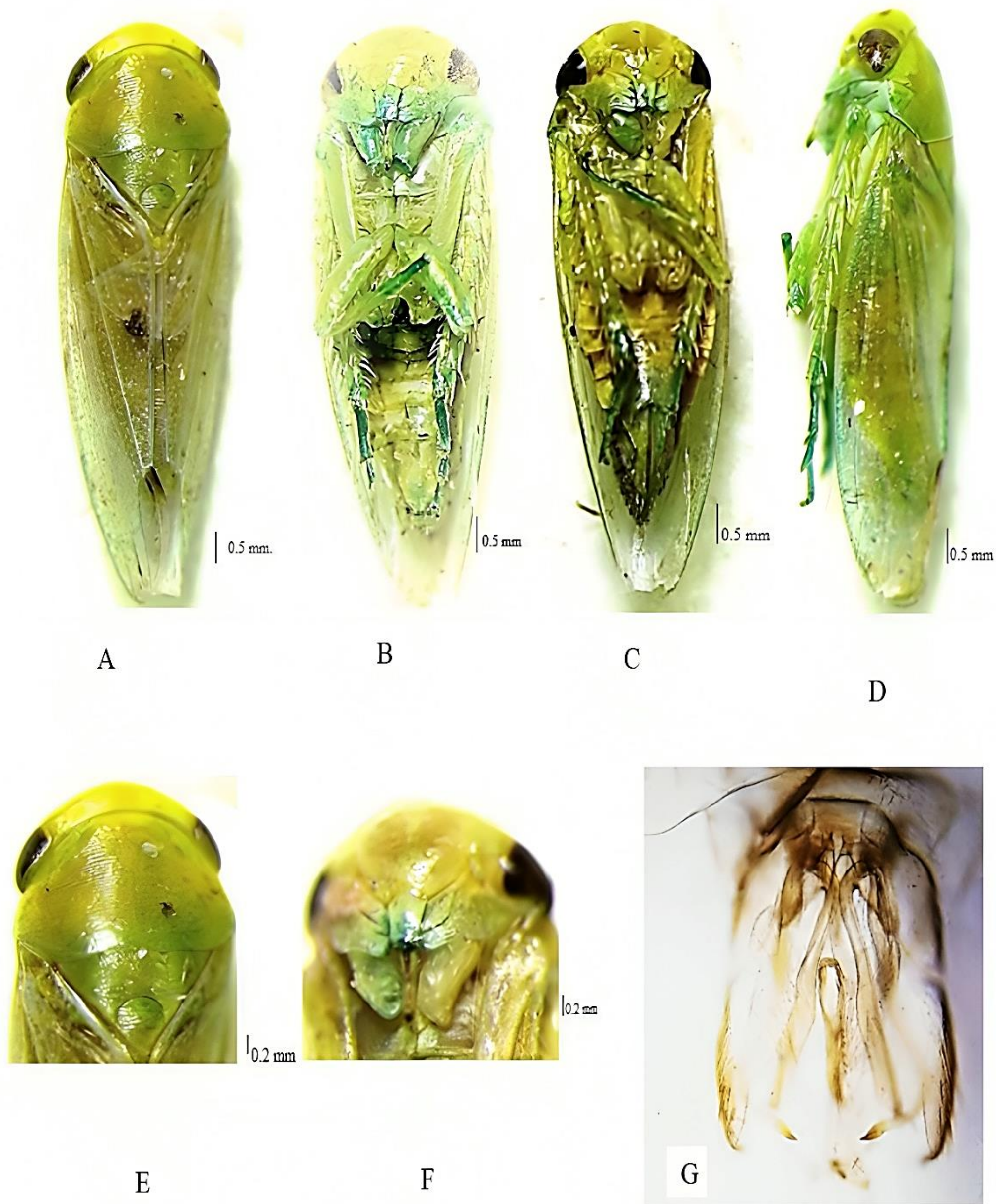


**Figure 1**: *Batracomorphus signatus*. A-D Habitus of adult, A. dorsal view; B. male ventral view; C. female venteral view, D. lateral view, E. Pronotum, scutlum, E. Face, G. Male genitalia (pygofer, subgenital plate, valve, styles and connective, aedeagus).

**2. Molecular studies:**

28SrDNA gene and mitochondrial Cytochrome Oxidase I region of the sample were successfully amplified using PCR. The sequences are summarized in Figures (1 and 2). The obtained sequences revealed that *B. signatus* species (EGY-ARC-1S) significantly had alignment with *B. chlorophana* (Accession number KX268280.1) with maximum identities of 99.70 %. *B. signatus* species (EGY-ARC-1C) significantly had alignment with *B. angustatus* (Accession number KM408151.1) with maximum identities of 100 %. Tables (2 and 3). These data were subsequently submitted to the (NCBI) with the corresponding accession numbers for both28SrDNA and COX genes, LC670604.1 and LC775122.1, respectively.

**EGY-ARC-1S**

```
  1 CCCTAAGCAG GTGGTAAACT CCATCTAAGG CTAAATATGA CCACGAGACC GATAGAAAAC
 61 AAGTACCGTG AGGGAAAGTT GAAAAGAACT TTGAAGAGAG AGTTCAAGAG TACGTGAAAC
121 CGTTCAGGGG TAAACGGAAA AGACTTTAAA CACCGAAAGG GGAGATTCAC GCTCTCTCGC
181 ATGAGTCGGC TCCCACGAGG TCAGATGGCA CTGTTCGCCC GCTCGGTGCA AGCCGCTGCG
241 GGGTCGTTTC GGTGATCACT CGGCGCTCGT GGGGGTTATG CCGGCCGCGG TGGGCCGCAC
301 TTCTCCCTCA GTAGGACGTC GGGACCCGTT GGACGACGGT CGACGGCCCC GGTGGGAGCC
361 CGTGTCGTGG GGAGGCTTGC CAAACCACGT CCGGACCCTG GGAGTCCTGG CCGATCGTCA
421 GACGGTATGA AATGCAGGTG CTGGACCGCC CCTTATTGGG CGTCCGGGCC GGTCGCAAGC
481 TCGTGCGTGC GCTCGGGATG ACGGACCTTA TGGCCCGGCT CCTGGCCCGT GCAGCTGTTG
541 GCGGCCGGTC CTCGGACGGG TCATGTAACA CCGGTCAGCG ATGTCAGTTT AAGGTACTTA
601 TCCGACCCGT CTTGAAACAC GGACCAAGGA GTCTAACATG TGCGCGAGTC ATTTGGGTAG
661 AAAACCTA
```

**Figure (1): The 28S rDNA subunit gene sequence of *Batracomorphus signatus* EGY-ARC-1S.**

**EGY-ARC-1C**

```
  1 ATAATATTAA GAATAATTAT CCGAATAGAA CTATCATCCT CAGGAATATT AATTAATAAT
 61 AGACAAACTT ATAATGTGAT TGTTACAGCA CATGCATTTA TTATAATTTT CTTTATAGTT
121 ATACCAATTA TAATTGGGGG GTTTGGGAAT TGACTTATTC CGATAATAAT TGGTGCTCCA
181 GATATAGCTT ATCCTCGAAT AAATAATATA AGATTCTGAT TATTACCACC ATCATTAACA
241 ATAATAATTA GAAGAAGTAT TACAGAAATA GGATCAGGAA CAGGATGAAC AGTTTACCCA
301 CCCCTTTCTA TAAATTCAGC TCATTCAGGA CCAAGAGTAG ATATATCAAT CTTCTCACTT
361 CATTTGGCTG GAATTTCATC AATTTTAGGA GCAATTAATT TTATTTCAAC AATTATAAAT
421 ATACGAAGAA TAGAAATAAA AATTGAACAA ATACAATTAT TTGTTTGATC AGTTCTAATT
481 ACAGCATTCC TTCTAATTTT ATCATTGCCG GTTTTAGCTG GCGCTATTAC AATATTACTT
541 ACTGATCGTA ATTTAAATAC ATCATTTTTT GATCCATCAG GTGGTGGGGA CCCAATTCTA
601 TATCAACATT TATTATAA
```

**Figure (2): The mitochondrial COX1 gene sequence of *Batracomorphus signatus* EGY-ARC-1S.**

**Table (2): Similarity percentage of 28S rRNA gene for *Batracomorphus signatus* generated by BLAST tools.**

| No | Isolate code | Significant Alignments | E value | Per. Ident | Retrieved Accession | Strains | Submitted accession no. |
|---|---|---|---|---|---|---|---|
| 1 | EGY-ARC-1S | *Batracomorphus signatus EGY-ARC-1S* | 0.0 | 100.00% | LC670604.1 | *Batracomorphus signatus EGY-ARC-1S* | LC670604.1 |
| | | *Batracomorphus chlorophana B16* | 0.0 | 99.70% | KX268280.1 | | |
| | | *Batracomorphus notatus B10-3* | 0.0 | 99.70% | KX268276.1 | | |
| | | *Batracomorphus lunatus B07* | 0.0 | 99.70% | KX268273.1 | | |
| | | *Batracomorphus laminocus B05* | 0.0 | 99.70% | KX268271.1 | | |
| | | *Batracomorphus rinkihonis B12* | 0.0 | 99.55% | KX268277.1 | | |

**Table (3): Similarity percentage of COX1 gene for *Batracomorphus signatus* generated by BLAST tools.**

| No | Isolate code | Significant Alignments | E value | Per. Ident | Retrieved Accession | Strains | Submitted accession no. |
|---|---|---|---|---|---|---|---|
| 1 | EGY-ARC-1C | *Batracomorphus signatus EGY-ARC-1C* | 0.0 | 100.00% | LC775122.1 | *Batracomorphus signatus EGY-ARC-1C* | LC775122.1:1 |
| | | *Batracomorphus angustatus cytochrome l* | 0.0 | 100.00% | KM408151.1 | | |
| | | *Batracomorphus angustatus KA2a* | 0.0 | 100.00% | MW621977.1 | | |
| | | *Batracomorphus angustatus KA2 l* | 0.0 | 100.00% | MW621976.1 | | |
| | | *Batracomorphus angustatus WW03471* | 0.0 | 98.36% | KF226795.1 | | |
| | | *Batracomorphus angustatus WW03300* | 0.0 | 98.36% | KF226800.1 | | |

For the precise determination of phylogenetic relationships between *Batracomorphus* species and to identify the presence of phylogenetic signals in the DNA sequences, the neighborhood joining (NJ) method of phylogenetic tree construction was chosen (Figures 3 and 4). Additionally, between each pair of sequences (Tables 4 and 5) the evolutionary distance of the COX and 28SrDNA sequences between *Batracomorphus* species was calculated. This information was then utilized to build the phylogenetic tree, which served as guidance for the final multiple alignments. Furthermore, the *Batracomorphus* species, EGY-ARC-1S and EGY-ARC-1C, were identified as *B. signatus* based on the analysis of the sequences of the COX and 28SrDNA genes. These data were submitted to NCBI with the accession numbers LC775122.1 and LC670604.1, respectively.

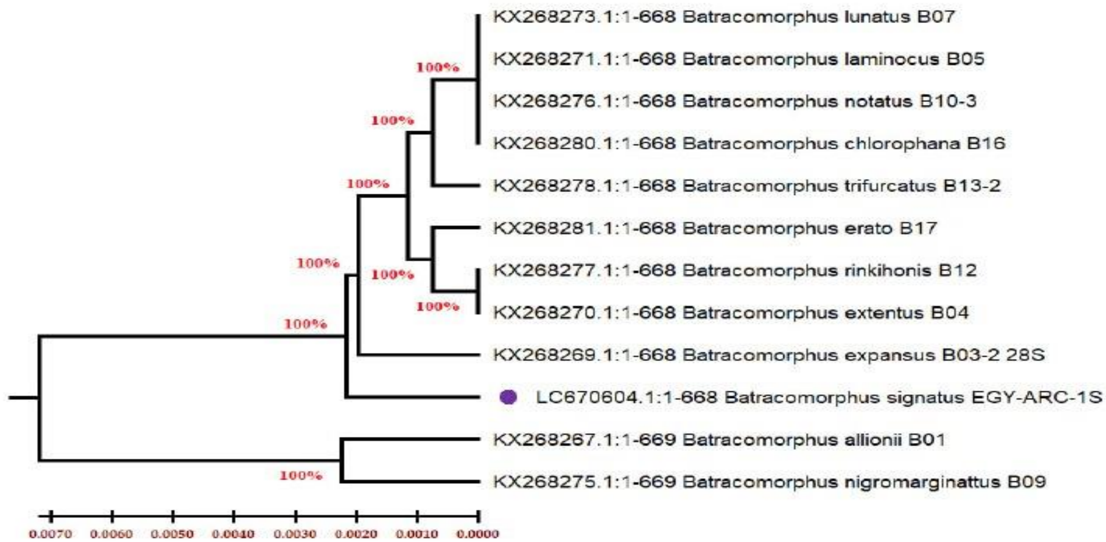


**Figure (3): NJ tree constructed using 28S gene sequence of *Batracomorphus signatus* species from Egypt, with related species.**

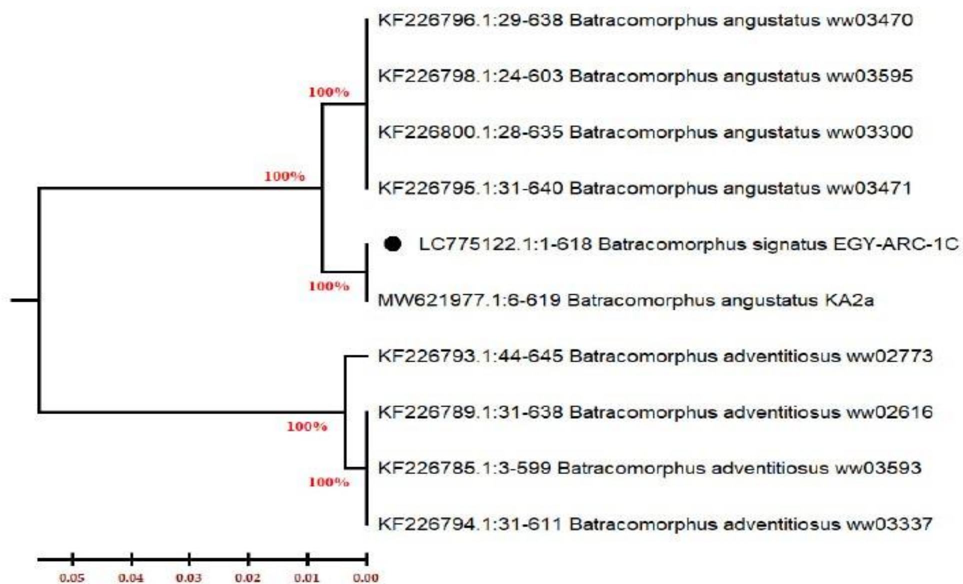


**Figure (4): NJ tree constructed using COX1 gene sequence of *Batracomorphus signatus* species from Egypt, with related *Batracomorphus* species.**

**Table (4): Estimates of Evolutionary Divergence of 28S rDNA sequences between *Batracomorphus*.**

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | LC670604.1:1-668_Batracomorphus_signatus_EGY-ARC-1S | | | | | | | | | | | |
| 2 | KX268280.1:1-668_Batracomorphus_chlorophana_B16 | 0.0030 | | | | | | | | | | |
| 3 | KX268276.1:1-668_Batracomorphus_notatus_B10-3 | 0.0030 | 0.0000 | | | | | | | | | |
| 4 | KX268273.1:1-668_Batracomorphus_lunatus_B07 | 0.0030 | 0.0000 | 0.0000 | | | | | | | | |
| 5 | KX268271.1:1-668_Batracomorphus_laminocus_B05 | 0.0030 | 0.0000 | 0.0000 | 0.0000 | | | | | | | |
| 6 | KX268277.1:1-668_Batracomorphus_rinkihonis_B12 | 0.0045 | 0.0015 | 0.0015 | 0.0015 | 0.0015 | | | | | | |
| 7 | KX268270.1:1-668_Batracomorphus_extentus_B04 | 0.0045 | 0.0015 | 0.0015 | 0.0015 | 0.0015 | 0.0000 | | | | | |
| 8 | KX268281.1:1-668_Batracomorphus_erato_B17 | 0.0060 | 0.0030 | 0.0030 | 0.0030 | 0.0030 | 0.0015 | 0.0015 | | | | |
| 9 | KX268278.1:1-668_Batracomorphus_trifurcatus_B13-2 | 0.0045 | 0.0015 | 0.0015 | 0.0015 | 0.0015 | 0.0030 | 0.0030 | 0.0045 | | | |
| 10 | KX268269.1:1-668_Batracomorphus_expansus_B03-2_28S | 0.0075 | 0.0045 | 0.0045 | 0.0045 | 0.0045 | 0.0030 | 0.0030 | 0.0045 | 0.0030 | | |
| 11 | KX268267.1:1-669_Batracomorphus_allionii_B01 | 0.0167 | 0.0136 | 0.0136 | 0.0136 | 0.0136 | 0.0121 | 0.0121 | 0.0136 | 0.0151 | 0.0121 | |
| 12 | KX268275.1:1-669_Batracomorphus_nigromarginattus_B09 | 0.0183 | 0.0151 | 0.0151 | 0.0151 | 0.0151 | 0.0136 | 0.0136 | 0.0151 | 0.0166 | 0.0136 | 0.0045 |

**Table (5): Estimates of Evolutionary Divergence of COX1 sequences between *Batracomorphus* species.**

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | LC775122.1:1-618_Batracomorphus_signatus_EGY-ARC-1C | | | | | | | | | |
| 2 | MW621977.1:6-619_Batracomorphus_angustatus_KA2a | 0.0000 | | | | | | | | |
| 3 | KF226795.1:31-640_Batracomorphus_angustatus_ww03471 | 0.0150 | 0.0150 | | | | | | | |
| 4 | KF226800.1:28-635_Batracomorphus_angustatus_ww03300 | 0.0150 | 0.0150 | 0.0000 | | | | | | |
| 5 | KF226796.1:29-638_Batracomorphus_angustatus_ww03470 | 0.0150 | 0.0150 | 0.0000 | 0.0000 | | | | | |
| 6 | KF226798.1:24-603_Batracomorphus_angustatus_ww03595 | 0.0149 | 0.0149 | 0.0000 | 0.0000 | 0.0000 | | | | |
| 7 | KF226789.1:31-638_Batracomorphus_adventitiosus_ww02616 | 0.1147 | 0.1147 | 0.1055 | 0.1055 | 0.1058 | 0.1078 | | | |
| 8 | KF226785.1:3-599_Batracomorphus_adventitiosus_ww03593 | 0.1175 | 0.1175 | 0.1083 | 0.1083 | 0.1085 | 0.1106 | 0.0000 | | |
| 9 | KF226794.1:31-611_Batracomorphus_adventitiosus_ww03337 | 0.1087 | 0.1087 | 0.1018 | 0.1018 | 0.1020 | 0.1041 | 0.0000 | 0.0000 | |
| 10 | KF226793.1:44-645_Batracomorphus_adventitiosus_ww02773 | 0.1267 | 0.1267 | 0.1150 | 0.1150 | 0.1153 | 0.1173 | 0.0075 | 0.0075 | 0.0075 |

The results of our investigation confirmed the *Batracomorphus signatus* species by GenBank using molecular identification based on the 28S DNA and COX1 genes. Table (6) lists the accession numbers for these sequences. Several *Batracomorphus* species may be reliably and accurately identified using this molecular barcoding technology, which has applications in conservation biology, pest management, and biodiversity analysis. Molecular methods, such as DNA sequencing and phylogenetic analysis have transformed the field of taxonomy and systematics. These modern techniques help examine the connections between species within the genus or assess the boundaries between species than the previous phylogenetic studies. This indicates the need for additional research using molecular techniques to examine the evolutionary relationships and taxonomic classification of *Batrachomorphus* species. There are similar studies to confirm the definition of species using DNA barcoding and the analysis of evolutionary relationships (Dietrich *et al.,* 2001, Zahniser and Dietrich, 2010 and 2013; Emam, 2016 and El-Hady and El-Sayed, 2024).

**Table (6): NCBI accession numbers of the studied *Batracomorphus signatus*, the accession numbers provided by NCBI for the submitted sequences.**

| No | Code | Strain | Subfamily | Accession number submitted to GenBank | |
|---|---|---|---|---|---|
| | | | | 28SrDNA | COX1 |
| **1** | EGY-ARC-15 | *Batracomorphus signatus* Lindberg | Iassinae | LC670604.1 | LC775122.1 |

The difficulty in differentiating between leafhopper species using only male genitalia and the instability of morphological characteristics that vary in response to host plant impacts and geographical associations are two drawbacks of traditional morphological approaches. Molecular barcoding with the 28S sequence provides a great alternative marker for characterizing, identifying, and confirming *Batracomorphus signatus* species collected from Egypt at any stage of their life cycle and for resolving several morphological identification problems.

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