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Article

Molecular Diagnosis of Sunn Pest (Eurygaster Integriceps)

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Abstract: Sun pests (Eurygaster integriceps) pose a significant threat to wheat and barley production, necessitating effective control strategies. Molecular tools like DNA barcoding and analysis of the cytochrome oxidase subunit 1 (COI) gene offer promising avenues for species identification. However, the specific application of these techniques for sunn pest identification remains underexplored. Addressing this gap, our research aimed to utilize DNA barcoding and COI gene analysis for accurate sunn pest species differentiation. Our results demonstrate that DNA barcoding is a suitable diagnostic approach, particularly through COI gene amplification, enabling reliable species identification compared to reference sequences from GenBank or Boldsystems. This finding not only fills a crucial knowledge void but also provides opportunities for tailored treatments targeting sunn pests, thereby enhancing crop protection measures.

Keywords: Sunn pest, Eurygaster integriceps, Scutelleridae family

1. Introduction

Eurygaster integriceps, or sunn pest, is a member of the Scutelleridae family in the Hemiptera order. Sunnpest is a dangerous pest that feeds on leaves, stems, and grains, causing significant quantitative and qualitative (gluten protein degradation) damage to crops (sometimes up to 100%)[1]. Sunn pest molecular gene identification can be a focus for creating new instruments in integrated pest control because there is a dearth of genetic data regarding this significant pest.

Molecular genetic techniques, including DNA barcoding and phylogenetic analysis, have gained significant traction in recent times as tools for determining an organism's taxonomic affiliation. It has been demonstrated that DNA barcoding is an effective method of identifying organisms[2]. A gene fragment is amplified, sequenced, and compared to the matching sequences in databases that are now available, such as GenBank and Boldsystems. Animal mitochondrial cytochrome c oxidase subunit I (COI) is the gene most frequently employed for barcoding). Crop pests may be quickly identified via DNA barcoding, which would enable crops to be treated differently.

The research is aims at utilization of molecular means in diagnosing and understanding the genetic characteristics of the Sunn insect species with emphasis on the analysis of the Eurygaster integriceps voucher VSU_Golub_003 cytochrome oxidase subunit 1 (COI) gene. The main reason for molecular diagnosis is that the identification and classification of insect species is more reliable based on molecular data. With regard to Sunn insects, which might have morphologically similar or cryptic species, the molecular techniques can be most reliable for species identification and differentiation.

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A. Description of sunn pest.

A frequent term for genuine members of the stink bug (Family: Pentatomidae) and shield bug (Family: Scutelleridae) families is "sunn pest." In many wheat-growing nations, the genus Eurygaster spp., which belongs to the Scutelleridae family, is one of the most economically significant pests of wheat.

Both nymphs and adults of the sunn pest inflict damage by feeding on various plant and grain sections, which directly lowers grain output According to Fathi et al. (2010), it breaks down the gluten in seeds, giving the flour poor baking qualities. The sunn pest was initially identified in 1920 in the northern part of Iraq. Reports of it have since come from Arbil, Dahok, Sulaimani, Nainawa, and the central and middle Euphrates provinces[3]. Body: semi-elongated oval shape, 10–12 mm in length, grey to creamy brown colour.

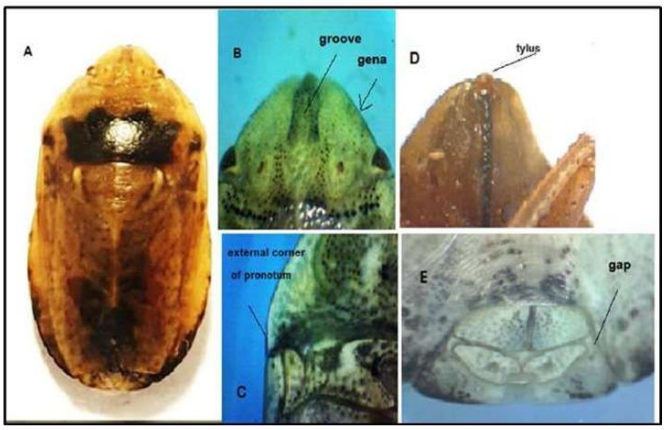


Figure 1. Sunn Pest

Head: semi-triangular in shape, rounded anteriorly, opisthognathous; the lobes on either side of the head, referred to as juga, exist anteriorly and are shorter than the tylus; the head's central depression, or groove, extends to two-thirds of the head, then narrows at the end of the clypus.

The thorax is made up of the pronotum which is shield-like and considerably broader than long. The humerus, which is the lower external corner of the pronotum, is rounded and exists at one level with the body. The prothorax joins the mesothorax at one level with the external corner of the pro-wing from the ventral side.

B. Life Cycle of Sunn Pest

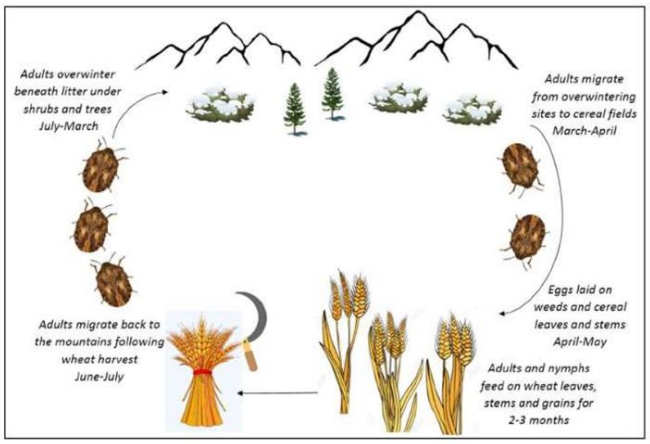


Figure 2. Life Cycle of Sunn Pest

According to Iranipour et al. (2010), Sunn Pest has a monovoltine life cycle (one generation per year) with two primary periods, each of which duration varies with geological location (Fig. 2). There are two phases to Sunn Pest life cycles: an inactive (non-

feeding) phase that begins after crop harvesting and the start of the hot, dry months, and an active (feeding) phase that is when the pests grow and develop in wheat and barley fields[4]. When the average daily temperature rises above 10 °C, over wintered adults migrate to the fields, signalling the start of the active period. In regions with lower temperatures and more frequent rain, this period, which lasts roughly 30 days, may extend.

On leaves, stalks, or weed plants, females lay 70–80 eggs after feeding on different areas of the crop. Five instars feed on wheat from the booting stage till the completion of the dough formation stage, which takes 6–28 days for the egg to develop. While the fifth instar and adults mostly eat growing grains, the first four instars graze on leaves and buds. In order to build up sufficient fat reserves for the overwintering season, adults continue to graze on grains.

When the wheat is harvested and there is no longer any food available and the fields get too hot, sunn pest adults return to mountains and higher elevations. In highlands that are cooler and wetter than the fields, adults aestivate beneath pasture plants like Artemisia spp. and Astragalus spp. as well as trees like oak and conifers[4]. The usual height of estimation sites is between 1700 and 2200 metres, with a preference for northern slopes.Sunn Pests hibernate between the soil's surface and the litter layer beneath the snow.

Adult sunna pests hibernate in the overwintering locations for nine to ten months before returning to the fields in the spring to start feeding[4]. The amount of fat reserves, minimum ambient temperatures, and density- dependent mortality variables all affect the overwintering adults' chances of surviving. Sunn pest management, chemical pesticides have been used for a long time to combat sunn pests, maybe because they are simple to use and effective.

In the Near East and West Asia, some 4 million acres are sprayed each year at a cost of -$150 million[5]. According to Muhammadipour et al. (2015), some of the most widely used registered pesticides in Iran for controlling sunn pests are deltamethrin, fenitrothion, trichlorfon, and lambdacyhalothrin.Depending on the SunnaPest species and stage of life, different insecticides have varying levels of toxicity and efficacy. For instance, report no resistance in E. Integriceps against fenitrothion and alpha-cypermethrin; however, they do record the emergence of resistance in E. Maura against alpha- cypermethrin in Anatolia, Turkey.

Natural enemies, although chemical and cultural treatments are still the mainstays of modern Sunn Pest management tactics, the use of natural enemies has advanced significantly in the last ten years.Numerous studies exist on the effective use of hymenopteran egg parasitoids, particularly those in the genus Trissolcus, as biological control agents for Sunn Pest populations[6].

Ninety percent of the Sunn Pest eggs obtained from Iranian wheatfields were parasitized by this species, according to research on the parasitism rates of T.grandis. Other egg parasitoids species, including T. rufiventris Mayr, T. vassilievi Mayr, and T. festivae, have also been identified as SunnaPest biological control agents[7]. Owing to the significance of Trissolcus spp. in Sunn Pest biological control, a great deal of research has been done on their geographic distribution, establishment rates, population growth in the fields, and the development of mass rearing methods using artificial diets[8].

2. Materials and Methods

Eurigaster Integriceps Put three sample were collected from Plant host wheat, Samples were collected in Iraq from three different areas in Tikrit, Sample one are collected from Hamrin District, sample two from Al-Alam District and sample three from Al-Dour district and samples were analyzed as follows.

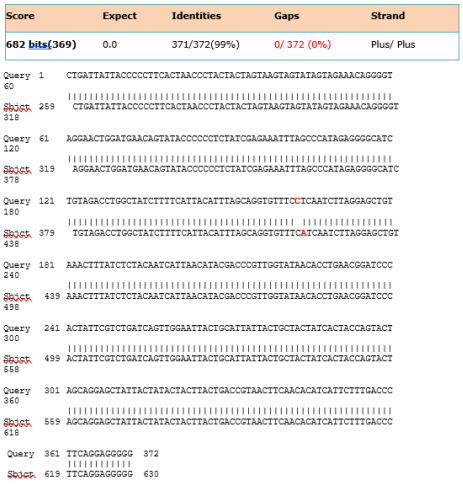
Table 1. Comparative Analysis 1

No.	Type of substitution	Location	Nucleotide	Sequence ID with compare	Source	Identities
1	Transversion	421	C/A	ID: KR105371.1	*Eurygaster integriceps* voucher VSU_Golub_003 cytochrome oxidase subunit 1 (COI) gene, partial cds;	99%
2	Transition	496	T/C	ID: KR105371.1	*Eurygaster integriceps* voucher VSU_Golub_003 cytochrome oxidase subunit 1 (COI) gene, partial cds;	99%
3	Transversion	421	C/A	ID: KR105371.1	*Eurygaster integriceps* voucher VSU_Golub_003 cytochrome oxidase subunit 1 (COI) gene	99%

According to (table 1) Each entry in the table represents one nucleotide substitution in the cytochrome oxidase subunit 1 (COI) gene, partial cds, of Eurygaster integriceps voucher KR105371.1 VSU_Golub_003. The "Identity" reflects a 99% similarity or match. The "Sequence ID" is ID with no further details. The "Nucleotide with compare" column displays the original nucleotide and the replaced nucleotide. The location column specifies the position of the substitution within the gene sequence. The "Type of substitution" indicates whether the substitution is atransversion (change between purine and pyrimidine) or a transition (change within the same type of nucleotide)[9].

Analysis of samples :

1. **Sample 1 C1_CF.**
   Eurygaster integriceps voucher VSU_Golub_003 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial Sequence ID: KR105371.1 Length: 658 Number of Matches: 1, Range 1: 259 to 630.



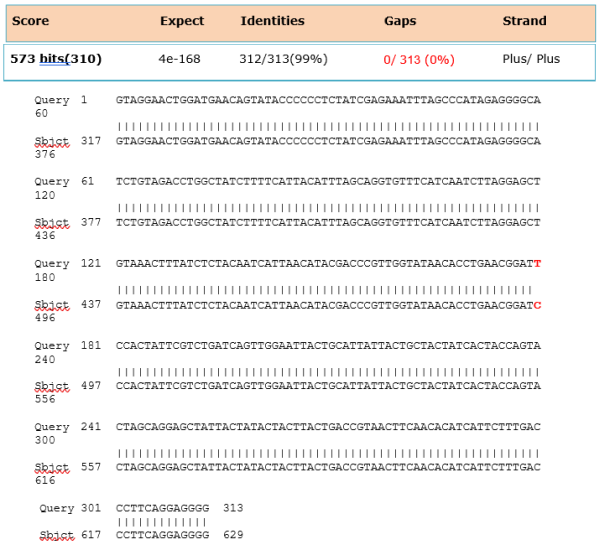
Score	Expect	Identities	Gaps	Strand
682 bits(369)	0.0	371/372(99%)	0/ 372 (0%)	Plus/ Plus

```
Query  1    CTGATTATTACCCCCTTCACTAACCCTACTACTAGTAAGTAGTATAGTAGAAACAGGGGT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  259  CTGATTATTACCCCCTTCACTAACCCTACTACTAGTAAGTAGTATAGTAGAAACAGGGGT  318

Query  61   AGGAACTGGATGAACAGTATACCCCCCTCTATCGAGAAATTTAGCCCATAGAGGGGCATC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  319  AGGAACTGGATGAACAGTATACCCCCCTCTATCGAGAAATTTAGCCCATAGAGGGGCATC  378

Query  121  TGTAGACCTGGCTATCTTTTCATTACATTTAGCAGGTGTTTCCTCAATCTTAGGAGCTGT  180
            |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Sbjct  379  TGTAGACCTGGCTATCTTTTCATTACATTTAGCAGGTGTTTCATCAATCTTAGGAGCTGT  438

Query  181  AAACTTTATCTCTACAATCATTAACATACGACCCGTTGGTATAACACCTGAACGGATCCC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  439  AAACTTTATCTCTACAATCATTAACATACGACCCGTTGGTATAACACCTGAACGGATCCC  498

Query  241  ACTATTCGTCTGATCAGTTGGAATTACTGCATTATTACTGCTACTATCACTACCAGTACT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  499  ACTATTCGTCTGATCAGTTGGAATTACTGCATTATTACTGCTACTATCACTACCAGTACT  558

Query  301  AGCAGGAGCTATTACTATACTACTTACTGACCGTAACTTCAACACATCATTCTTTGACCC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  559  AGCAGGAGCTATTACTATACTACTTACTGACCGTAACTTCAACACATCATTCTTTGACCC  618

Query  361  TTCAGGAGGGGG  372
            ||||||||||||
Sbjct  619  TTCAGGAGGGGG  630
```

Figure 3. C1_CF Analysis of Eurygaster Integriceps Voucher

This sample, given as C1_CF 386, refers to the VSU_Golub_003 voucher of Eurygaster integriceps, which is cytochrome oxidase subunit 1 (COI) gene. In the case of this sequence, it has a length of 658 nucleotides and shows a high similarity score of 682 bits. There is a match with only one other sequence, with 99% identity. No gaps were observed, and the strand is in the plus direction[10].

2. **Sample 2 C2_CF.**
   Eurygaster integriceps voucher VSU_Golub_003 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial Sequence ID: KR105371.1 Length: 658 Number of Matches: 1, Range 1: 317 to 629.



Score	Expect	Identities	Gaps	Strand
573 bits(310)	4e-168	312/313(99%)	0/ 313 (0%)	Plus/ Plus

```
Query  1    GTAGGAACTGGATGAACAGTATACCCCCCTCTATCGAGAAATTTAGCCCATAGAGGGGCA  60
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  317  GTAGGAACTGGATGAACAGTATACCCCCCTCTATCGAGAAATTTAGCCCATAGAGGGGCA  376

Query  61   TCTGTAGACCTGGCTATCTTTTCATTACATTTAGCAGGTGTTTCATCAATCTTAGGAGCT  120
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  377  TCTGTAGACCTGGCTATCTTTTCATTACATTTAGCAGGTGTTTCATCAATCTTAGGAGCT  436

Query  121  GTAAACTTTATCTCTACAATCATTAACATACGACCCGTTGGTATAACACCTGAACGGATT  180
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  437  GTAAACTTTATCTCTACAATCATTAACATACGACCCGTTGGTATAACACCTGAACGGATC  496

Query  181  CCACTATTCGTCTGATCAGTTGGAATTACTGCATTATTACTGCTACTATCACTACCAGTA  240
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  497  CCACTATTCGTCTGATCAGTTGGAATTACTGCATTATTACTGCTACTATCACTACCAGTA  556

Query  241  CTAGCAGGAGCTATTACTATACTACTTACTGACCGTAACTTCAACACATCATTCTTTGAC  300
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  557  CTAGCAGGAGCTATTACTATACTACTTACTGACCGTAACTTCAACACATCATTCTTTGAC  616

Query  301  CCTTCAGGAGGGG  313
            |||||||||||||
Sbjct  617  CCTTCAGGAGGGG  629
```

Figure 4. C2_CF. Analysis Eurygaster Integriceps Voucher

This sample (C2_CF 327) also corresponds to the Eurygaster integriceps voucher VSU_Golub_003 cytochrome oxidase subunit 1 (COI) gene. The sequence has a length of 658 nucleotides and shows a high similarity score of 573 bits. There is a match with one other sequence, which gives an identity of 99%. No gap was noted, and the strand is in the plus direction.

3. Sample 3 C3_CF.

Eurygaster integriceps voucher VSU_Golub_003 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial Sequence ID: KR105371.1 Length: 658 Number of Matches: 1, Range 1: 287 to 630.

Score	Expect	Identities	Gaps	Strand
630 bits(341)	0.0	343/344 (99%)	0/ 344 (0%)	Plus/ Plus

```
Query  1    CTACTAGTAAGTAGTATAGTAGAAACAGGGGTAGGAACTGGATGAACAGTATACCCCCCT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  287  CTACTAGTAAGTAGTATAGTAGAAACAGGGGTAGGAACTGGATGAACAGTATACCCCCCT  346

Query  61   CTATCGAGAAATTTAGCCCATAGAGGGGCATCTGTAGACCTGGCTATCTTTTCATTACAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  347  CTATCGAGAAATTTAGCCCATAGAGGGGCATCTGTAGACCTGGCTATCTTTTCATTACAT  406

Query  121  TTAGCAGGTGTTTCCTCAATCTTAGGAGCTGTAAACTTTATCTCTACAATCATTAACATA  180
            |||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  407  TTAGCAGGTGTTTCATCAATCTTAGGAGCTGTAAACTTTATCTCTACAATCATTAACATA  466

Query  181  CGACCCGTTGGTATAACACCTGAACGGATCCCACTATTCGTCTGATCAGTTGGAATTACT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  467  CGACCCGTTGGTATAACACCTGAACGGATCCCACTATTCGTCTGATCAGTTGGAATTACT  526

Query  241  GCATTATTACTGCTACTATCACTACCAGTACTAGCAGGAGCTATTACTATACTACTTACT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  527  GCATTATTACTGCTACTATCACTACCAGTACTAGCAGGAGCTATTACTATACTACTTACT  586

Query  301  GACCGTAACTTCAACACATCATTCTTTGACCCTTCAGGAGGGGG  344
            |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  587  GACCGTAACTTCAACACATCATTCTTTGACCCTTCAGGAGGGGG  630
```

Figure 5. C3_CF. Eurygaster Integriceps Voucher

Comment: This sample (C3_CF 356) is referring to Eurygaster integriceps voucher VSU_Golub_003, which is the cytochrome oxidase subunit 1 (COI) gene. The length is 658 nucleotides, which corresponds to a high similarity score of 630 bits. There is one matching sequence and a 99% identity. No gaps were observed, and the strand is in the plus direction[11].

3. Results and Discussion

The results presented in the document provide useful information regarding the molecular diagnosis of the Sunn insect, ascertaining analyses within the COI gene. Specifically, the analyzed Eurygaster integriceps voucher VSU_Golub_003 cytochrome oxidase subunit 1 (COI) gene has led to the identification of nucleotide substitutions and their class (transversion and transition). The presence of substitutions of C to A at the 421 nucleotide position and T to C at the 496 nucleotide position indicates genetic variation within the COI gene of the Sunn insect population. Such genetic variations can provide the basis for the identification of the molecular diagnostic technique based on the species identification and analysis of population structure.

Transversion and transition substitutions are common types of genetic variation that contribute to genetic diversity in a species. This type of substitution may arise as a result of different processes, such as evolution, mutational events, or exposure to environmental influences. When one looks at these substitutions, it points out that someone will know what might be the genetic structure or the dynamics of the population within this insect species. The high percentages of similarity observed (99%) in the results clearly showed that most of the samples and the reference sequence (Eurygaster integriceps voucher KR105371.1 VSU_Golub_003 cytochrome oxidase subunit 1 (COI) gene) were very similar. This suggests that the analyzed samples are probably from the same species or closely related taxa.

Molecular diagnosis is, therefore, essential in pest management and biodiversity conservation studies. It helps in the classification of insect species, among other things, because with DNA sequencing and analyses, it can provide highly accurate identification and classification that is particularly important when dealing with morphologically similar or cryptic species. The COI gene has been applied as a DNA barcode marker for species identification since the specific conservation of segments and variable regions can allow species to be differentiated.

The results of the document may be taken in the sense of paving a way to molecular knowledge about the Sunn insect and perhaps research may be furthered to understand genetic diversity and relationships within populations and evolutionary history of this particular insect. In this case, the increase of the sample, analysis of more markers, and integration of phylogenetic analysis in the future studies will give more details in the search of the structure and relationships within the Sunn insect population.

In general, the presented results highlight the importance of molecular diagnostic work regarding understanding the genetic characteristics and diversity of the Sunn insect. That knowledge will have practical applications in pest management strategies, biodiversity conservation, and evolutionary studies.

4. Conclusion

The findings of the research further emphasize the significance of the application of molecular data in the identification and classification of insect species, particularly for those who could be cryptically similar or have morphologically little to distinguish them, like sunn pests. Therefore, DNA barcoding has been proved effective in quick and precise identification of crop pests by using gene sequencing and comparing with databases such as GenBank or Boldsystems. This allows one to devise and apply specific treatment strategies for different pest species for good pest control and crop safety. Besides, this research has also shed light on the life cycle and behavioral facts of sunn pests.

It referred to two phases of their life cycle: an inactive phase occurring during hot, dry months, where the pests remain dormant, and an active phase where the pests feed and develop in fields of wheat and barley. It was underlined how temperature and rainfall help determine the duration of these phases. It discussed different management strategies against sunn pests. It has widely been reported to use the chemical insecticides that are known to control the pests but in some species, they have developed resistance that is raising concern. Their natural enemies, insect parasitoids of the Hymenopteran Trissolcus genus, offer excellent results for biological control.

Efforts have been made in the past to explore their distribution, establish rates, population growth, and mass rearing techniques. In summary, the research shows that the use of molecular techniques, especially DNA barcoding, plays an important role in the identification and classification of sunn pests. This knowledge about the genetic characteristics and behavior of these pests aids in formulating effective strategies for pest management, which involves targeted treatments and biological control methods.

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