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MOLECULAR CHARACTERIZATION AND DNA BARCODING OF GROUNDNUT BRUCHID *CARYEDON GONAGRA* (COLEOPTERA: BRUCHIDAE) FROM ANDHRA PRADESH, INDIA

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ABSTRACT

Six different populations of groundnut bruchid, *Caryedon gonagra*, were collected from groundnut growing districts of Andhra Pradesh, India viz., Anantapuram, Guntur, Chittoor and Kurnool. Molecular characterization was carried out using partial mitochondrial cytochrome B (mtCO1) gene sequences at Department of Entomology, S.V. Agricultural College, Tirupati. The obtained sequences were submitted to the NCBI database and showed 97 to 98% homology with *C. gonagra* sequences from Africa and Mexico. Taxonomic confirmation was further carried out by the examination of male genitalia of holotype specimens, verifying the identity of *C. gonagra* in all six bruchid populations. The sequences were aligned and a phylogenetic tree was constructed using the neighbor-joining method with bootstrap analysis, revealing genetic relationships among the populations. The six *C. gonagra* sequences were deposited in the NCBI GenBank with accession numbers MZ397879 (Atp-1), MZ397880 (Atp-2), MZ397881 (Atp-3), MZ397882 (Bpt), MZ397883 (Tpt), and MZ397884 (Adh). DNA barcodes were generated for the first time from the Andhra Pradesh, India.

Keywords: Groundnut, *Caryedon gonagra*, Molecular characterization, Accession numbers

Introduction

Groundnut (*Arachis hypogaea* L.), the major oilseed crop, is a self-pollinated annual legume crop, widely grown for its high-quality edible oil and food use in the tropical and subtropical regions of the world. The stored groundnut either pods or kernels are very much susceptible to insects, fungi and mites. It was estimated that 6-10 per cent losses in groundnut was due to storage pests (Ahir *et al.*, 2018). More than 100 species of insects infesting the stored groundnuts (RangaRao *et al.*, 2010). *Caryedon* spp. are the most potential and economically important insect pests contributing significant post-harvest losses and deterioration of seed quality. It is the only insect species that can infest kernels inside the intact pod. In 1920, Bridwell identified a seed beetle infesting *Prosopis juliflora* seeds in Hawaii as *Caryoborus gonagra* (F.). Several specimens of a species of *Caryedon* also identified as *C. gonagra* (F.) were reared from *Tamarindus indica* seeds. Subsequent reports of the bruchid in the new World were under the name *C. serratus* (Romero & Johnson 2002). *Caryedon gonagra* and *C. serratus* appear as sister

species deriving from an ancestor that may have fed on both Mimosoideae and Caesalpinioideae (Delobel *et al.*, 2003). Accurate identification is important to carryout timely and specific control measures. Traditional taxonomy, which is mostly focused on external morphology is used to identify the insects (Rebijith *et al.*, 2012). However, taxonomic keys are frequently supplied for only specific life stages or genders; phenotypic differences in taxonomically relevant features can also make species identification problematic (Ball and Armstrong, 2006). The taxonomic analysis found that there is a significant amount of morphological variation between species, which makes it difficult to identify insects, particularly stored grain pests (Singh and Singh, 2014).

DNA barcoding, a modern approach has the potential to reduce the difficulties associated with insect pest identification (Hanner *et al.*, 2009 and Sethusa *et al.*, 2014). As compared to traditional approaches, DNA barcoding employing Barcode index numbers (BINs) provides improved taxonomic resolution in specimen identification up to the species level. The Barcode of Life Data Systems (BOLD) is a

publicly accessible domain providing a reference library and analytical capabilities for DNA barcode projects. Deep genetic divergences within a species are viewed as a potential indicator of cryptic species and molecular diversity studies can be an important first step in identifying cryptic species (Hereward *et al.*, 2017). Phylogeographic studies are viewed as a valuable tool for pest species management and to be taken up to know the diversity of insects in a particular region (Porretta *et al.*, 2007).

Materials and Methods

Collection of bruchid adults

Adult insects of *Caryedon gonagra* were collected from different storage godowns located at different groundnut growing areas of Andhra Pradesh, India (Table 1). The collected bruchids were transferred to 70 per cent ethyl alcohol and were stored in deep freezer (-20 °C).

Table 1: Groundnut bruchid, *Caryedon gonagra*.collected from different geographical locations of Andhra Pradesh, India

S. No.	Location	Latitude	Longitude	Population code
1.	Reddipalli, Anantapur (dt), Andhra Pradesh, India	14.1765 ⁰ N	78.0367 ⁰ E	Atp-1
2.	Rekulakunta, Anantapur (dt), Andhra Pradesh, India	14.6938 ⁰ N	77.6696 ⁰ E	Atp-2
3.	Anantapur, Anantapur (dt), Andhra Pradesh, India	14.6819 ⁰ N	77.6006 ⁰ E	Atp-3
4.	Bapatla, Guntur (dt), Andhra Pradesh, India	15.9039 ⁰ N	80.4671 ⁰ E	Bpt
5.	Tirupati, Chittoor (dt), Andhra Pradesh, India	13.6288 ⁰ N	79.4192 ⁰ E	Tpt
6.	Adhoni, Kurnool (dt), Andhra Pradesh, India	15.6319 ⁰ N	77.2759 ⁰ E	Adh

Extraction of Genomic DNA and PCR Amplification of mtCO1 Gene in groundnut bruchid *Caryedon gonagra*:

Total genomic DNA was extracted from individual adult bruchid by using modified CTAB method (Murray and Thomson, 1980) and dissolved in 50µl of sterile distilled water. The DNA samples stored at -20 °C and subjected to Polymerase chain reaction (PCR) amplification by using *Caryedon* specific primer related to partial mtCO1 region i.e., Forward - 5'-TATGTACTACCATGAGGACAAATATC-3' and Reverse - 5'-ATTACACC TCCTAATTTATTAGG AAT-3' (Sembene, 2006). PCR reaction was performed in 25µl volume of mix containing the components of 10x PCR reaction buffer, 2.5 mM of MgCl₂, 10 mM of dNTPs, 10 pM of each primer, 2.5 units of Taq DNA polymerase and 100 ng of DNA template. The amplification was performed in a PCR machine and the conditions for amplification of target DNA are 1 cycle of 94 °C for 4min for initial denaturation, 94 °C for 30s denaturation, 48 °C for 45s annealing, 72 °C for 1 min extension (35 cycles) and 1 cycle of 72°C for 10 min final extension. The obtained PCR products were analysed on 1 per cent agarose gel stained with 6X bromophenol blue loading dye (Fermentas, USA) in 1x TBE buffer. The PCR amplified DNA fragments were extracted from the excised gel slices using QIA quick columns (Qiagen, Germany) kit. The eluted PCR product was sequenced at automated DNA sequencing facility (Eurofin Genomics India Pvt. Ltd., Bangaluru).

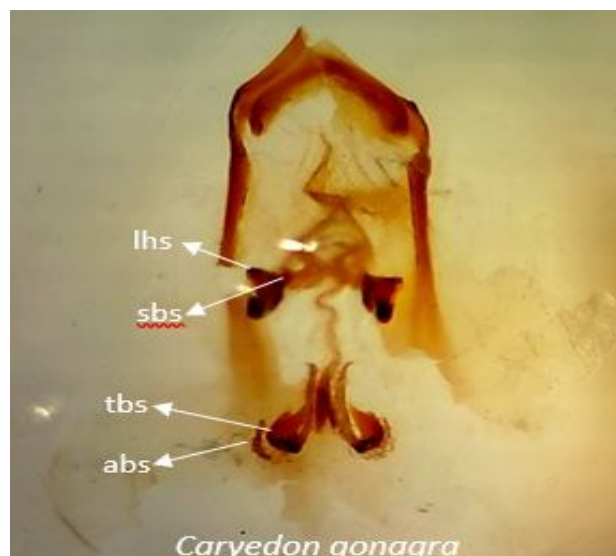
Phylogenetic Analysis and DNA barcode generation:

Sequence assembling and nucleotide alignment were done with Bio Edit version 7.0 software (Hall, 1999). Sequences obtained were analyzed using BLAST program in National center for Biotechnology Information (NCBI) GenBank database to confirm identification of *Caryedon gonagra*. Percent identity matrix table was constructed with Bio Edit version 7.0 software (Hall, 1999) by comparing the nucleotide sequences with other *Caryedon* spp. sequences mined from NCBI GenBank and a phylogram was constructed from aligned sequences using neighbour-joining method with a boot strap value of 1000 replicates using Mega 7.0 software (Kumar *et al.*, 2016). The sequences were deposited in NCBI GenBank and accession numbers were obtained. DNA Barcodes were generated using variable sequences in all six populations in Barcode of Life Data System (BOLD)

Results and Discussion

Taxonomic Characterization of Groundnut Bruchid, *Caryedon gonagra*

Taxonomic studies were conducted to confirm the groundnut bruchid, *Caryedon gonagra*. The male genitalia were dissected and observed under Olympus SZX7 Stereozoom Trinocular research microscope attached to camera. The photos were taken with camera attached to microscope with computer system with image analysis software. The results obtained are presented here under (Fig. 1).



lhs: large fishhook-like sclerite, **sbs:** small basal sclerite, **tbs:** thin bent sclerite, **abs:** apical bent sclerite.

Fig. 1 : Different life stages and Male genitalia of *C. gonagra*.

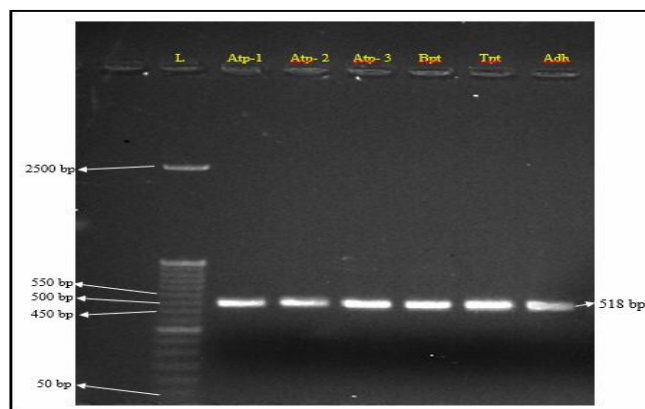
Internal sac of male genitalia of *C. gonagra* indicated that the first pair of sclerites (tbs). First pair is in the form of large hooks (lhs). Second pair is small and located near the base of first pair. Third pair (tbs) of sclerite is enlarged at base with almost parallel sided stem and is slightly curved before the blunt apex. The base of fourth pair of sclerites (abs) is hardly enlarged and gradually thinned out. The distinct characters of male genitalia confirmed as *C. gonagra*. (Delobel *et al.*, 2003).

Molecular Characterization of *C. gonagra*:

The extracted DNA from six samples of groundnut bruchids were amplified with partial mtCO1 primers and PCR product was obtained at 518 bp (Fig. 2). Comparison of mtCO1 gene sequences of six populations (Table 2) revealed that, Anantapuram-Reddipalli (Atp-1) population shared 98.5 per cent nucleotide similarity with both Anantapuram-Rekulakunta (Atp-2) and Anantapuram local (Atp-3) population and 97.6 per cent similarity with both Guntur-Bapatla (Bpt), Chittoor-Tirupati (Tpt) and Kurnool-Adhoni (Adh) population. Based on the above results, we concluded that, among the six populations Anantapuram populations were closely related to each other while Chittoor-Tirupati (Tpt) and Kurnool-Adhoni (Adh) populations are considered as same population, as they exhibited 100 per cent similarity.

The multiple nucleotide sequence analysis of mitochondrial gene sequences of six populations under study with other *Caryedon* spp. in NCBI database revealed that, Anantapuram-Reddipalli (Atp-1) population shared maximum similarity (98.1 per cent) with *C. gonagra*-Africa population (AY449489) while

97.8 per cent similarity with *C. gonagra*-Mexico (KP216600 and KP216601) populations. Mitochondrial sequence of Atp-2 population shared 97.6 per cent and 97.1 per cent similarity with *C. gonagra* of Africa (AY449489) and Mexico (KP216600) populations, respectively.



ATP-1: Anantapuram, Reddipalli; ATP-2: Anantapuram, Rekulakunta; ATP-3: Anantapuram local; Bpt: Guntur, Bapatla; Tpt: Chittoor, Tirupati; Adh: Kurnool, Adhoni; L = 50 bp Ladder.

Fig. 2 : Agarose gel electrophoresis of PCR amplified DNA of mitochondrial partial cytochrome B region of six populations of groundnut bruchid, *Caryedon gonagra* collected from different locations of Andhra Pradesh, India.

Atp-3 (Anantapuram local) shared 98.5 per cent and 97.1 per cent similarity with *C. gonagra* of Africa (AY449489) and Mexico (KP216600) populations, respectively. While all three populations Guntur-Bapatla (Bpt), Chittoor-Tirupati (Tpt) and Kurnool-Adhoni (Adh) shared highest similarity (97.6%) with *C. gonagra*-Africa population (AY449489).

Table 2. Similarity matrix of mitochondrial sequences of six *Caryedon* populations with other *Caryedon* spp retrieved from NCBI (GenBank)

S. No.	Population name	1	2	3	4	5	6	7	8	9	10	11	12	13
1	Anantapuram-Reddipalli, A.P, India	ID	0.985	0.985	0.976	0.976	0.976	0.981	0.978	0.978	0.976	0.910	0.910	0.908
2	Anantapuram-Rekulakunta, A.P, India	0.985	ID	0.985	0.988	0.988	0.988	0.976	0.969	0.971	0.966	0.917	0.917	0.915
3	Anantapuram local, A.P, India	0.985	0.985	ID	0.976	0.976	0.976	0.985	0.969	0.971	0.966	0.906	0.906	0.903
4	Guntur-Bapatla, A.P, India	0.976	0.988	0.976	ID	0.995	0.995	0.976	0.969	0.971	0.966	0.913	0.917	0.915
5	Chittoor-Tirupati, A.P, India	0.976	0.988	0.976	0.995	ID	1	0.976	0.969	0.971	0.966	0.913	0.917	0.915
6	Kurnool- Adhoni, A.P, India	0.976	0.988	0.976	0.995	1	ID	0.976	0.969	0.971	0.966	0.913	0.917	0.915
7	AY449489-Caryedon gonagra: Africa	0.981	0.976	0.985	0.976	0.976	0.976	ID	0.973	0.976	0.971	0.908	0.908	0.905
8	KP216601-Caryedon gonagra: Mexico	0.978	0.969	0.969	0.969	0.969	0.969	0.973	ID	0.997	0.997	0.901	0.905	0.908
9	KP216600-Caryedon gonagra: Mexico	0.978	0.971	0.971	0.971	0.971	0.971	0.976	0.997	ID	0.995	0.903	0.908	0.910
10	KP216599-Caryedon gonagra: Mexico	0.976	0.966	0.966	0.966	0.966	0.966	0.971	0.997	0.995	ID	0.898	0.903	0.905
11	JF957757-Caryedon serratus: West Africa	0.910	0.917	0.906	0.913	0.913	0.913	0.908	0.901	0.903	0.898	ID	0.992	0.988
12	JF957754-Caryedon serratus: West Africa	0.910	0.917	0.906	0.917	0.917	0.917	0.908	0.905	0.908	0.903	0.992	ID	0.990
13	JF957760-Caryedon serratus: West Africa	0.908	0.915	0.903	0.915	0.915	0.915	0.905	0.908	0.910	0.905	0.988	0.990	ID
14	JF957753-Caryedon serratus: West Africa	0.908	0.915	0.903	0.915	0.915	0.915	0.905	0.908	0.910	0.905	0.983	0.990	0.995
15	JF957748-Caryedon serratus: West Africa	0.908	0.915	0.903	0.915	0.915	0.915	0.905	0.908	0.910	0.905	0.990	0.997	0.992
16	AY449487-Caryedon serratus: Africa	0.898	0.900	0.893	0.900	0.900	0.900	0.900	0.900	0.902	0.898	0.936	0.929	0.924
17	JF957759-Caryedon serratus: West Africa	0.906	0.913	0.901	0.913	0.913	0.913	0.903	0.905	0.908	0.903	0.985	0.988	0.997
18	JF957755-Caryedon serratus: West Africa	0.903	0.910	0.899	0.910	0.915	0.915	0.901	0.903	0.905	0.901	0.985	0.992	0.983
19	JF957764-Caryedon serratus: West Africa	0.901	0.908	0.901	0.903	0.903	0.903	0.898	0.894	0.896	0.891	0.978	0.971	0.967
20	JF957758-Caryedon serratus: West Africa	0.901	0.908	0.896	0.908	0.908	0.908	0.898	0.901	0.903	0.898	0.988	0.990	0.985
21	JF957780-Caryedon serratus: West Africa	0.896	0.899	0.892	0.899	0.899	0.899	0.898	0.898	0.901	0.896	0.950	0.943	0.938
22	JF957781-Caryedon serratus: West Africa	0.894	0.896	0.889	0.896	0.896	0.896	0.896	0.896	0.898	0.894	0.948	0.941	0.936
23	AY449480-Caryedon serratus: Africa	0.886	0.898	0.886	0.898	0.893	0.893	0.883	0.879	0.881	0.876	0.938	0.936	0.931
24	JF957778-Caryedon serratus: West Africa	0.892	0.899	0.887	0.894	0.894	0.894	0.889	0.889	0.891	0.887	0.955	0.948	0.943
25	AY449483-Caryedon serratus: Africa	0.884	0.895	0.884	0.895	0.891	0.891	0.881	0.876	0.879	0.874	0.934	0.931	0.927
26	JF957779-Caryedon serratus: West Africa	0.889	0.896	0.884	0.896	0.901	0.901	0.891	0.891	0.894	0.889	0.952	0.945	0.941

ID= Identity matrix

Table 2 Contd....

Table 2. Similarity matrix of mitochondrial sequences of six *Caryedon* populations with other *Caryedon* spp retrieved from NCBI (GenBank)

S. No.	Population name	14	15	16	17	18	19	20	21	22	23	24	25	26
1	Anantapuram-Reddipalli, A.P, India	0.908	0.908	0.898	0.906	0.903	0.901	0.901	0.896	0.894	0.886	0.892	0.884	0.889
2	Anantapuram-Rekulakunta, A.P, India	0.915	0.915	0.900	0.913	0.910	0.908	0.908	0.899	0.896	0.898	0.899	0.895	0.896
3	Anantapuram local, A.P, India	0.903	0.903	0.893	0.901	0.899	0.901	0.896	0.892	0.889	0.886	0.887	0.884	0.884
4	Guntur-Bapatla, A.P, India	0.915	0.915	0.900	0.913	0.910	0.903	0.908	0.899	0.896	0.898	0.894	0.895	0.896
5	Chittoor-Tirupati, A.P, India	0.915	0.915	0.900	0.913	0.915	0.903	0.908	0.899	0.896	0.893	0.894	0.891	0.901
6	Kurnool- Adhoni, A.P, India	0.915	0.915	0.900	0.913	0.915	0.903	0.908	0.899	0.896	0.893	0.894	0.891	0.901
7	AY449489-Caryedon gonagra: Africa	0.905	0.905	0.900	0.903	0.901	0.898	0.898	0.898	0.896	0.883	0.889	0.881	0.891
8	KP216601-Caryedon gonagra: Mexico	0.908	0.908	0.900	0.905	0.903	0.894	0.901	0.898	0.896	0.879	0.889	0.876	0.891
9	KP216600-Caryedon gonagra: Mexico	0.910	0.910	0.902	0.908	0.905	0.896	0.903	0.901	0.898	0.881	0.891	0.879	0.894
10	KP216599-Caryedon gonagra: Mexico	0.905	0.905	0.898	0.903	0.901	0.891	0.898	0.896	0.894	0.876	0.887	0.874	0.889
11	JF957757-Caryedon serratus: West Africa	0.983	0.990	0.936	0.985	0.985	0.978	0.988	0.950	0.948	0.938	0.955	0.934	0.952
12	JF957754-Caryedon serratus: West Africa	0.990	0.997	0.929	0.988	0.992	0.971	0.990	0.943	0.941	0.936	0.948	0.931	0.945
13	JF957760-Caryedon serratus: West Africa	0.995	0.992	0.924	0.997	0.983	0.967	0.985	0.938	0.936	0.931	0.943	0.927	0.941
14	JF957753-Caryedon serratus: West Africa	ID	0.992	0.920	0.992	0.983	0.962	0.985	0.934	0.931	0.927	0.938	0.922	0.936
15	JF957748-Caryedon serratus: West Africa	0.992	ID	0.927	0.990	0.990	0.969	0.988	0.941	0.938	0.934	0.945	0.929	0.943
16	AY449487-Caryedon serratus: Africa	0.920	0.927	ID	0.922	0.931	0.952	0.934	0.985	0.988	0.957	0.976	0.952	0.974
17	JF957759-Caryedon serratus: West Africa	0.992	0.990	0.922	ID	0.981	0.964	0.983	0.936	0.934	0.929	0.945	0.924	0.938
18	JF957755-Caryedon serratus: West Africa	0.983	0.990	0.931	0.981	ID	0.969	0.988	0.945	0.943	0.934	0.950	0.929	0.952
19	JF957764-Caryedon serratus: West Africa	0.962	0.969	0.952	0.964	0.969	ID	0.971	0.967	0.964	0.950	0.971	0.945	0.969
20	JF957758-Caryedon serratus: West Africa	0.985	0.988	0.934	0.983	0.988	0.971	ID	0.948	0.945	0.931	0.952	0.927	0.950
21	JF957780-Caryedon serratus: West Africa	0.934	0.941	0.985	0.936	0.945	0.967	0.948	ID	0.997	0.952	0.985	0.948	0.988
22	JF957781-Caryedon serratus: West Africa	0.931	0.938	0.988	0.934	0.943	0.964	0.945	0.997	ID	0.950	0.988	0.945	0.985
23	AY449480-Caryedon serratus: Africa	0.927	0.934	0.957	0.929	0.934	0.950	0.931	0.952	0.950	ID	0.957	0.995	0.960
24	JF957778-Caryedon serratus: West Africa	0.938	0.945	0.976	0.945	0.950	0.971	0.952	0.985	0.988	0.957	ID	0.952	0.988
25	AY449483-Caryedon serratus: Africa	0.922	0.929	0.952	0.924	0.929	0.945	0.927	0.948	0.945	0.995	0.952	ID	0.955
26	JF957779-Caryedon serratus: West Africa	0.936	0.943	0.974	0.938	0.952	0.969	0.950	0.988	0.985	0.960	0.988	0.955	ID

ID= Identity matrix

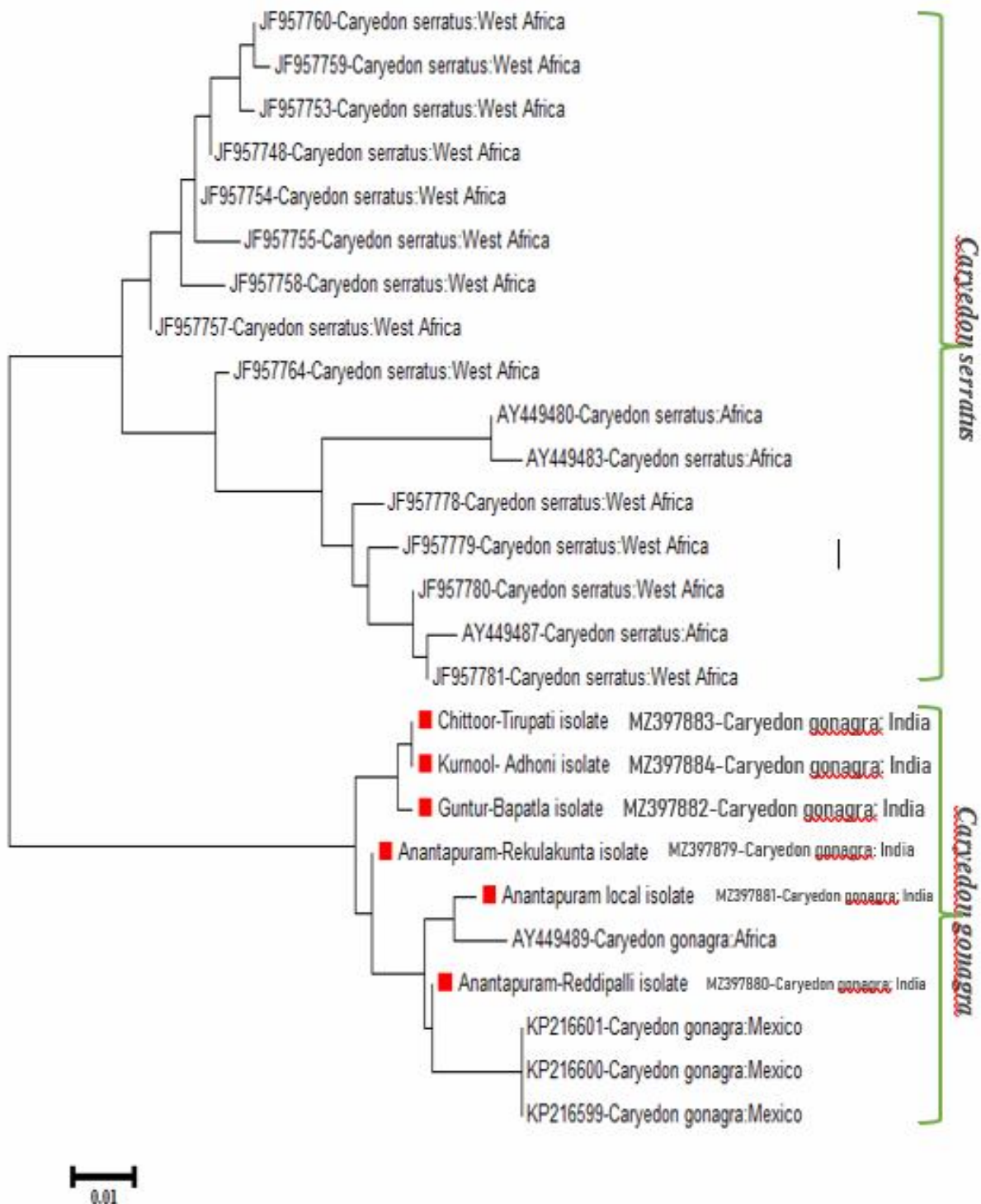


Fig. 3 : Phylogenetic tree of partial mtCO1 gene of *Caryedon gonagra* collected from different districts of Andhra Pradesh, India was constructed with other *Caryedon* spp. mined from NCBI by bootstrap test (1000 replicates) phylogeny using neighbour-joining method of MEGA 7 software to depict the genetic relationships derived from mitochondrial partial cytochrome B region.

■ **Note:** Six populations under present investigation are marked as red

A phylogenetic tree using mtCO1 sequences of *Caryedon gonagra* of Anantapuram-Reddipalli, Anantapuram-Rekulakunta, Anantapuram-local, Guntur-Bapatla, Chittoor-Tirupati and Kurnool-Adhoni

populations was constructed along with other population sequences mined from NCBI GenBank by using neighbour-joining method (Fig. 3). In phylogenetic tree, two major clusters were formed i.e.,

one for *C. gonagra* and another for *C. serratus*, and all populations under study formed a single cluster with *C. gonagra* populations of Mexico and Africa. Anantapuram-Reddipallipopulation formed sub-cluster with Mexico populations (KP216600, KP21661 and KP216599) and Anantapuram-local population formed sub-cluster with *C. gonagra*-Africa population (AY449489).

The populations Chittoor-Titupati and Kurnool-Adhoni have shown close relationship with each other and formed a cluster with Guntur-Bapatla population. Based on the phylogenetic analysis it is concluded that the three populations from Anantapur district are similar while Chittoor, Kurnool and Guntur populations might be same. Moreover, all the six populations are closely related to *C. gonagra* than the *C. serratus*.

Sembene *et al.* (2010) amplified the cytochrome B gene using primers CB1 and CB2, producing a 518 bp fragment. In the present study, we used the same primers and obtained an identical amplicon size (518 bp) in *Caryedon* species. Sequence analysis of samples collected from different districts of Andhra Pradesh

showed highest similarity with *C. gonagra*. However, both *C. serratus* and *C. gonagra* were previously reported by Manjunath *et al.* (2020) in the Rayalaseema region of Andhra Pradesh. Their study also indicated genetic differentiation among individuals from different storage locations. Similarly, Sembene *et al.* (2008) found significant genetic differentiation between native and groundnut-feeding forms of *C. serratus*, influenced more by host plant than by geographical distance. In our study, minor genetic differences were observed among populations from different districts, but these were not strongly associated with geographic distance. These findings are in line with the findings of Ndong *et al.* (2011) and Sembene *et al.* (2008), suggesting that host plant plays a more significant role in shaping genetic structure than geography

The *C. gonagra* sequences were submitted to NCBI and accession numbers for six *C. gonagra* populations were obtained and presented in Table 3. DNA Barcodes were generated for all the six populations using BOLD database and presented in Fig 4.

Table 3 : Accession numbers acquired from NCBI for groundnut bruchid, *C. gonagra* collected from different districts of Andhra Pradesh, India

S. No	Population code	Accession number	Latitude	Longitude
1	Atp-1	MZ397879	14.1765 ⁰ N	78.0367 ⁰ E
2	Atp-2	MZ397880	14.6938 ⁰ N	77.6696 ⁰ E
3	Atp-3	MZ397881	14.6819 ⁰ N	77.6006 ⁰ E
4	Bpt	MZ397882	15.9039 ⁰ N	80.4671 ⁰ E
5	Tpt	MZ397883	13.6288 ⁰ N	79.4192 ⁰ E
6	Adh	MZ397884	15.6319 ⁰ N	77.2759 ⁰ E

1. Atp-1 (*Caryedon gonagra*, Reddipalli, Anantapuram, Andhra pradesh, India); GenBank Accession number- – MZ397879



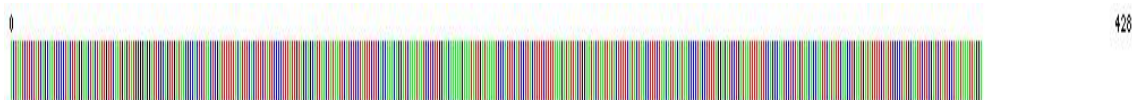
2. Atp-2 (*Caryedon gonagra*, Rekulakunta, Anantapuram, Andhra pradesh, India); GenBank Accession number- – MZ397880



3. Atp-3 (*Caryedon gonagra*, Anantapuram-local, Andhra pradesh, India); GenBank Accession number- – MZ397881



4. **Bpt (*Caryedon gonagra*, Bapatla, Guntur, Andhra pradesh, India); GenBank Accession number- – MZ397882**



5. **Tpt (*Caryedon gonagra*, Tirupati, Chittoor, Andhra pradesh, India); GenBank Accession number- – MZ397883**



6. **Adh (*Caryedon gonagra*, Adhoni, Kurnool, Andhra pradesh, India); GenBank Accession number- – MZ397884**

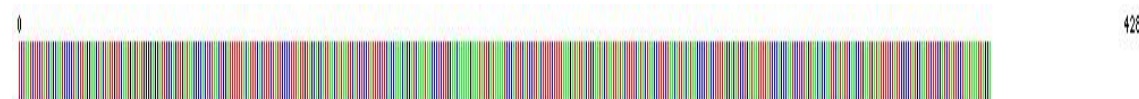


Fig. 4 : DNA Barcode images of six Groundnut bruchid, *C. gonagra* populations collected from different districts of Andhra Pradesh, India.

Conclusions

This study confirms the species of groundnut bruchid, *Caryedon gonagra* in Andhra Pradesh, feeding on groundnut based on Taxonomic and Molecular characterization studies. The genetic diversity of bruchid populations was less and not strongly linked to their geographic locations. This is the first study to generate DNA barcodes of groundnut from Andhra Pradesh, India.

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