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# Phylogeny of Trichoplusia ni (Lepidoptera: Noctuidae) 18S and 5S ribosomal RNA gene sequence

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## ABSTRACT

Trichoplusia ni Hübner, a cabbage looper belonging to the Noctuidae family of Lepidoptera, is a highly varied agricultural pest. It is essential to use partial sequences of nuclear DNA, 18S, and 5S ribosomal genes to isolate and analyze the phylogenetic relationships within T. ni and other insect species families and orders using distinct gene markers. T. DNA. After being extracted and sequenced using primers, Clustal W alignment and NCBI BLAST were completed. Members of the 18S BLAST lineage sequences are classified according to the order Coleoptera and the family Coccinellidae. A total of 2461 positions are occupied by the 33 sequences that were chosen for the final dataset. In the final dataset, 19 nucleotide sequences totaling 1935 positions were chosen from the taxonomy asset of 5S BLASTed lineages, which included members of the order Hymenoptera and the family Chalcidoidea. For both genes, phylogenetic analyses were performed using the Maximum Composite Likelihood model (ML), which aids in the construction of phylogenetic relationships at various taxonomic levels of trees, provides phylogenetic insights for understanding relationships between closer clades, and explains some significant regions of substitution rates appropriate for changing evolutionarily distant taxa.

**Contribution/Originality:** Molecular phylogenetic studies and evolutionary biology have garnered more attention recently. They contribute to the knowledge of the traits of foraging behavior and insect resurgence, such as host specificity, transmission patterns, genetic diversity, and natural enemies. There aren't many known phylogenetic analyses of insect ribosomal RNA searches.

## 1. INTRODUCTION

In Egypt, 160 different crops and vegetables, including cotton, are vulnerable to attacks by the highly polyphagous cabbage looper, Trichoplusia ni Hübner (Lepidoptera: Noctuidae), which is regarded as a significant agricultural pest of tomato, eggplant, zucchini, and other members of the Cruscus family [1]. Larvae consume vast amounts of plant leaves and fruits, which leads to population growth and crop damage [2]. The potential for control failure is caused by the economic damage at the lower threshold. The most common issue with this pest is the variation in the intensity of insecticide resistance development by location. Some substitutes, such as pyrethroids, Bacillus thuringiensis, and neem, are still effective, particularly when used in the early stages of larval development, when the high pest resistance status indicates polygenic inheritance or multiple resistance mechanisms [3, 4]. The recently suggested insecticide products for the control of cabbage loopers necessitate careful planning for resistance

management strategies, choices regarding acquisition, and the preservation of the new insecticide's effectiveness [5]. Five to seven generations of cabbage loopers are produced annually. Temperature and thresholds impact development and reproduction; in winter, reproduction is lower (10–12 °C), but in summer, it is higher (40°C), where this pest overwinters as a pupa. For two to three weeks, larvae feed on leaves and fruit, but tomato crop damage is uncommon. Weekly scouting to find a 5 percent defoliation threshold is necessary for efficient management, followed by effective insecticide, Natwick and Lopez [6]. Large DNA sequence data sets for biological research on the pattern of mitochondrial and nuclear gene evolution in animal, insect, or plant genomes are made available by advances in molecular biology techniques and sequencing. These developments also aid in the construction of phylogenetic relationships at various taxonomic levels [7]. A popular tool for examining the molecular evolution of multigene families is ribosomal DNA (rDNA). The two primary types of rDNA genes found in eukaryotes are 5S rDNA and 45S rDNA repeats, which encode rRNA and are highly conserved gene products found in all cells. According to Zhao et al. [8], the 5S rDNA gene is a unit made up of a gene transcription region (120 bp) and a non-transcribed spacer (NTS), whereas the 45S rDNA in animals contains 18S, 5.8S, 28S, and spacers (IGS, ITS1, and ITS2) [8]. Additionally, many insects' mitochondrial genes, including 16S and COI, have been studied [9, 10], The availability of the 18S rRNA molecule is used to provide phylogenetic gestures for perceptive closer clade relationships and to explain some significant regions of substitution rates appropriate for changing evolutionarily distant taxa, Wu, et al. [11]. The study of evolutionary relationships between species, taxa, individuals, or genes/proteins is known as phylogeny. In order to predict phylogenetic affiliations between molecular characters of the nucleotide data and align appropriate sequences to achieve similarity, it is crucial to determine homology as soon as possible [12-14]. Individual base positions are aligned to maximize overall position resemblance across the sequence [15]. More than a thousand different insect species can be found in Egypt. They all belong to a limited number of orders that share certain traits, despite their differences in size and shape [16, 17]. The previous T organization system. The kingdom Animalia, phylum Arthropoda, subphylum Hexapoda, class Insecta, order Lepidoptera, family Noctuidae, subfamily Plusiinae, and tribe Argyrogrammatini are among the hierarchical categories that comprise insect taxonomic orders. Therefore, it is urgent to create phylogenetic trees and align multiple sequences using software in order to perform evolutionary relationship or phylogeny analysis on some sequences of various holometabolous insect species that are divided into three assemblages: (Neuropterida) includes neuroptera, megalopteran, raphidoptera, strepsiptera, and coleoptera, and (Hymenopterioida) includes hymenoptera and panopridae includes (Siphonaptera, diptera, trichoptera, lepidoptera, and mecoptera.) Additionally, it is necessary to estimate evolutionary distances, build trees, test tree reliability, work with genes and domains, test for selection, manage taxa with groups, compute sequence statistics, and construct likelihood trees, and compare the results using all five different methods [14, 18]. These techniques include Minimum-Evolution, Neighbour-Joining, and Maximum Likelihood (ML). Nevertheless, UPGMA was one of two cluster analysis methods Sayers et al. [19].

## 2. MATERIALS AND METHODS

### *2.1. Insect Sources and DNA Extraction of Larvae*

A few *Trichoplusia ni* Hubner larvae were transferred to Cairo University's molecular biology labs after being collected from tomato plants. About 0–5 ml of CTAB buffer was prepared in accordance with the CTAB DNA extraction protocol, which was used to complete the extraction process according to Doyle and Doyle [20].

### 2.2. Polymerase Chain Reaction Procedure Preparations

After thawing, gently vortex and quickly centrifuge DreamTaq Green PCR Master Mix (2X). Additionally, for each 50 µl reaction, place a thin-walled PCR tube on ice and add the following ingredients: DreamTaq Green PCR Master Mix (2X) 25 µl, Forward primer 2 µl, Reverse primer 2 µl, Template DNA 4 µl, Nuclease-free water 17 µl, and Total volume 50 µl. After adjusting the volume, sample vortexing and gentle spinning were performed. The following suggested thermal cycling conditions were used for the PCR procedures: initial denaturation at 95°C for 5 minutes for one cycle, denaturation at 95°C for 30 seconds for 40 cycles, annealing at 65°C for 30 seconds for 40 cycles, extension at 72°C for 30 seconds for 40 cycles, and final extension at 72°C for 10 minutes for one cycle.

Table 1 Sequences of the primers used for PCR amplification and sequencing.

**Table 1.** Primer used in the amplification.

| Gene | Primer name | Primer sequence | Length bp | GC | MW | Annealing temp |
|---|---|---|---|---|---|---|
| 18S | | 5- AGTACGGTGAAACCGCGAAA Reverse: TTTCGCGGTTTCACCGTACT | 20 | 50 % | 6246 | 56.7 |
| 5S | | 5-AAGTGTACTCATTCCGATTACGG Reverse: CCGTAATCGGAATGAGTACACTT | 23 | 43.47 % | 7101 | 54.4 |

### 2.3. Sequencing Procedure

As previously mentioned, two primers were used to sequence the purified PCR. The Big Dye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems, USA) was used for the sequencing process. The Macrogen company's Applied Biosystems model 3730 automated DNA sequencing system was used to resolve the sequencing products.

### 2.4. Phylogenetic Analysis: Sequence Alignment and Phylogenetic Tree

The National Center for Biotechnology Information's (NCBI) BLAST algorithm databases were used to compare the *T ni* 18s and 5s rRNA nucleotide sequences obtained from the sequencing company with those of other insects (Tables 2,3). aligning multiple sequences with the Thompson et al. [21] was completed, after which Mega 11 software built phylogenetic trees and carried out all phylogenetic analysis [22].

## 3. RESULTS AND DISCUSSIONS

### 3.1. The 18S, 5s rRNA Phylogenetic Analysis

Partial nuclear DNA, 18S, and 5S ribosomal gene sequences were used to investigate the evolutionary relationships between lineage groups of the cabbage looper *T. ni*. In a separate analysis, phylogenetic relationships between insect lineage families and orders were inferred. Members of the taxonomy of 18S blasted lineage sequences belong to the family Coccinellidae and the order Coleoptera. Additionally, the member of the 5S blasted lineage belonged to the family Chalcidoidea, order Hymenoptera. The examination of T's 18S rRNA molecular data set included 33 nucleotide sequences from the ClustalW alignment that were then blasted against the PCR-generated native sequence. The final dataset contained 2646 positions in total. First, second, third, and noncoding codon positions were included. There were nineteen nucleotide sequences in the 5S rRNA analysis. The final dataset contained 1935 positions in total. For both genes, phylogenetic analyses were performed using the (ML) (Table 6). Every sequence pair's ambiguous positions were eliminated. Alignment and evolutionary analyses were performed to calculate different statistical quantities of nucleotide sequences. Table 2 lists the species information for the 18S rRNA sequences that have been aligned, and Table 3 lists the 5S rRNA sequences that have been aligned.

The NCBI blast plus identity and E value were displayed in Tables 2 and 3, along with the accession number, distances, homogeneity, divergence, and insect species names and orders. The Disparity Index test (Homogeneity of Substitution Patterns test Between Sequences or the net composition bias) is one of the results of phylogeny analysis [23, 24]. It is used to understand shifts in mutational patterns and selective pressures if sequences and species evolve with heterogeneous patterns, as well as the role of base composition biases between sequences and the probability that the null hypothesis will be rejected if sequences with the same pattern have evolved of substitution. P-values less than 0.05 are considered significant, according to the Monte Carlo test's estimations (500 replicates). According to Tajima and Nei [25], the number of nucleotide substitutions per site between two homologous DNA sequences is an estimate of the evolutionary change of DNA sequences caused by nucleotide substitution, deletion, and insertion. The pattern of change accumulation was calculated using the evolutionary distance, and the amino acid sequence data indicate that deletions and insertions are significantly less common in the coding regions of globin genes than in the noncoding regions. Actually, there is no issue when there are few nucleotide substitutions per site. In order to measure the impact of insertion and deletion on the evolutionary change of DNA sequences, Gamma was introduced.

**Table 2.** List of different insect 18S ribosomal RNA sequences (Practically from the order Coleoptera, family Coccinellidae) obtained from NCBI-BLAST sequences, identity from 99.55 to 97.81, accession number, homogeneity, divergence, and distance between all taxa and *T.ni* PCR-generated sequence.

| N. | Species name | E-value | Identity | Length | Accession n. | Distance | Hom | Div.h |
|---|---|---|---|---|---|---|---|---|
| 1 | Rodolia sp. | 3.00E-112 | 99.55 | 1857 | KP829195.1 | 0.919 | 0.226 | 0.273 |
| 2 | Monocoryna sp. | 3.00E-112 | 99.55 | 1866 | KP829165.1 | 0.910 | 0.190 | 0.294 |
| 3 | Epilachna borealis | 3.00E-112 | 99.55 | 1848 | KP123077.1 | 0.900 | 0.240 | 0.234 |
| 4 | Toxotoma forsteri | 3.00E-112 | 99.55 | 1848 | KP123017.1 | 0.900 | 0.192 | 0.234 |
| 5 | Neoneuromus ignobilis | 3.00E-112 | 99.55 | 7345923 | CP092098.1 | 0.900 | 0.172 | 0.328 |
| 6 | Rhyzobius hilura | 3.00E-112 | 99.55 | 1823 | EF209861.1 | 0.923 | 0.346 | 0.099 |
| 7 | Neohermes californicus | 3.00E-112 | 99.55 | 1676 | EU815261.1 | 0.919 | 0.232 | 0.206 |
| 8 | Parainocellia bicolor | 3.00E-112 | 99.55 | 2097 | EU815245.1 | 0.913 | 0.204 | 0.266 |
| 9 | Austroneurorthus brunneipennis | 3.00E-112 | 99.55 | 2461 | EU815229.1 | 0.925 | 0.202 | 0.237 |
| 10 | Strigocis opacicollis | 3.00E-112 | 99.55 | 820 | FM877872.1 | 0.925 | 0.198 | 0.279 |
| 11 | Hyperaspidini sp. | 3.00E-112 | 99.55 | 1768 | EU145620.1 | 0.916 | 0.296 | 0.148 |
| 12 | Negha meridionalis | 3.00E-112 | 99.55 | 2071 | AY521865.1 | 0.881 | 0.008 | 1.578 |
| 13 | Meropathus zelandicus | 3.00E-105 | 97.82 | 601 | LT990835.1 | 0.920 | 0.226 | 0.262 |
| 14 | Neostylopyga rhombifolia | 3.00E-105 | 98.24 | 1842 | KP986337.1 | 0.916 | 0.302 | 0.154 |
| 15 | Ametastegia equiseti | 4.00E-111 | 99.1 | 25643224 | OZ022392.1 | 0.888 | 0.000 | 1.643 |
| 16 | Libethra strigiventris | 3.00E-105 | 98.23 | 1823 | MN925370.1 | 0.921 | 0.220 | 0.258 |
| 17 | Adelphydraena orchymonti | 3.00E-105 | 97.82 | 601 | HM588578.1 | 0.918 | 0.176 | 0.333 |
| 18 | Meropathus sp. | 3.00E-105 | 97.82 | 1791 | EF214162.1 | 0.900 | 0.012 | 1.356 |
| 19 | Neuroperlopsis patris | 3.00E-105 | 97.41 | 1996 | EF622699.1 | 0.894 | 0.000 | 1.695 |
| 20 | Desertaclopus lucasi | 1.00E-104 | 97.81 | 1845 | MG924409.1 | 0.921 | 0.174 | 0.341 |
| 21 | Mikado sp. | 1.00E-104 | 97.81 | 620 | LR742672.1 | 0.916 | 0.132 | 0.424 |
| 22 | Hydraena paeminosa | 1.00E-104 | 98.65 | 622 | LR742669.1 | 0.933 | 0.182 | 0.286 |
| 23 | Scirtoidea sp. | 1.00E-104 | 97.81 | 1921 | KX092894.1 | 0.949 | 0.166 | 0.331 |
| 24 | Belohina inexpectata | 1.00E-104 | 97.81 | 1917 | OR754010.1 | 0.921 | 0.112 | 0.430 |
| 25 | Satonius sp. | 1.00E-104 | 97.81 | 1067 | KP419279.1 | 0.913 | 0.122 | 0.448 |

| N. | Species name | E-value | Identity | Length | Accession n. | Distance | Hom | Div.h |
|---|---|---|---|---|---|---|---|---|
| 26 | Nycteus infumatus | 1.00E-104 | 97.81 | 1868 | KP419194.1 | 0.913 | 0.120 | 0.448 |
| 27 | Clytra quadripunctata | 1.00E-104 | 97.81 | 1883 | KP762945.1 | 0.923 | 0.120 | 0.542 |
| 28 | Labidostomis lucida | 1.00E-104 | 97.81 | 1916 | KP762940.1 | 0.913 | 0.108 | 0.448 |
| 29 | Lachnaia gallaeca | 1.00E-104 | 97.81 | 1915 | KP762933.1 | 0.913 | 0.108 | 0.448 |
| 30 | Smaragdina rufimana | 1.00E-104 | 97.81 | 1883 | KP762874.1 | 0.913 | 0.168 | 0.448 |
| 31 | Trigonogenium angulosum | 1.00E-104 | 97.81 | 1865 | KM364119.1 | 0.926 | 0.158 | 0.445 |
| 32 | Paragrilus aeraticollis | 1.00E-104 | 97.81 | 1748 | KM364075.1 | 0.933 | 0.130 | 0.464 |

**Note:** Div=Interspecific genetic divergence, H=heterogeneity and Hom=Homogeneity.

**Table 3.** List of different insect 5s ribosomal RNA sequences (Practically from order Hymenoptera, family Calcidoidae) attained from NCBI-blast information, identity from 89.72 to 88.79, accession number, distance, homogeneity and divergence.

| N. | Species name | E-value | % Identity | Length | Accession n. | Dist | Homo | Div. |
|---|---|---|---|---|---|---|---|---|
| 1 | Phymastichus coffea | 3.00E-28 | 89.72 | 1915 | XR_009302357.1 | 0.441 | 0.022 | 0.647 |
| 2 | Nasonia vitripennis | 1.00E-26 | 88.79 | 1915 | XR_004228347.1 | 0.559 | 0.010 | 0.772 |
| 3 | Coruna clavata | 1.00E-26 | 88.79 | 897 | KY887836.1 | 0.559 | 0.012 | 0.772 |
| 4 | Asaphes vulgaris | 1.00E-26 | 88.79 | 897 | KY887833.1 | 0.559 | 0.004 | 0.772 |
| 5 | Alloxysta victrix | 1.00E-26 | 88.79 | 893 | KY887822.1 | 0.559 | 0.018 | 0.772 |
| 6 | Aphelinus varipes | 1.00E-26 | 88.79 | 892 | KY887812.1 | 0.559 | 0.018 | 0.772 |
| 7 | Praon necans | 1.00E-26 | 88.79 | 903 | KY873372.1 | 0.559 | 0.014 | 0.772 |
| 8 | Ephedrus plagiator | 1.00E-26 | 88.79 | 904 | KY873354.1 | 0.559 | 0.012 | 0.772 |
| 9 | Kerria yunnanensis | 1.00E-26 | 88.79 | 568 | JQ365156.1 | 0.559 | 0.006 | 0.772 |
| 10 | Ephuta sp. | 1.00E-26 | 88.79 | 737 | EF473894.1 | 0.559 | 0.010 | 0.772 |
| 11 | Sphaeropthalma coaequalis | 1.00E-26 | 88.79 | 730 | EF473891.1 | 0.559 | 0.016 | 0.772 |
| 12 | Odontophotopsis melicausa | 1.00E-26 | 88.79 | 731 | EF473889.1 | 0.559 | 0.006 | 0.772 |
| 13 | Dasymutilla subhyalina | 1.00E-26 | 88.79 | 731 | EF473888.1 | 0.559 | 0.010 | 0.772 |
| 14 | Aprostocetus purpureus | 1.00E-26 | 88.79 | 1775 | JQ359003.1 | 0.559 | 0.018 | 0.772 |
| 15 | Trichogramma platneri | 1.00E-26 | 88.79 | 1900 | JN623531.1 | 0.500 | 0.012 | 0.713 |
| 16 | Podagrion sp. | 1.00E-26 | 88.79 | 1895 | JN623524.1 | 0.559 | 0.016 | 0.772 |
| 17 | Tetracampe sp. | 1.00E-26 | 88.79 | 1268 | JN623512.1 | 0.559 | 0.022 | 0.772 |
| 18 | Foersterella reptans | 1.00E-26 | 88.79 | 1898 | JN623511.1 | 0.559 | 0.012 | 0.772 |
| 19 | Signiphora dipterophaga | 1.00E-26 | 88.79 | 1901 | JN623482.1 | 0.441 | 0.022 | 0.647 |

Table 5 displays the maximum composite ML of the nucleotide substitution pattern as well as the rates of various transitional and transversional substitutions for both genes. A = 24.06 percent, T/U = 24.22 percent, C = 23.92 percent, and G = 27.79 percent are the nucleotide frequencies of the 18S alignment. For purines, the transition/transversion rate ratio is k1 = 23.918; for pyrimidines, it is K2 = 291.494. R = [A*G*k1 + T*C*k2]/[(A+G) * (T+C)] is the overall transition/transversion bias, or R = 73.714. However, the estimated ratio (R) value for 5S is 0.53, which is the number of transitional substitutions to that of transversional substitutions. Rates and patterns of substitution were calculated using the Tamura et al. [26] as well as the Takahashi and Nei [27]. A = 25.00 percent, T/U = 25.00 percent, C = 25.00 percent, and G = 25.00 percent are the nucleotide frequencies. A tree topology was automatically calculated for ML value estimation. The gamma shape parameter was 139.2826 and the ML for the 18-second computation was -14371.636; for the 5-second computation, the ML was -4097.380. A total of 1935 positions and 19 nucleotide sequences were included in the final dataset for this analysis.

The compositional distance is correlated with the number of differences between sequences. Non-uniformity of evolutionary rate differences among sites was modeled by using a discrete gamma distribution (+G) with 5 rate categories and by assuming that a certain fraction of sites is evolutionarily invariable (+I). Mean evolutionary rates in these categories were 0.88, 0.95, 1.00, 1.04, and 1.12 substitutions per site. The sites showing a rate < 1 are evolving slower than average, and those with a rate > 1 are evolving faster than average. The gamma distribution (ʎ) can be specified by the coefficient of variation of the substitution rate (CV), where the smaller the CV, the higher ʎ. Actually, analysis of ML results that gave the lowest BIC scores (Bayesian Information Criterion) is considered to describe the best substitution pattern (Table 6). For each model, AICc value (Akaike Information Criterion, corrected), ML value (lnL), and the number of parameters, including branch lengths are also presented in Table 6. Estimates of the gamma shape parameter, fraction of invariant sites, and transition/transversion bias (R) are shown for each model. The nucleotide frequencies of the 18s rRNA found in Table 6 and rates of base substitutions (r) for each nucleotide pair found in Table 6, with the sum of r-values made equal to 100. The difference in base composition bias per site (relative frequencies of the four nucleotides) is in Table 6; also, the twenty amino acid residues (amino acid composition) were attained computationally.

**Table 4.** Different in ML models of the 18s and 5s rRNA gene using one of the most parsimonious trees topology fits.

| No. | Blasred 18s r RNA sequences ML | | | | | Blasted 5s sequences ML | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Model | BIC | AICc | lnL | R | Model | BIC | AICc | lnL | R |
| 1 | TN93+G | 27737.48 | 27123.59 | -13492.70 | 1.262 | K2+G | 8502.82 | 8207.70 | -4066.78 | 2.670 |
| 2 | TN93+G+I | 27748.45 | 27125.67 | -13492.74 | 1.265 | T92+G | 8511.61 | 8208.51 | -4066.18 | 2.668 |
| 3 | GTR+G | 27757.79 | 27117.21 | -13486.51 | 1.260 | K2+G+I | 8514.44 | 8211.34 | -4067.60 | 2.586 |
| 4 | GTR+G+I | 27768.75 | 27119.28 | -13486.54 | 1.263 | T92+G+I | 8523.24 | 8212.17 | -4067.01 | 2.585 |
| 5 | K2+G | 27781.00 | 27202.69 | -13536.26 | 1.231 | HKY+G | 8526.68 | 8207.64 | -4063.74 | 2.687 |
| 6 | T92+G | 27782.15 | 27194.94 | -13531.39 | 1.240 | TN93+G | 8526.71 | 8199.69 | -4058.77 | 2.688 |
| 7 | T92+G+I | 27793.02 | 27196.92 | -13531.38 | 1.242 | HKY+G+I | 8538.56 | 8211.54 | -4064.69 | 2.600 |
| 8 | K2+G+I | 27803.93 | 27216.73 | -13542.28 | 1.492 | TN93+G+I | 8538.90 | 8203.91 | -4059.87 | 2.600 |
| 9 | HKY+G | 27808.17 | 27203.18 | -13533.50 | 1.259 | GTR+G | 8550.61 | 8199.68 | -4055.75 | 2.664 |
| 10 | HKY+G+I | 27819.04 | 27205.15 | -13533.49 | 1.261 | GTR+G+I | 8566.43 | 8207.53 | -4058.67 | 2.296 |
| 11 | JC+G | 28021.11 | 27451.69 | -13661.77 | 0.500 | K2+I | 8604.27 | 8309.14 | -4117.51 | 2.287 |
| 12 | JC+G+I | 28044.68 | 27466.37 | -13668.10 | 0.500 | T92+I | 8613.08 | 8309.98 | -4116.92 | 2.288 |
| 13 | GTR+I | 28904.97 | 28264.40 | -14060.10 | 1.078 | TN93+I | 8625.82 | 8298.80 | -4108.32 | 2.348 |
| 14 | TN93+I | 28911.16 | 28297.27 | -14079.55 | 1.212 | JC+G | 8626.20 | 8339.04 | -4133.46 | 0.500 |
| 15 | K2+I | 28989.98 | 28411.67 | -14140.76 | 1.050 | HKY+I | 8628.42 | 8309.38 | -4114.61 | 2.289 |
| 16 | T92+I | 28996.19 | 28408.99 | -14138.41 | 1.053 | JC+G+I | 8639.04 | 8343.91 | -4134.89 | 0.500 |
| 17 | HKY+I | 29024.60 | 28419.61 | -14141.72 | 1.056 | GTR+I | 8652.88 | 8301.94 | -4106.88 | 2.071 |
| 18 | JC+I | 29202.05 | 28632.63 | -14252.24 | 0.500 | K2 | 8678.14 | 8390.98 | -4159.43 | 2.095 |
| 19 | GTR | 29733.37 | 29101.69 | -14479.75 | 0.996 | T92 | 8686.72 | 8391.59 | -4158.73 | 2.096 |
| 20 | TN93 | 29742.70 | 29137.70 | -14500.76 | 1.113 | TN93 | 8701.04 | 8381.99 | -4150.92 | 2.098 |
| 21 | K2 | 29823.80 | 29254.38 | -14563.12 | 1.137 | HKY | 8702.36 | 8391.28 | -4156.57 | 2.096 |
| 22 | T92 | 29834.34 | 29256.03 | -14562.94 | 1.136 | JC+I | 8717.71 | 8430.56 | -4179.22 | 0.500 |
| 23 | HKY | 29860.28 | 29264.18 | -14565.01 | 1.137 | GTR | 8725.76 | 8382.80 | -4148.31 | 2.107 |
| 24 | JC | 30015.88 | 29455.36 | -14664.60 | 0.500 | JC | 8786.54 | 8507.36 | -4218.62 | 0.500 |

**Note:** LnL = log likelihood, LRT= likelihood ratio test.

### *3.2. Parsimony Analysis, Sequence Variation and Nucleotide Composition*

The principle of parsimony states that the most straightforward explanation for the data should be chosen. Parsimony in phylogenetic analysis refers to the likelihood that a relationship hypothesis that calls for the fewest character changes will be accurate. Additionally, Mega computer software supports the maximum-parsimony (MP) method of reconstructing phylogenetic trees. Equal branch lengths in the tree, consistency of substitution rates between nucleotides, and consistency of rates across nucleotide sites are among the very stringent assumptions about the sequence evolution process that parsimony appears to entail. Few species in the data, relatively long interior branches, and similar substitution rates among lineages (the existence of an approximate molecular clock) are necessary for the practical analysis of data

in order to meet the requirement of equal branch lengths. However, a small amount of evolution is not necessary for the method to work.

**Table 5.** Number of taxa, characters, and nucleotide frequency and substitution matrix of the 18s and 5s r RNA gene of the most parsimonious trees topology fits.

| Gene | N. of sequence | A% | T% | C% | G% | Variable site | Pars. informative site |
|---|---|---|---|---|---|---|---|
| Neocleotid frequency 18s | 33 | 0.2435 | 0.2445 | 0.246 | 0.2655 | 2646 | 68 |
| Neocleotid frequency 5s | 19 | 0.251 | 0.2525 | 0.2385 | 0.258 | 1935 | 40 |
| Substitution rate 18s | | 3.84 | 45.6 | 44.9 | 4.2 | | |
| Substitution rate 5s | | 12.66 | 21.4 | 19.17 | 13.44 | | |

### *3.3. Variation in 18s and 5s Genes rDNA Sequence*

The intraspecific distance of all species is given in Tables 2 and 3. The minimum intraspecific pairwise distance variation was observed between *T.ni* tested sequence and *Trigonogenium angulosum* (Accession number is KM364119.1 and 1865 in length) was 0.547, and the maximum intraspecific distance was calculated between *T.ni* tested sequence and *Monocoryna sp.* (Accession number KP829165.1 and 1866 in length) was 2.7. Similarly, evolutionary genetic divergence for species discrimination estimated between sequences refers to the number of base substitutions per site between sequences, which are in Tables 2 and 3. The maximum interspecific genetic divergence ranged from 98.78 for *Meropathus sp.* (Accession number EF214162.1 and 1791 length) to 228.7

5 for *Rhyzobius hilura* (Accession number EF209861.1 and 1823 length). To attain the phylogenetic position of *T. ni*, the phylogenetic tree was constructed, and topology differences were obtained from some trees constructed in this study for 18S and 5S blasted sequences in Figures 1 and 2. Details of the gene alignment of *T. ni* from the Egypt population sequenced and some from China with their sequence variable sites and parsimony informative sites were observed. The molecular diversity analysis revealed minute intraspecific variation with pairwise genetic distance ranging from 0 to 0%. Pattern heterogeneity ranged from 1.0 to 1.0. Interspecific genetic divergence between the two groups (14 Chinese sequences and the target Egyptian one) was 0.02. The 15-nucleotide sequence alignment result included 391 selected sites, including 247 complete (no gaps, no N), 180 variable (72.9% of complete), and 0 informative (0.0% of complete).

**Table 6.** Test of the homogeneity of substitution patterns between sequences.

| Position | 18s r RNA | | | | 5s rRNA | | | |
|---|---|---|---|---|---|---|---|---|
| | A | C | G | T | A | C | G | T |
| All position | 24.218 | 23.922 | 24.048 | 27.810 | 25.436 | 22.764 | 25.121 | 26.677 |
| First position | 25.084 | 24.423 | 23.729 | 26.761 | 27.363 | 20.963 | 25.822 | 25.850 |
| Second position | 24.004 | 23.493 | 24.620 | 27.881 | 24.197 | 22.585 | 24.030 | 29.186 |
| Third position | 23.566 | 23.849 | 23.793 | 28.790 | 24.746 | 24.746 | 25.510 | 24.996 |

**Note:** Heterogeneous base composition across species was tested with x2 test statistic.
Base composition broken down by codon position.

Molecular phylogenetics of lepidopteran datasets created using 18S rRNA nucleotide alignments produced by ClustalW between the main Noctuidae lineages, Figure 1. The tree with the highest log likelihood (-44601.14) is shown. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. This analysis involved 43 nucleotide sequences of familiar (Agricultural pests) and total of 2083 positions in the final dataset. The nucleotide frequencies are A = 25.00%, T/U = 25.00%, C = 25.00%, and G = 25.00%. The estimated Transition/Transversion bias (R) is 0.52. BIC=90076.5, AIC=89333.6. And

confidence intervals on phylogenies by the number of bootstrap replicates with 10,000 [28] estimated and tree constructed based on the protein-coding amino acid sequences (Figure 3, 4). Nonetheless molecular phylogeny of 5s alignment with different orders analysis involved 28 nucleotide sequences and a total of 607 positions. InL=2813.6, BIC=5738.8 and BIC= 6084.3. Synonymous and non-synonymous substitution (Silent and amino acid alteration) were also calculated according to Nei and Gojobori [29]. It is obtained by counting silent and amino acid-altering nucleotide differences. Where the silent amino acid substitution is much higher than the altered and looks similar for different genes. The small value refers to there is no more than one nucleotide difference between each pair of homologous codons, but the high value refers to the differences is complicated.

Another alignment with some members of the subfamily Plusiinae 18s rRNA sequences and phylogenetic analysis by the ML method was done to generate the evolutionary history. Results indicate that the topology was similar and the pairwise genetic distance is the same Figure 5. Many literature producers' efforts have been made regarding the subject of phylogeny as well as insect taxonomy, insect detoxifications, and important insect molecules like specific target function encoding proteins such as [30, 31]. Many scientists have proposed some high-quality *T. ni* genome assembly, which contains 14,384 protein-coding genes of the assembly across 31 chromosomes, as Chen et al. [32] and Talsania et al. [33].

**Table 7.** The alignment of many insect orders with the species name and accession number indicates varying degrees of homogeneity, distance, and divergence of substitution.

| N. | Species name | Order | Family | E value | % Identity | Length | Accession n | Dist. | Homo | Div. |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Aedes_aegypti | Diptera | Culicidae | 4.0E-38 | 82.81 | 825 | HE613439 | 0.036 | 1.000 | 0.000 |
| 2 | Aphis_gossypii | Hemiptera | Aphidae | 1.0E-40 | 96.43 | 1163 | KF018922 | 0.036 | 1.000 | 0.000 |
| 3 | Bemisia_tabaci | Homoptera | Aleyrodidae | 0.009 | 28.57 | 506 | JQ995259 | 0.153 | 1.000 | 0.000 |
| 4 | Bombyx_mori | Lepidoptera | Bombicidae | 5.0E-43 | 82.19 | 1124 | KF982847 | 2.629 | 0.014 | 1.871 |
| 5 | Cephus_pygmaeuss | Coleoptera | Cephidae | 2.0E-46 | 98.44 | 1860 | GQ410588 | 3.357 | 0.006 | 2.629 |
| 6 | Chilo_suppressalis | Lepidoptera | Crambidae | 1.0E-41 | 80.82 | 1914 | GQ265912 | 0.017 | 1.000 | 0.000 |
| 7 | Chrysoperla_carnea | Neuroptera | Chrysopidae | 2.0E-46 | 98.44 | 989 | KT204369 | 0.031 | 1.000 | 0.000 |
| 8 | Drosophila_melanogaster | Diptera | Drosophilidae | 2.0E-36 | 79.17 | 1994 | NR_133559 | 0.031 | 1.000 | 0.000 |
| 9 | Eniclases_pseudoluteolus | Ccoleoptera | Lycidae | 8.0E-46 | 96.88 | 221 | MG871684 | 0.031 | 1.000 | 0.000 |
| 10 | Frankliniella_occidentalis | Thysanoptera | Thyripidae | 2.0E-43 | 90.91 | 228 | KC512959 | 0.352 | 1.000 | 0.000 |
| 11 | Helicoverpa_zea | Lepidoptera | Noctuidae | 4 | 81.6 | 21 | KT946004 | 3.065 | 0.006 | 2.324 |
| 12 | Heliothis_subflexa | Lepidoptera | Noctuidae | 4 | 81,5 | 21 | KT946000 | 0.368 | 1.000 | 0.000 |
| 13 | Hypera_postica | Coleoptera | Curculionidae | 1.0E-43 | 89.39 | 1609 | AF389058 | 0.866 | 0.306 | 0.105 |
| 14 | Locusta_migratoria | Orthoptera | Acrididae | 1.0E-44 | 92.42 | 1893 | KM853191 | 0.085 | 1.000 | 0.000 |
| 15 | Lycorma_delicatula | Hemiptera | Erebidae | 4.0E-44 | 95.08 | 230 | KC413787 | 0.425 | 1.000 | 0.000 |
| 16 | Macroglenes sp. | Hymenoptera | Pirenidae | 1E-42 | 95.31 | 678 | JN623447.1 | 0.425 | 1.000 | 0.000 |
| 17 | Musca_domestica | Diptera | Muscidae | 3E-34 | 81.25 | 1943 | KC177313 | 3.980 | 0.000 | 3.251 |
| 18 | Nala_lividipes | Dermaptera | Labidoridae | 4 | 69.6 | 55 | AY707362 | 5.385 | 0.000 | 4.623 |
| 19 | Nasonia_vitripennis | Hymenoptera | Pteromalidae | 2E-11 | 100 | 573 | MF583329 | 1.494 | 0.106 | 0.749 |
| 20 | Neohermes_californicus | Megaloptera | Corydalidae | 1E-47 | 100 | 223 | CP092098.1 | 5.235 | 0.000 | 4.494 |
| 21 | Neoneuromus_ignobilis | Megaloptera | Corydalidae | 1E-47 | 100 | 223 | CP092098.1 | 1.980 | 0.046 | 1.235 |
| 22 | Nilaparvata_lugen | Hemipteran | Delphacidae | 4 | 57.6 | 135 | JN662398 | 1.729 | 0.098 | 0.947 |
| 23 | Nomada_panzeri | Hymenoptera | Apidae | 0.002 | 100 | 784 | KF512686 | 1.729 | 0.078 | 0.996 |
| 24 | Ostrinia_nubilalis | Lepidoptera | Crambidae | 4 | 82.4 | 17 | X576726 | 1.833 | 0.068 | 1.059 |
| 25 | Tuta absoluta | Lepidoptera | Gelichidae | 4 | 68.2 | 47 | MH644412 | 1.761 | 0.052 | 1.041 |
| 26 | Parainocellia bicolor | Raphidoptera | Inocellidae | 1E-47 | 100 | 223 | EU815245 | 0.049 | 1.000 | 0.000 |
| 27 | Phthorimaea_operculella | Lepidoptera | Gelichidae | 4 | 54.5 | 99 | OL655414 | 0.757 | 0.388 | 0.008 |
| 28 | Phytoseiulus_persimilis | Acari | Phytosidae | 4 | 59.5 | 42 | U39916 | 3.429 | 0.004 | 2.704 |
| 29 | Pieris_brassicae | Lepidoptera | Pieridae | 4e-46 | 83.56% | 248 | XR_006754962 | 1.243 | 0.178 | 0.506 |
| 30 | Pieris_napi | Lepidoptera | Pieridae | 1E-44 | 92.42 | 1893 | XR_007118579 | 1.640 | 0.094 | 0.879 |
| 31 | Planococcus_ficus | Hemipteran | Coccoidae | 4 | 56.2 | 80 | MF952600 | 1.826 | 0.058 | 1.085 |
| 32 | Plutella_xylostella | Lepidoptera | Plutillidae | 4 | 66.7 | 33 | JN410814 | 0.684 | 1.000 | 0.000 |
| 33 | Pteromalus_albipennis | Hymenoptera | Pteromalidae | 4 | 68,2 | 44 | KC008498 | 1.798 | 0.084 | 1.028 |
| 34 | Pulvinaria_psidii | Hemiptera | Coccoidae | 1E-15 | 96 | 139 | JQ651034 | 1.239 | 0.178 | 0.494 |
| 35 | Rodolia_cardinalis | Coleopteran | Coccinillidae | | | | GU073726 | 1.437 | 0.146 | 0.684 |

| N. | Species name | Order | Family | E value | % Identity | Length | Accession n | Dist. | Homo | Div. |
|---|---|---|---|---|---|---|---|---|---|---|
| 36 | Signiphora_dipterophaga | Hymenoptera | Calcidoidae | 2E-45 | 95.31 | 1901 | N623482 | 3.644 | 0.004 | 2.899 |
| 37 | Spodoptera_exigua | Lepidoptera | Noctuidae | | | | FJ041111 | 1.081 | 0.226 | 0.340 |
| 38 | Spodoptera_litura | Lepidotera | Noctuidae | | | | JX041469 | 4.393 | 0.000 | 3.640 |
| 39 | Tenebrio_molitor | Coleoptera | Tenebrionidae | 2E-45 | 95.31 | 2083 | X07801 | 1.408 | 0.130 | 0.614 |
| 41 | Tetranychus_urticae | Acari | Tetranychidae | 4 | 74.3 | 27 | KP642052 | 1.413 | 0.150 | 0.652 |
| 42 | Thrips_tabaci | Thysanoptera | Thripidae | 2.0 | 20.2 | 1545 | KM877307 | 3.862 | 0.002 | 3.097 |
| 43 | Chinese_Trichoplusia_ni | Lepidoptera | Noctuidae | 2E-46 | 94.15 | 247 | KY514086.1 | 2.870 | 0.010 | 2.105 |

Table 7 Different levels of homogeneity, distance, and divergence of substitution rate are indicated by the alignment of many insect orders with the species name and accession number.

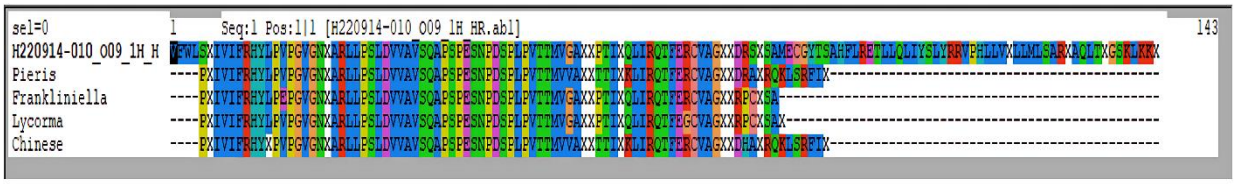


**Figure 1.** Showed alignment of the most identical sequence to the attained sequence of *T. ni* 18S rRNA protein.

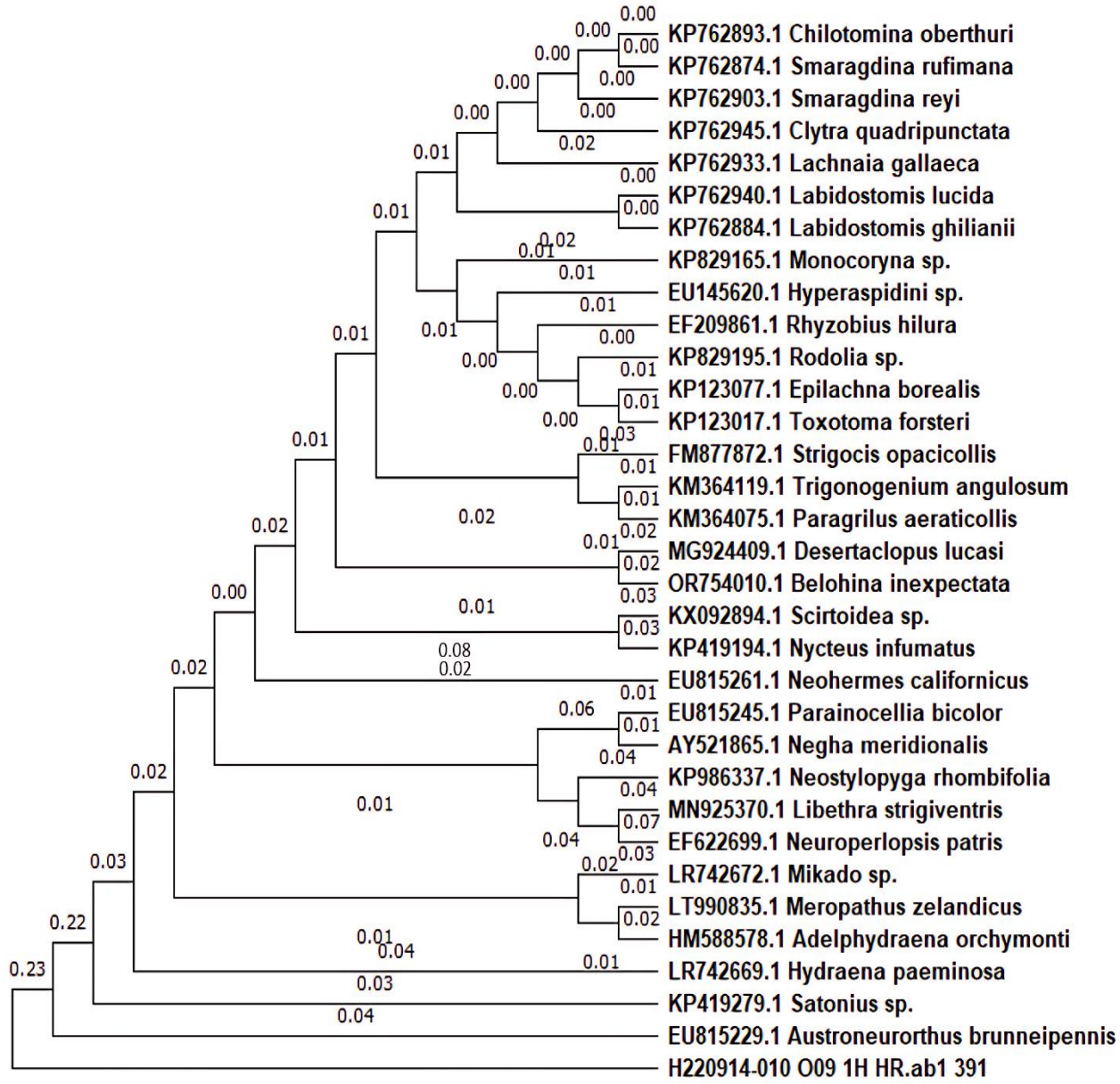


**Figure 2.** Phylogram characterize the phylogenetic relationships among major evolutionary lineages of *T. ni* were investigated using partial sequences of nuclear DNA, 18S. Numbers above branches represent bootstrap values; numbers below branches are posterior probability values.

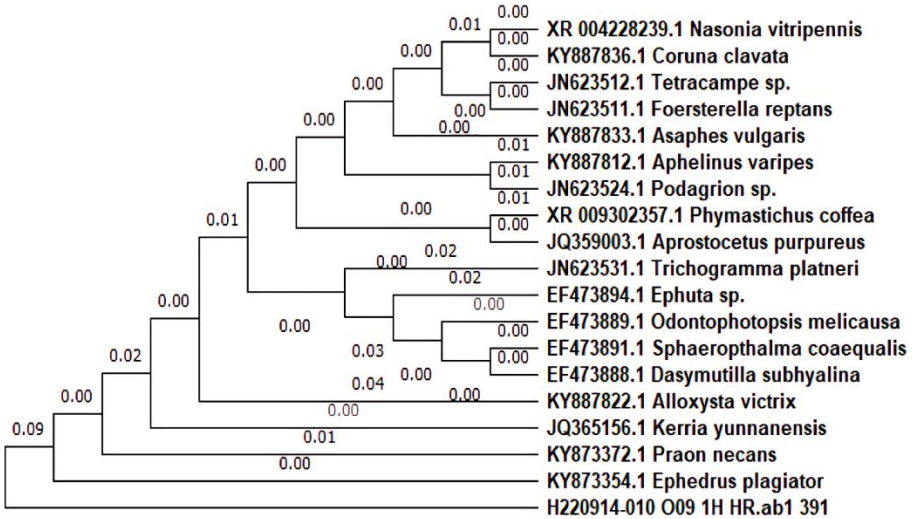


**Figure 3.** Phylogram characterize the phylogenetic relationships among major evolutionary lineages of *T. ni* were investigated using partial sequences of nuclear DNA, 5S ribosomal protein. Numbers above branches represent bootstrap values; numbers below branches are posterior probability values.

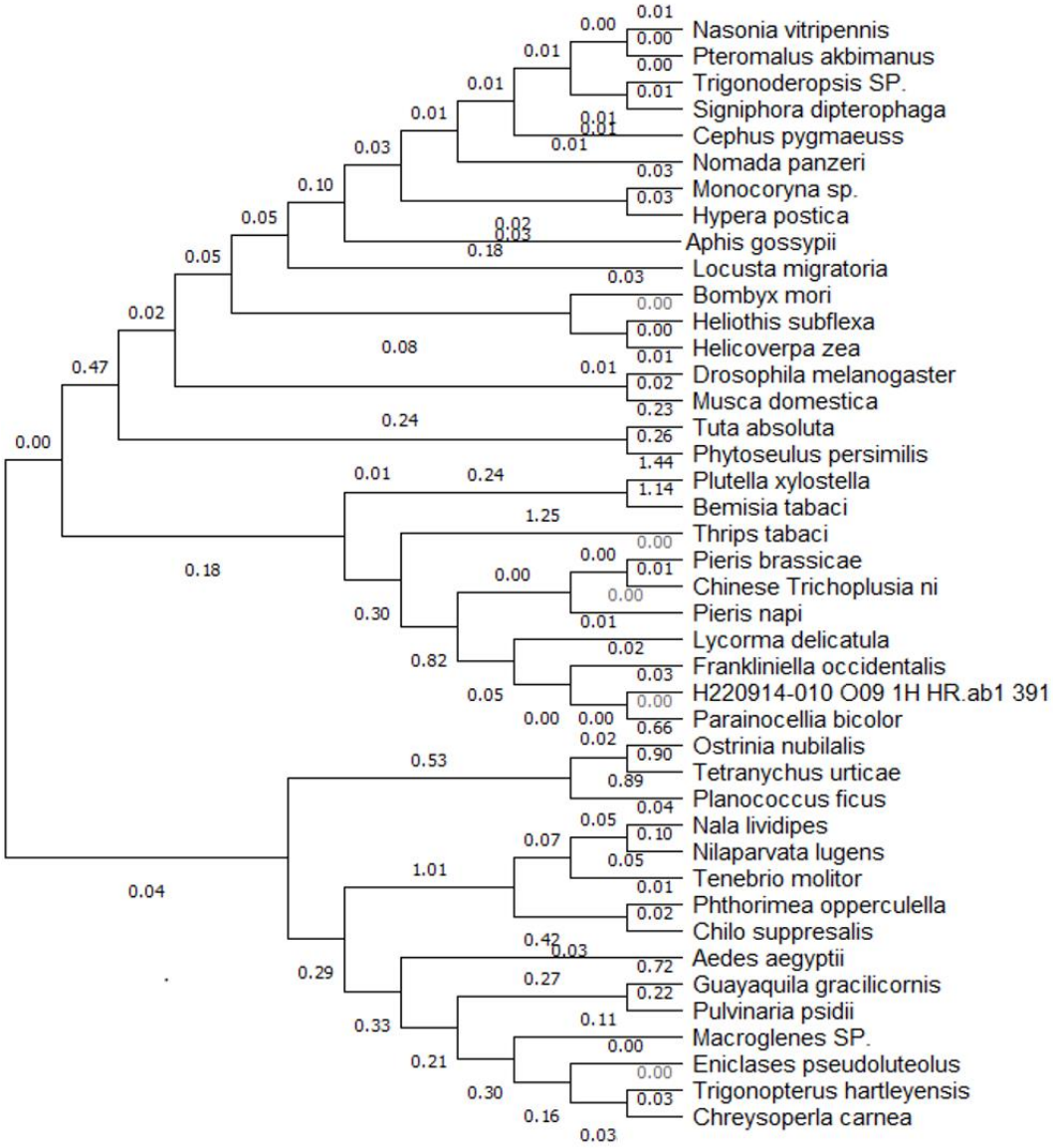


**Figure 4.** Phylogram of 18s rRNA of Egyptian *T.ni* sequence aligned with other insect orders best ML tree of phylogenetic analysis. Numbers above branches represent bootstrap values; numbers below branches are posterior probability values.

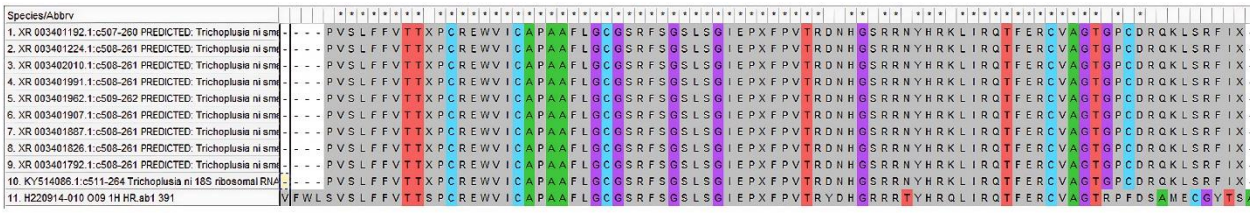


**Figure 5.** Aligned translated sequence of some Chinese *T. ni* with Egyptian *T.ni* sequence.

Figure 6. The best ML tree of phylogenetic analysis shows the phylogram of the Egyptian T . ni sequence's 5s rRNA aligned with other insect orders. Bootstrap values are represented by numbers above branches, while posterior probability values are represented by numbers below branches.

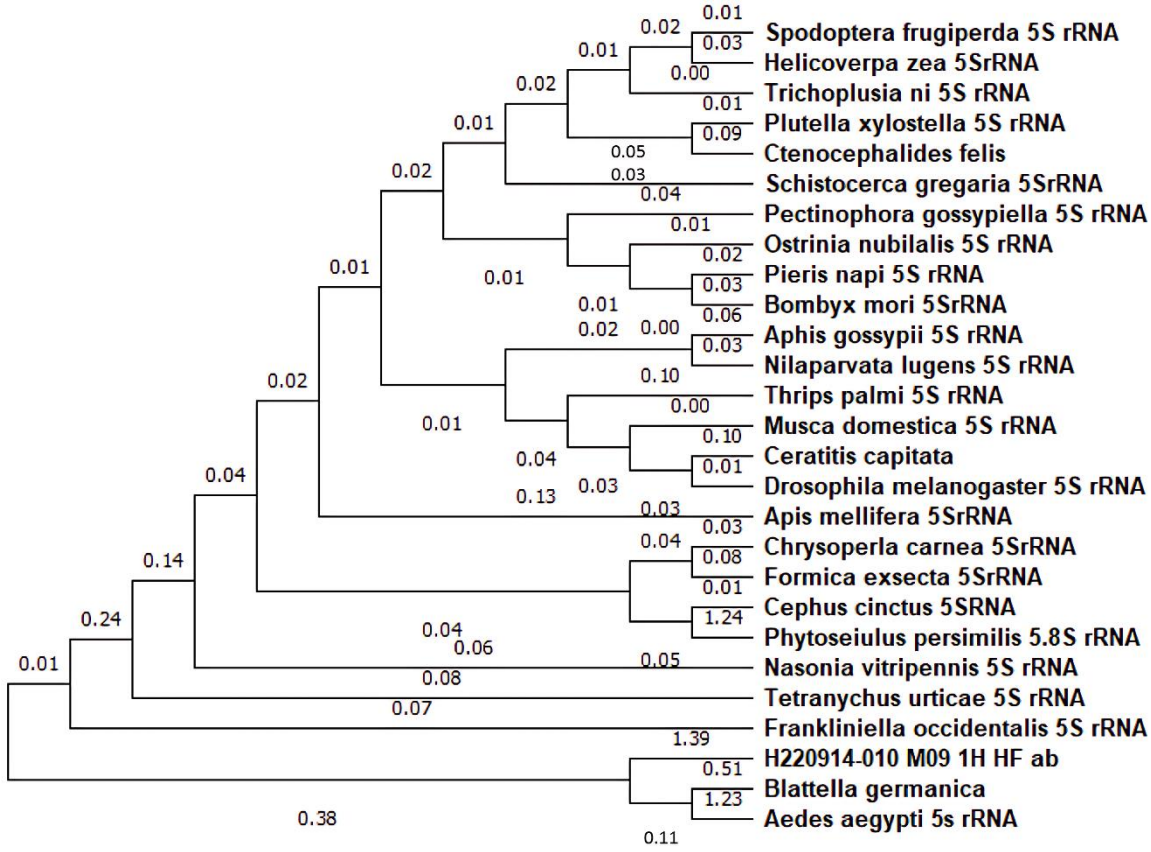


**Figure 6.** Phylogram of 5s rRNA of Egyptian *T.ni* sequence aligned with other insect orders best ML tree of phylogenetic analysis. Numbers above branches represent bootstrap values; numbers below branches are posterior probability values

## 4. CONCLUSIONS

The main goal of this study is to realize the phylogenetic relationships among *T. ni* species and others and between evolutionarily closer clades based on 18S rRNA gene markers. From the results, it can be concluded that the 18S rRNA gene is useful for differentiation between a variety of insects and the agricultural economic moth group. The phylogenetic analysis tool exhibits ease of data handling and provides a clear illustration of this subject of molecular research, depending on the structures, the position of substitutions, and the necessity of aligning sequences, which are characters determining similarity and homology.

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**Competing Interests:** The author declares that there are no conflicts of interests regarding the publication of this paper.

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