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Phylogenetic Reconstruction and Genetic Divergence of Catantopinae (Acrididae) from Western Himalaya using Mitochondrial Markers

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Abstract: The subfamily Catantopinae (Orthoptera: Acrididae) comprise a diverse group of short-horned grasshopper with significant ecological and agricultural importance. Despite their wide distribution and economic relevance, molecular phylogenetic studies on this group, particularly from the Western Himalaya region remain scarce. In this study mtDNA (COI) gene were used to examine the molecular diversity and phylogenetic relationship among selected Catantopinae species collected from lower Western Himalaya of Himachal Pradesh, India. The nucleotide composition revealed a strong AT composition 64.2%. Interspecific divergence among species such as *Diabolocantops innotabilis*, *Stenocantops splendens*, and *Xenocantops humilis* was found to be low (0.0 % - 0.05%), indicating genetic homogeneity across geographic regions. Interspecific divergences ranged from 1.0% to 9.0%, confirming clear genetic boundaries among congeneric taxa. Phylogenetic reconstruction using the Maximum Likelihood Method revealed three well-supported clades corresponding to genera *Diabolocantops*, *Stenocantops* and *Xenocantops* with *Eucrotopa exigua* used as an outgroup.

Keywords: Catantopinae, COI, mtDNA, Phylogenetic tree, Orthoptera

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Introduction

Orthoptera represent most ancient extant insect lineages, comprises 30251 recognized species (Cigliano *et al.*, 2025). The subfamily Catantopinae (Acridoidea, Orthoptera) represents a prominent lineage of grasshoppers which are considered as major agricultural pest (Uvarov,

1966). They inhabit within grassland and serve as crucial function in sustaining the food web (Belovsky and Slade, 1993; Joern *et al.*, 2006). In addition to its ecological and economic importance, the family Acrididae possesses a biogeography history, with an origin to the early

Cenozoic Era, subsequently diversification throughout the mid to late Cenozoic period (Song *et al.*, 2015). Over the past century, the rare orthopteran specimens have many taxonomic challenges and controversies on systematic concept (Eades, 2000; Song, 2010). However, the precise classification of Acrididae orthopterans is difficult due to sexual dimorphism (Simpson and Sword, 2009). Molecular methodologies, such as DNA barcoding have been extensively integrated into systematic investigation to resolve issues of species identification as well other biological inquiries (Hebert *et al.*, 2003; Kundu *et al.*, 2020). Several molecular studies have been undertaken to clarify species identification (Walton *et al.*, 1997; Chapco *et al.*, 1997; Yin *et al.*, 2003; Zhang *et al.*, 2005; Huang *et al.*, 2013; Nazir *et al.*, 2014; Husemann *et al.*, 2015; Song *et al.*, 2018; Kundu *et al.*, 2020; Zhang *et al.*, 2025). However, no study has been conducted on the molecular marker (COI gene) of family Catantopinae from the lower western Himalayan region of Himachal Pradesh, India. Therefore, the mtDNA COI gene has been used in this study to explain the phylogenetic relationship of family Catantopinae in lower western Himalayan region of Himachal Pradesh, India.

Materials and Methods

Sample collection and preparation

The study was conducted on regular basis from 2022 to 2024 at the different sites of lower western Himalayan region of Himachal Pradesh. Collection was made through methods including sweep nets method and direct hand picking to collect the specimens from the different selected sites. Short horned Grasshoppers were generally collected during morning or evening hours. Some specimens were reared in an ethyl acetate glass jar from grassland, crop land, litters, and tea garden, whereas some specimens were kept in 70% ethanol for further molecular studies. The collected specimens were stretched on wooden stretch box with the help of entomological pins. After proper stretching the specimens were stored inside wooden insect box for further identification

along with information of sample site, voucher name and GPS coordinates. Photographs were taken through iPhone 14 Pro max. Specimens were identified in lab through taxonomic key given Kirby (1914) and The Fauna of British India, <https://orthoptera.speciesfile.org/>. Final confirmation of collected samples has been done by Dr. Kamal Saini Scientist B at Zoological Survey of India, Solan, India.

DNA Barcoding

DNA was isolated from samples from hind leg muscle by Macherey-Nagel (NucleoSpin) Tissue DNA kit following the directives provided by the manufacturer. DNA fragments of COI gene was amplified by using Thermocycler (Veriti Model; Applied Biosystems, USA). PCR amplification was performed in 12.5 µl, containing genomic DNA 2.5 µl, premix Ex Taq TAKARA 6.25 µl, Forward primer 0.25 µl, reverse primer 0.25 µl and nuclease free water 3.25 µl. LC01490 GGTCAACAAATCATAAAGATATTGG and HCO-2198 TAAACTTCAGGGTGACCAAAAAATCA primers were used for COI gene amplification (Geller *et al.*, 2013). Following conditions were used to perform PCR. Heated lid 112 °C initially hot start at 95 °C for 5 min then PCR products were subjected to 40 cycles followed by Denaturation at 95°C for 45 sec, annealing at 45°C for 45 sec, elongation at 72°C for 90 sec followed by final extension of 72 °C for 7 min. ExoSAP-IT™ PCR product cleanup reagent (Thermo Fisher) were used to purify the PCR products which were further purified with cleanup reagent and further PCR amplicon sequenced. Sequencer machine 3500 Genetic analyzers (Thermo Fisher) were used for sequencing. The obtained sequenced were deposited to GenBank under accession number PQ463995, PQ466783, PQ466788, PQ467096, PQ468464, PV786827, PV787252, PV786611 and PV786612 (Table 1).

Results and Discussion

Nucleotide Base Composition

Nucleotide composition in the sequences of subfamily Catantopinae is found to be A = 30.2, T=34.0, C=19.3 and G=16.6 which indicate high AT

Table 1: List of specimens of subfamily Catantopinae used for COI gene analysis along with respective GenBank Accession no.

Voucher name	Species	Location	Latitude	Longitude	GenBank Accession no. COI
CPU-7	<i>Diabolocatantops innotabilis</i> (Walker, 1870)	Chamyog, (Hamirpur)	31° 37' 22.728" N	76° 37' 15.7728" E	PQ463995
JG-5	<i>Diabolocatantops innotabilis</i> (Walker, 1870)	Jogindernagar (Mandi)	31° 56' 46.356" N	76° 47' 46.4892" E	PQ466783
BP-8	<i>Diabolocatantops innotabilis</i> (Walker, 1870)	Bharari, (Bilaspur)	31° 34' 44.922" N	76° 40' 1.0056" E	PQ466788
JG-11	<i>Xenocatantops humilis</i> (Serville, 1838)	Chamyog, (Hamirpur)	31° 36' 8.2044" N	76° 39' 4.1688" E	PQ467096
KTH-8	<i>Xenocatantops humilis</i> (Serville, 1838)	Thural khas, (Kangra)	31°54' 36.8424" N	76° 28' 8.1516" E	PQ468464
PLNK@5.1	<i>Xenocatantops karnyi</i> (Kirby, 1910)	Thural khas, (Kangra)	31°54' 34.8048" N	76° 28' 7.0032" E	PV786827
UNKS@2.1	<i>Xenocatantops karnyi</i> (Kirby, 1910)	Jol, Una	31° 32' 5.1324" N	76° 18' 21.9708" E	PV787252
KTH@4.1	<i>Stenocatantops splendens</i> (Thunberg, 1815)	Thural khas, (Kangra)	31°54' 34.8048" N	76° 28' 7.0032" E	PV786611
KTH@4.2	<i>Stenocatantops splendens</i> (Thunberg, 1815)	Thural khas, (Kangra)	31°54' 34.8048" N	76° 28' 7.0032" E	PV786612

Table 2: Base composition of different taxa of Subfamily Catantopinae

S. No.	Taxa	T(U)	C	A	G	Total
1.	<i>Diabolocatantops innotabilis</i> PQ466788	33.0	19.0	31.4	16.5	636.0
2.	<i>Diabolocatantops innotabilis</i> PQ463995	33.0	19.0	31.4	16.5	636.0
3.	<i>Diabolocatantops innotabilis</i> PQ466783	33.1	19.0	31.2	16.6	631.0
4.	<i>Xenocatantops humilis</i> PQ467096	34.2	19.9	29.5	16.4	597.0
5.	<i>Xenocatantops humilis</i> PQ468464	34.2	19.9	29.5	16.4	597.0
6.	<i>Xenocatantops karnyi</i> PV786827	34.5	19.6	29.7	16.3	603.0
7.	<i>Xenocatantops karnyi</i> PV787252	34.5	19.6	29.7	16.3	603.0
8.	<i>Stenocatantops splendens</i> PV786611	34.9	19.2	30.0	15.9	573.0
9.	<i>Stenocatantops splendens</i> PV786612	35.0	19.3	29.8	15.9	571.0
10.	<i>Diabolocatantops pinguis pinguis</i> FJ571154	33.5	18.6	31.3	16.7	636.0
11.	<i>Stenocatantops splendens</i> FJ571155	34.9	18.4	29.2	17.5	636.0
12.	<i>Diabolocatantops pinguis</i> KC139976	34.2	19.4	30.6	15.8	576.0
13.	<i>Stenocatantops splendens</i> KC139978	34.6	18.2	29.7	17.5	636.0
14.	<i>Diabolocatantops innotabilis</i> KJ672135	32.7	19.5	30.8	17.0	636.0
15.	<i>Stenocatantops vitripennis</i> MG888111	32.9	19.5	30.3	17.3	630.0
16.	<i>Xenocatantops humilis</i> MT325832	34.2	19.9	29.5	16.4	597.0
17.	<i>Xenocatantops brachycerus</i> KC139982	34.7	19.6	29.0	16.8	597.0
	Avg.	34.0	19.3	30.2	16.6	611.2

Table 3: Estimate of Substitution Matrix by Maximum Likelihood

	A	T/U	C	G
A	-	3.94	3.94	17.11
T/U	3.94	-	17.11	3.94
C	3.94	17.11	-	3.94
G	17.11	3.94	3.94	-

The maximum Log likelihood for this computation was -1936.358, which involved 17 sequences of nucleotide. A total of 655 positions reported in the final dataset.

Table 4: Estimate of the Pattern of Nucleotide Substitution by Maximum Composite Likelihood

	A	T	C	G
A	-	6.21	3.48	5.35
T	5.44	-	17.49	2.96
C	5.44	31.16	-	2.96
G	9.82	6.21	3.48	-

The nucleotide frequencies are 16.37% [G], 34.31% [T/U], 19.25% [C], and 30.06% [A]. The k_1 value for purines is 1.805 and k_2 value for pyrimidine is 5.02 transition/transversion rate ratios whereas the overall transition/transversion bias is $R = 1.691$, "where $R = [A \cdot G \cdot k_1 + T \cdot C \cdot k_2] / [(A+G) \cdot (T+C)]$ ". This study encompassed 17 nucleotide sequences. All uncertain positions were excluded for each sequence pair utilizing the pair-wise deletion methodology. The final dataset comprised a total of 655 positions (Kimura, 1980; Kumar *et al.*, 2016).

content 64.2 in the subfamily Catantopinae (Table 2).

Intraspecific divergence among species of subfamily Catantopinae

Intraspecific divergence has been calculated for species of subfamily Catantopinae: *Diabolocatantops innotabilis*, *Xenocatantops karnyi*, *Xenocatantops humilis*, *Stenocatantops splendens*, and *Diabolocatantops pinguis pinguis*. *Diabolocatantops innotabilis* (658bp) from India (present study) shows 0.05% and 0.01% divergence with *Diabolocatantops innotabilis* (659bp) from Pakistan (Nazir *et al.*, 2020--sequence retrieved from GenBank data). While it is 0.01% for *Diabolocatantops pinguis pinguis* (685bp) from China (Zhao *et al.*, 2009) and *Diabolocatantops pinguis* (577bp) from China (Huang *et al.*, 2014). It is calculated as 0.0% between *Stenocatantops splendens* from India (present study) while it is 0.04% between the *Stenocatantops splendens* (658bp) from India

(present study) and *Stenocatantops splendens* (659bp) from China (Zhao *et al.*, 2009; Huang *et al.*, 2014). For the specimens of *Stenocatantops splendens* intraspecific distance ranges from 0.05 to 0.04%. Intraspecific distance for *Xenocatantops humilis* (658bp) from India (present study) and *Xenocatantops humilis* (598bp) (Kundu *et al.*, 2020) is found to be 0.0%. The analysis of *Xenocatantops karnyi* (present study) has been studied for the first time as well COI gene analysis on *Xenocatantops karnyi* is the first report worldwide. Therefore, *Xenocatantops karnyi* is the noble contribution for NCBI database.

Interspecific divergence among species of subfamily Catantopinae

Interspecific distance has been calculated for the tribe Catantopini (subfamily Catantopinae; Genus: *Diabolocatantops*). The range of interspecific divergence among *Diabolocatantops innotabilis* (present study) and *Diabolocatantops pinguis pinguis* (Zhao *et al.*, 2009; Huang *et al.*, 2014) is

Table 5: List of taxa of subfamily Catantopinae retrieved from GenBank

S. No.	Taxa	Accession No.	Country	Length of COI gene	Reference
Tribe-Catantopini					
1.	<i>Diabolocatantops innotabilis</i>	KJ672135	Pakistan	659bp	Nazir <i>et al.</i> (2014)
2.	<i>Diabolocatantops pinguis pinguis</i>	FJ571154	China	685bp	Zhao <i>et al.</i> (2009)
3.	<i>Diabolocatantops pinguis</i>	KC139976	China	577bp	Huang <i>et al.</i> (2013)
4.	<i>Xenocatantops brachycerus</i>	KC139982	China	598bp	Huang <i>et al.</i> (2013)
5.	<i>Xenocatantops humilis</i>	MT325832	India	598bp	Kundu <i>et al.</i> (2020)
6.	<i>Stenocatantops splendens</i>	KC139978	China	659bp	Huang <i>et al.</i> (2013)
7.	<i>Stenocatantops splendens</i>	FJ571155	China	689bp	Zhao <i>et al.</i> (2009)
8.	<i>Stenocatantops vitripennis</i>	MG888111	USA	649bp	Song <i>et al.</i> (2018)

Table 6: Intraspecific distance among species of subfamily Catantopinae

S. No.	Taxa	Location	Taxa	Location	% Intraspecific Divergence
1	<i>Diabolocatantops innotabilis</i> PQ463995	India	<i>Diabolocatantops innotabilis</i> PQ466783	India	0
2	<i>Diabolocatantops innotabilis</i> PQ463995	India	<i>Diabolocatantops innotabilis</i> KJ672135	Pakistan	5
3	<i>Diabolocatantops innotabilis</i> PQ466783	India	<i>Diabolocatantops innotabilis</i> KJ672135	Pakistan	5
4	<i>Diabolocatantops innotabilis</i> PQ466788	India	<i>Diabolocatantops innotabilis</i> PQ463995	India	0
5	<i>Diabolocatantops innotabilis</i> PQ466788	India	<i>Diabolocatantops innotabilis</i> PQ466783	India	0
6	<i>Diabolocatantops innotabilis</i> PQ466788	India	<i>Diabolocatantops innotabilis</i> KJ672135	Pakistan	5
7	<i>Diabolocatantops pinguis pinguis</i> FJ571154	China	<i>Diabolocatantops pinguis</i> KC139976	China	1
8	<i>Stenocatantops splendens</i> FJ571155	China	<i>Stenocatantops splendens</i> KC139978	China	2
9	<i>Stenocatantops splendens</i> PV786611	India	<i>Stenocatantops splendens</i> PV786612	India	0
10	<i>Stenocatantops splendens</i> PV786611	India	<i>Stenocatantops splendens</i> FJ571155	China	4
11	<i>Stenocatantops splendens</i> PV786611	India	<i>Stenocatantops splendens</i> KC139978	China	4
12	<i>Stenocatantops splendens</i> PV786612	India	<i>Stenocatantops splendens</i> FJ571155	China	4
13	<i>Stenocatantops splendens</i> PV786612	India	<i>Stenocatantops splendens</i> KC139978	China	4
14	<i>Xenocatantops humilis</i> PQ467096	India	<i>Xenocatantops humilis</i> PQ468464	India	0
15	<i>Xenocatantops humilis</i> PQ467096	India	<i>Xenocatantops humilis</i> MT325832	India	0
16	<i>Xenocatantops humilis</i> PQ468464	India	<i>Xenocatantops humilis</i> MT325832	India	0
17	<i>Xenocatantops karnyi</i> PV786827	India	<i>Xenocatantops karnyi</i> PV787252	India	0

between 1 to 5. Interspecific divergence for *Stenocatantops splendens* (present study; Zhao *et al.*, 2009; Huang *et al.*, 2014) and *Stenocatantops vitripennis* (Song *et al.*, 2018) it ranges from 7 to

9.0 and for *Xenocatantops humilis* (India) (present study; Kundu *et al.*, 2020) and *Xenocatantops brachycerus* (Huang *et al.*, 2014) it is 2. For the specimens of *Xenocatantops karnyi* and

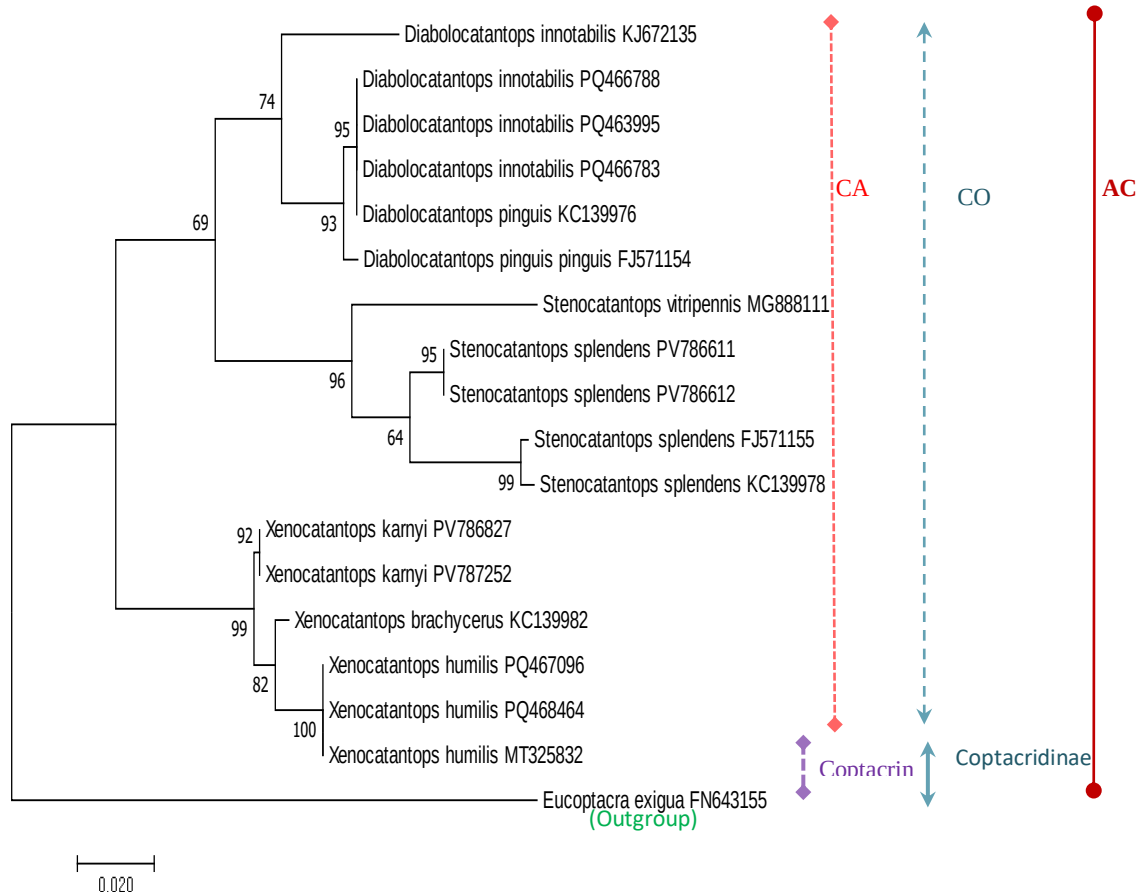


Fig. 1: Molecular Phylogenetic exploration by utilizing the Maximum Likelihood (ML) technique (CA=Catantopini; CO- Catantopinae; AC= Acrididae).

Xenocatantops brachycerus (Huang *et al.*, 2014) it ranges from 1 to 2.

Phylogenetic analysis

A molecular phylogenetic tree (Fig. 1) was constructed by using mtDNA COI gene sequences among selected genera within the subfamily Catantopinae. The “Maximum Likelihood method, accompanied by 1,000 bootstrap replicates” to evaluate nodal support has been used. Kumar *et al.* (2016) and Kimura (1980). The species *Eucoptacra exigua* served as an outgroup to root the phylogenetic tree. A total of III clades have been constructed for the genus *Diabolocatantops*, *Stenocatantops*, and *Xenocatantops*. Clade I – Genus *Diabolocatantops* delineated a distinct and robustly supported monophyletic clade, which includes two species: *D. innotabilis* and *D. pinguis*. *D. innotabilis* exhibited tight clustering with

elevated bootstrap values (95), thereby indicating minimal genetic divergence and substantial intraspecific consistency. *D. pinguis* (KC139976, FJ571154) was identified as a sister lineage to *D. innotabilis*, suggesting moderate divergence. Clade II – Genus *Stenocatantops* showcased close clustering of *S. splendens* (PV786611, PV786612, FJ571155, KC139978) accompanied by high bootstrap support (95), indicating significant genetic cohesion. Conversely, *S. vitripennis* (MG888111) branched independently implying the possibility of cryptic divergence. In clade III – Genus *Xenocatantops* was distinctly resolved into three separate species clusters: *X. karnyi*, *X. brachycerus*, and *X. humilis*. The *Xenocatantops humilis* clade (PQ467096, PQ468464, MT325832) shows monophyly with a bootstrap value of 100. While *Xenocatantops karnyi* (PV786827, PV787252) was recovered with high support (92),

reflecting a clearly distinct lineage. *X. brachycerus* (KC139982) was positioned as a sister taxon to *X. humilis*, with bootstrap support (82), thereby confirming the phylogenetic monophyly among the species. These results are consistent with the preceding research conducted by Wang *et al.* (2023) and Zhang *et al.* (2025). The outgroup *Eucoptacra exigua* established a separate basal lineage, confirming its phylogenetic distance from the ingroup taxa.

Conclusion

The phylogenetic analysis of Family Acrididae and subfamily Catantopinae by using mt DNA COI gene sequences with *Eucoptacra exigua* as an outgroup, unveiled well-supported monophyletic groupings within each genus (*Diabolocatantops*, *Stenocatantops*, and *Xenocatantops*) of Catantopinae, with sustainable bootstrap value, thereby validating current taxonomic classifications and accentuating both intra- and interspecific divergence. Notably, the divergence observed within *Stenocatantops* may necessitate further taxonomic and nuclear gene markers examination. The present study on the subfamily Catantopinae genus (*Diabolocatantops*, *Stenocatantops*) has been reported for the first time from India. However, the phylogenetic analysis of *Xenocatantops karnyi* has been reported for the first time and COI gene analysis on *Xenocatantops karnyi* is the first report worldwide. Therefore, *Xenocatantops karnyi* is the noble contribution for NCBI database.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Department of Biosciences, Division Zoology, Career Point University, Hamirpur, Himachal Pradesh, India.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors assert the absence of any conceivable conflicts of interest pertaining to the publication of this work.

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