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## Faecal Microbiota Profiling of Captive Gyr Falcons (*Falco rusticolus*) and Hybrids in Saudi Arabia

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### Abstract

Falcons have been used in falconry for centuries in the Kingdom of Saudi Arabia and other countries in the area. In many of these countries, various falcon species are captive-bred, with the goal of selecting for hunting ability. Purebred Gyr falcons (*Falco rusticolus*), the largest species of falcon, are highly regarded by falconers for their hunting abilities. Gyr falcons that have been captive-bred may live, reproduce, and hunt for longer periods of time, making them more stable. Aspergillosis is a non-contagious fungal disease of wild and domestic birds caused by the *Aspergillus* fungus. This fungal illness is commercially important since it is the leading cause of mortality in captive birds, particularly falcons. This study examined the effect of nutrition on the intestinal bacterial ecology of Aspergillosis-affected Gyr falcons using faecal culture, as well as the potential for the isolated organisms to transfer to humans and avian species, an important aspect in the One-Health approach which is presently adopted universally. An infected male Gyr and a hybrid female (Gyr and Peregrine - *F. peregrinus*) were fed meat and a fresh chicken-rich diet, and the results revealed an equal distribution of gram-positive and gram-negative bacteria in fecal analysis that were less hazardous. The bacteria discovered here could be temporary flora acquired through food and, under normal conditions, are unlikely to colonize and become permanent inhabitants of the raptorial enteral tract. Thus, supplying falcons with different healthy foods is a better option in captivity, which may assist falconers keep their birds free from infection.

### Key words

*Captive, Gyr falcons, Aspergillosis, Peregrine, faecal culture.*

## Introduction

Falcons (Falconidae family) are noted for their amazing speed and agility, which they utilize to pursue other birds and small mammals (Fox *et al.*, 1976). There are over 40 species of falcons, including peregrines, Gyr falcons, hobbies, and kestrels (Oberprieler *et al.*, 2009). The Gyrfalcon (*Falco rusticolus*), sometimes known as the “Arctic Falcon,” has a vast distribution in the Arctic and sub-Arctic regions of North America, Europe, and Asia. It is considered the world’s largest falcon, with females much larger than males. Adult males weigh 800-1300 g and are 48-61 cm long, whilst females weigh 1200-2200 g and are 51-65 cm long. The Gyrfalcon is classified as a species of “Least Concern” by the International Union for Conservation of Nature (IUCN). Throughout the Middle Ages, monarchs would catch and train the Gyrfalcon to be a hunting partner because it was regarded as the bird of kings in traditional falconry. The practice of teaching a falcon to hunt and return to its handler is called falconry. The peregrine falcon, commonly known as the “peregrine” or *Falco peregrinus* is a bird that is widely spread and renowned for its swiftness. The peregrine falcon is a highly prized bird in falconry worldwide because of its exceptional hunting skills, outstanding trainability, adaptability, high success rate in captive breeding, and resulting ease of supply.

Due to stress, wild Gyr falcons kept in captivity are more likely to have illnesses such as Aspergillosis. Gyr falcons raised in captivity are more resilient and have a longer lifespan for hunting and breeding. Without affecting wild populations, captive breeding will produce a steady supply. Many efforts have been made in Saudi Arabia to enhance the management of the Gyrfalcon, despite the fact that the species poses numerous difficulties in terms of infections and captive care. When husbandry conditions expose birds to disease, a fungal disease of both wild and domestic birds, caused by the fungus *Aspergillus* species, can arise as a flock concern. Decomposing organic matter, especially hay, compost, or wood, contains a lot of the fungus’s spores. The danger of infection is increased by inadequate cleanliness and poor ventilation. The fungus is characterized by its major involvement of the respiratory tract, the formation of yellow cheesy plaques, and hard nodular masses in the lungs and air sacs, while it can also commonly impact other organs. Isolating social animals, combining flocks of birds,

or beginning training are all possible stressors that could result in immunosuppression and infections. For falconers involved in rehabilitation, bringing sick or injured wild falcons into captivity is a known risk factor for Aspergillosis. One of the main causes of bird mortality in captivity is Aspergillosis, which has important economic ramifications. The purpose of this study was to investigate the intestinal bacterial flora of these raptors through faecal culturing of Aspergillosis affected Gyr falcons. The impact of food on faecal bacteria was also taken into account, and the possibility that isolated organisms could infect people and birds was explored.

## Materials And Methods

Aspergillosis was discovered in pet Gyr falcons and Gyr-Peregrine hybrid falcons in a Riyadh, Saudi Arabian bird market according to the study conducted by Rahim *et al.*, 2013. After feeding on meat and fresh chicken-rich meals, an infected male Gyr and a hybrid female (Gyr and Peregrine - *Falco peregrinus*) were examined, and samples of their faeces were taken for additional analysis. The samples were examined under a microscope after Gram staining to ascertain whether they were gram-positive or gram-negative. The bacterial culture was maintained at 40°C on a nutrient agar medium using the spread plate method. Microbial colony’s appearance and colour were used to diagnose the infection. The test organism was then kept in the refrigerator for later use after being separated from colonies using the streak plating technique. After that, the cells’ pure genomic DNA was extracted. Origin Genomic DNA Kit was used for DNA extraction and isolation. Using the PCR method, almost two Nano grams of genomic DNA were amplified. The PCR procedure included a 5-minute denaturation step, 30 cycles of 10 seconds at 95°C, 30 seconds at 55°C, and 45 seconds at 72°C, and a final 3-minute phase at 72°C. To verify the amplification of the target gene, the PCR products were resolved on a 2% TAE-agarose gel. The Mo Bio UltraClean PCR Cleanup Kit (Mo Bio Laboratories, Inc., California) was used to column purify the PCR product. SciGenom Labs Private Ltd. in Cochin performed the sequencing of the purified PCR product. After chromatograms were examined to assess the quality of the obtained sequence, Clustal W was used to combine the forward and reverse sequences, and the consensus was collected

for additional analysis. The resultant sequence was analysed for similarity using NCBI's BLAST ([www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/)). MEGA10 software was used to plot the phylogenetic tree using the neighbor-joining method.

## Results

These falcons' faecal samples yielded the bacterial isolates shown in Table 1 for samples 1-OR413817 (Figure 1), 2-OR413816 (Figure 2), 3-OR413804 (Figure 3), and 4-OR413813 (Figure 4). Table 2 lists the biochemical assays that were performed on the samples. Figures 1, 2, 3, and 4 represents the quadrant streak colony of the bacterial isolates.



Figure 1. The colony of *Enterococcus faecalis*



Figure 2. The colony of *Enterobacter sichuanensis*



Figure 3. The colony of *Bacillus tropicus*

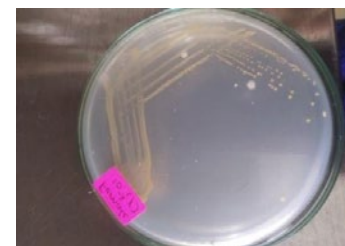


Figure 4. The colony of *Serratia marcescens*

**Table 1- The bacterial isolates identified.**

Species	Bacteria isolated	Bacteria identified	Nature of the bacteria
Gyr falcon			
Male	Gram-positive	<i>Enterococcus faecalis</i> (sample 1- OR413817)	Opportunistic bacteria used as probiotic
	Gram-negative	<i>Enterobacter sichuanensis</i> (sample 2- OR413816)	Potential pathogen in humans
Hybrid Female (Gyr and Peregrine - <i>F. peregrinus</i> )	Gram-positive	<i>Bacillus tropicus</i> (sample 3- OR413804)	Feather-degrading bacteria, a potential pathogen in humans
	Gram-negative	<i>Serratia marcescens</i> (sample 4- OR413813)	Opportunistic pathogen causing nosocomial infections

Table 2- The biochemical tests conducted

Biochemical test	Bacteria isolated			
	<i>Enterococcus faecalis</i>	<i>Enterobacter sichuanensis</i>	<i>Bacillus tropicus</i>	<i>Serratia marcescens</i>
Citrate	-	+	+	+
Carbohydrate	+	+	-	+
Catalase	-	+	-	+
Indole	-	-	-	-
Oxidase	-	-	+	-
Methyl-red	-	-	-	-
Starch	+	-	+	+
Urease	-	-	-	-
Voges-Proskauer test	+	+	-	+

Figure 1. Showing OR413817 *Enterococcus faecalis* strain ATCC 19433 16S ribosomal RNA, partial sequence

Sequence ID: NR_115765.1, Length: 1483Number of Matches: 1				
Score	Expect	Identities	Gaps	Strand
2560 bits(1386)	0.0	1423/1440(99%)	6/1440(0%)	Plus/Plus

Query	1	TGCAAGTCGAACGCTTCTTTCTCCCGAGGGCTTGCACTCAATTGGAAAGAGGAGTGGCG	60
Sbjct	29	TGCAAGTCGAACGCTTCTTTCTCCCGAGTGCTTGCACTCAATTGGAAAGAGGAGTGGCG	88
Query	61	GACGGGTGAGTAACACGTGGGTAACCTACCCATCAGAGGGGGATAACACGTTGGCAAACA	120
Sbjct	89	GACGGGTGAGTAACACGTGGGTAACCTACCCATCAGAGGGGGATAACAC-TTGG-AAACA	146
Query	121	GGTGCTAATACCGCTATAACAGCTTTATGCCGCATGGCATAAGAGTGAAAGGCGTTTCG	180
Sbjct	147	GGTGCTAATACCGC-ATAACAG-TTTATGCCGCATGGCATAAGAGTGAAAGGCGTTTCG	204
Query	181	GGTGTCGCTGATGGATGGACCCGCGTGCATTAGCTAGTTGCGTGAGGTAACGGCTCACC	240
Sbjct	205	GGTGTCGCTGATGGATGGACCCGCGTGCATTAGCTAGTTG-GTGAGGTAACGGCTCACC	263
Query	241	AAGGCCACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGG	300
Sbjct	264	AAGGCCACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGG	323
Query	301	CCCAGACTCCTACGGGAGGCAGCACTACGGAATCTTCGGCAATGGACGAAAGTCTGACCG	360
Sbjct	324	CCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCG	383
Query	361	AGCAACGCCGCGTGAGTGAAGAAAGTTTTCGGATCGTAAAACCTCTGTTGTTAGAGAAGAA	420
Sbjct	384	AGCAACGCCGCGTGAGTGAAGAAAGTTTTCGGATCGTAAAACCTCTGTTGTTAGAGAAGAA	443
Query	421	CAAGGACGTTAGTAACTGAACGTCCCCTGACGGTATCTAACCAGAAAGCCACGGCTAACT	480
Sbjct	444	CAAGGACGTTAGTAACTGAACGTCCCCTGACGGTATCTAACCAGAAAGCCACGGCTAACT	503
Query	481	ACGTGCCAGCAGCCGCGTAATACGTACGTGGCAAGCGTTGTCCGATTATTGGGCGTA	540





**Figure 2. Showing OR413816 *Enterobacter sichuanensis* strain WCHECL1597 16S ribosomal RNA, partial sequence  
Sequence ID: NR 179946.1, Length: 1528Number of Matches: 1**

Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
1965 bits (1064)	0.0	1115/1140 (98%)	3/1140 (0%)	Plus/Plus

Query	1	TGCCTGATGGAGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGA	60
Sbjct	124	TGCCTGATGGAGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGA	183
Query	61	CCAAAGAGGGGGACCTTCGGGCCTCTTGCCCTCAGATGTGCCAGATGGGATTAGCTAGT	120
Sbjct	184	CCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCAGATGGGATTAGCTAGT	243
Query	121	AGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCC	180
Sbjct	244	AGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCC	303
Query	181	ACACTGGAAGTACGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC	240
Sbjct	304	ACACTGGAAGTACGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC	363
Query	241	AATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAACGCCTTCGGGTTGTAAA	300
Sbjct	364	AATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAA	423
Query	301	GTACTTTCAGCGGGGAAGAAAGGTGTTGTGGTTAATAACCACAGCAATTGACGTTACCCG	360
Sbjct	424	GTACTTTCAGCGGGG-AGGAAGGTGTTGAGGTTAATAACCTCAGCAATTGACGTTACCCG	482
Query	361	CAGAAGAAGCACC GGCTAACTCCGTGCCAGCAGCCGCGTAATACAGAGGGTGCAAGCGT	420
Sbjct	483	CAGAAGAAGCACC GGCTAACTCCGTGCCAGCAGCCGCGTAATACAGAGGGTGCAAGCGT	542
Query	421	TACTCGGATTACTGGGCGTAAAGCGCACACAGGCGGTCTGTCAAGTCAGATGTGAAATC	480
Sbjct	543	TAATCGGAATTACTGGGCGTAAAGCGCACAGCAGGCGGTCTGTCAAGTCAGATGTGAAATC	602
Query	481	CCCGGGCTCAACCTGGGAACTGCATTGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGG	540
Sbjct	603	CCCGGGCTCAACCTGGGAACTGCATTGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGG	662
Query	541	TAGAATTCCAGGTGTAGCTGTGATATGCGTAGAGATCTGGAAGAATACCGGTGGCGAACG	600
Sbjct	663	TAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGG	722
Query	601	CGGCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAG	660
Sbjct	723	CGGCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAG	782
Query	661	ATACCCTGGTAGTCCACGCCGTAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTG	720
Sbjct	783	ATACCCTGGTAGTCCACGCCGTAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTG	842
Query	721	GCTTCCGGAGCTAACCGGTTAAGTCGACCGCTGGGGAGTACGGCCGAAGGTTAAAACT	780
Sbjct	843	GCTTCCGGAGCTAACCGGTTAAGTCGACCGCTGGGGAGTACGGCCGAAGGTTAAAACT	902



**Figure 3. Showing OR413804 *Bacillus tropicus* strain MCCC 1A01406 16S ribosomal RNA, partial sequence  
Sequence ID: NR\_157736.1, Length: 1509Number of Matches: 1**

Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
2495 bits(1351)	0.0	1370/1379 (99%)	1/1379 (0%)	Plus/Plus

Query	3	AGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGACGGGTGAGTAAC	62
Sbjct	53	AGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGACGGGTGAGTAAC	112
Query	63	ACGTGGGTAACCTGCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGAT	122
Sbjct	113	ACGTGGGTAACCTGCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGAT	172
Query	123	AACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGCACTTATGGATGG	182
Sbjct	173	AACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGCACTTATGGATGG	232
Query	183	ACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTACCAAGGCAACGATGCGTAGCC	242
Sbjct	233	ACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTACCAAGGCAACGATGCGTAGCC	292
Query	243	GACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGG	302
Sbjct	293	GACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGG	352
Query	303	CAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGA	362
Sbjct	353	CAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGA	412
Query	363	TGAAGGCTTTCGGGTCGTAAAACCTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAG	422
Sbjct	413	TGAAGGCTTTCGGGTCGTAAAACCTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAG	472
Query	423	CTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGG	482
Sbjct	473	CTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGG	532
Query	483	TAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTT	542
Sbjct	533	TAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTT	592
Query	543	TCTTAAGTCTGATGTGAAAGCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGA	602
Sbjct	593	TCTTAAGTCTGATGTGAAAGCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGA	652
Query	603	CTTGAGTGCAGAAGAGGAAAGTGAATTCATGTGTAGCGGTGAAATGCGTAGAGATATG	662
Sbjct	653	CTTGAGTGCAGAAGAGGAAAGTGAATTCATGTGTAGCGGTGAAATGCGTAGAGATATG	712
Query	663	GAGGAACACCAGTGGCGAAGGCGACTTCTGGTCTGTAAGTACTGACTGAGGCGCGAAAGC	722
Sbjct	713	GAGGAACACCAGTGGCGAAGGCGACTTCTGGTCTGTAAGTACTGACTGAGGCGCGAAAGC	772
Query	723	GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAG	782
Sbjct	773	GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAG	832
Query	783	TGTTAGAGGGTTTCCGCCCTTATGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGA	842
Sbjct	833	TGTTAGAGGGTTTCCGCCCTTATGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGA	892
Query	843	GTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGCCCGCACAAAGCGGTGGAGCA	902
Sbjct	893	GTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGCCCGCACAAAGCGGTGGAGCA	952
Query	903	TGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTTCTGACAACC	962



**Figure 4. Showing OR413813 *Serratia marcescens* strain KRED 16S ribosomal RNA, partial sequence Sequence ID: NR\_036886.1, Length: 1532Number of Matches: 1**

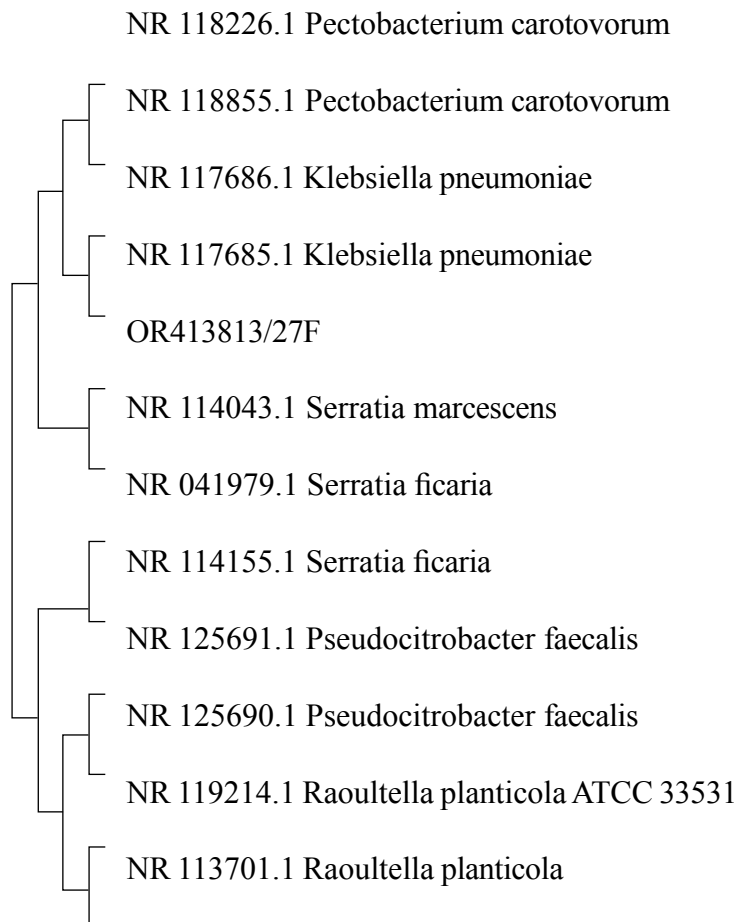
Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
2538 bits(1374)	0.0	1379/1381(99%)	1/1381(0%)	Plus/Plus

Query	1	ATGC-AGTCGAGCGGTAGCACAGGGGAGCTTGCTCCCTGGGTGACGAGCGGCGGACGGGT	59
Sbjct	49	ATGCAAGTCGAGCGGTAGCACAGGGGAGCTTGCTCCCTGGGTGACGAGCGGCGGACGGGT	108
Query	60	GAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAACGGTAGCTAATA	119
Sbjct	109	GAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAACGGTAGCTAATA	168
Query	120	CCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCC	179
Sbjct	169	CCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCC	228
Query	180	CAGATGGGATTAGCTAGTAGGTGGGGTAATGGCTCACCTAGGCGACGATCCCTAGCTGGT	239
Sbjct	229	CAGATGGGATTAGCTAGTAGGTGGGGTAATGGCTCACCTAGGCGACGATCCCTAGCTGGT	288
Query	240	CTGAGAGGATGACCAGCCACACTGGAAGTCTGAGACACGGTCCAGACTCCTACGGGAGGCAG	299
Sbjct	289	CTGAGAGGATGACCAGCCACACTGGAAGTCTGAGACACGGTCCAGACTCCTACGGGAGGCAG	348
Query	300	CAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGA	359
Sbjct	349	CAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGA	408
Query	360	AGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGGAAGGTGGTGAACCTAATACGTTCA	419
Sbjct	409	AGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGGAAGGTGGTGAACCTAATACGTTCA	468
Query	420	TCAATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAAT	479
Sbjct	469	TCAATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAAT	528
Query	480	ACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGT	539
Sbjct	529	ACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGT	588
Query	540	AAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTTGAAACTGGCAAGCTAG	599
Sbjct	589	AAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTTGAAACTGGCAAGCTAG	648
Query	600	AGTCTCGTAGAGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGG	659
Sbjct	649	AGTCTCGTAGAGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGG	708
Query	660	AATACCGGTGGCGAAGGCGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGG	719
Sbjct	709	AATACCGGTGGCGAAGGCGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGG	768
Query	720	GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGTCGATTGGAGGT	779
Sbjct	769	GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGTCGATTGGAGGT	828
Query	780	TGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCTGGGGAGTACG	839
Sbjct	829	TGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCTGGGGAGTACG	888
Query	840	GCCGCAAGGTTAAAACCTCAAATGAATTGACGGGGGCCGACAAGCGGTGGAGCATGTGG	899
Sbjct	889	GCCGCAAGGTTAAAACCTCAAATGAATTGACGGGGGCCGACAAGCGGTGGAGCATGTGG	948
Query	900	TTAATTCGATGCAACGCGAAGAACCTTACTACTCTTGACATCCAGAGAACCTTCCAGA	959
Sbjct	949	TTAATTCGATGCAACGCGAAGAACCTTACTACTCTTGACATCCAGAGAACCTTCCAGA	1008



Query	960	GATGGATTGGTGCCTTCGGGA	ACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTG	1019
Sbjct	1009		GATGGATTGGTGCCTTCGGGA	1068
Query	1020	TTGTGAAATGTTGGGTTAAGTCCC	GCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGG	1079
Sbjct	1069		TTGTGAAATGTTGGGTTAAGTCCC	1128
Query	1080	TTCGGCCGGGA	ACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGT	1139
Sbjct	1129		TTCGGCCGGGA	1188
Query	1140		CAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGTATACAAAGA	1199
Sbjct	1189		CAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGTATACAAAGA	1248
Query	1200		GAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTACGTCGTAGTCCGGATTGGAGTC	1259
Sbjct	1249		GAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTACGTCGTAGTCCGGATTGGAGTC	1308
Query	1260		TGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGA	1319
Sbjct	1309		TGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGA	1368
Query	1320		ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAAGAA	1379
Sbjct	1369		ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAAGAA	1428
Query	1380	G	1380	
Sbjct	1429	G	1429	

## Phylogenetic tree



## Discussion

Numerous internal and external factors, including host genetics, nutrition, ambient conditions, behavioural patterns, social interactions, age, and sex, affect the makeup of the faecal microbiota. The organisms found in these birds' faeces are therefore determined by a variety of factors, such as geographic location, environmental exposure, and nutrition. It makes sense that the organisms in these birds' excrement would be influenced by a variety of factors, such as geographic location, environmental exposure, and nutrition. The faecal samples taken from the unhealthy captive Gyr falcons fed on a diet high of chicken demonstrated an equivalent distribution of gram-positive and gram-negative bacteria, highlighting the importance of nutrition in the composition of the microbiome. Gram-negative bacteria, however, were the most prevalent in the faeces of 47 healthy raptors kept in captivity in a different study (Bangert et al., 1988) on raptors given professionally cooked chicken. The two gram-positive bacteria, *Enterococcus faecalis*, are used as probiotics, and *Bacillus tropicus* is a feather-degrading bacterium. The other two gram-negative bacteria *Enterobacter sichuanensis* and *Serratia marcescens* can cause nosocomial infections if not properly controlled in captive areas. The results of both studies were similar in that they found a variety of bacterial isolates that were potential pathogens. However, another study (Bangert et al., 1988) on raptors fed professionally prepared chicken found that the most common bacteria in the faeces of 47 healthy raptors housed in captivity were gram-negative. The two gram-positive bacteria, *Bacillus tropicus*, which breaks down feathers, and *Enterococcus faecalis*, are employed as probiotics. If the other two gram-negative bacteria are not adequately managed in captivity, they may result in nosocomial illnesses. Both research' findings were similar in that they identified a range of bacterial isolates that might be harmful.

## Conclusion

Numerous bacterial, fungal, and viral illnesses are commonly seen in falcons. In confined falcons, the environment and food quality have a close relationship with nutritional deficiencies and metabolic disorders. Birds raised intensively may become infected with Aspergillosis and other common illnesses due to a variety of circumstances, including cage layout, food

preparation and storage, hygiene, stress, and other factors. Nearly every disease can be partially avoided with appropriate care, better personal hygiene, a healthy diet, and routine examinations. If the intestinal biota of the infected falcon enters the humans through the faecal contamination, it may result in serious illnesses, which has significance in the One Health and zoonotic disease perspective.

## Acknowledgement

We acknowledge the falcon hospitals, falcon clinics and falcon shops in the Kingdom of Saudi Arabia for permitting us to collect samples.

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# A Baseline Survey of Freshwater Fish Fauna in the Krishna River of Northern Western Ghats up to Wai tehsil

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## Abstract

Indian freshwater supports diverse Ichthyofauna (more than 1000 species; as per FishBase) and thus broadly classified as “freshwater eco-region”. This study aimed to assess the freshwater fish diversity of the Krishna River at Wai and the upstream Dhom Reservoir was assessed during January 2021 to December 2022, revealing a total of 56 species distributed across 14 families and 33 genera. Among these, 13 species are endemic to the Western Ghats of India, while two species are exclusive to the Krishna river system. The study identified five globally threatened species *Hypselobarbus curmuca*, *Labeo potail*, *Schismatorhynchus nukta*, *Tor khudree* and *Parapsilorhynchus discophorus* with populations ranging from moderate to rare. The fish fauna of this region is under significant threats from five introduced species, *Clarias gariepinus*, *Cyprinus carpio*, *Hypophthalmichthys molitrix*, *Oreochromis mossambicus* and *Oreochromis niloticus*. Anthropogenic activities such as overfishing, habitat degradation, and pollution from organic and inorganic sources impacted to great extent on diversity and distribution of fish. To mitigate these threats, site-specific conservation strategies are essential to safeguard rare and endangered fish species in this ecosystem.

## Keywords

Biodiversity hotspot, endemism, conservation, Dhom Reservoir, anthropogenic activities, introduced species

## Introduction

The Western Ghats of India is recognized as a global biodiversity hotspot due to its exceptional species richness and high endemism across various taxonomic groups, including amphibians and freshwater fish (Myers et al., 2000). This region harbors approximately 320 freshwater fish species, distributed across 11 orders, 35 families, and 112 genera, with ongoing discoveries of new taxa (Dahanukar & Raghavan, 2013). Among the major river systems originating in the northern Western Ghats, the Krishna River plays a pivotal role in sustaining aquatic biodiversity. The Krishna River originates from Mahabaleshwar in the Satara District of Maharashtra and follows an eastward course spanning approximately 290 km through the districts of Satara, Sangli, and Kolhapur before traversing into Karnataka and Telangana. The river's hydrological network is marked by several infrastructural interventions, with the Dhom Dam serving as the first major impoundment and Wai being the first prominent urban settlement along its trajectory. Despite the ecological importance of this riverine system, studies addressing freshwater fish diversity within the Krishna River at Wai and Dhom Reservoir remain sparse. The ichthyofaunal diversity of the Deccan Plateau has been historically documented since Sykes (1839) with more focused investigations on the Krishna and Godavari River systems conducted by David (1963). However, these studies did not furnish species-specific inventories for distinct river sections. A more comprehensive evaluation of the Krishna River's fish assemblages was performed by Jayaram (1995), though this assessment lacked an exclusive checklist for tributaries. Research efforts have predominantly centered on the tributaries within the Satara District.

Annandale (1919) recorded 18 fish species in the Yenna River at Medha, while Silas (1953) documented 14 species from Mahabaleshwar and Wai. Arunachalam et al. (2002) contributed further by identifying 14 species from the Dhom Reservoir. A more extensive ichthyofaunal survey was conducted by (Jadhav et al., 2011) who documented 58 species from the Koyna tributary. A study by Kharat et al. (2012) subsequently provided a significant update, reporting 51 species from the Krishna River at Wai and Dhom Reservoir. Despite these valuable contributions, research on the fish fauna of Krishna river at Wai has been scarce in recent years, with no updated studies published since 2012.

Additionally, the Krishna river has undergone

substantial changes in the last decade, driven by growing tourism, industrialization, and recreational activities (Joshi and Chakravarty, 2025). These alterations, coupled with the increasing human footprint on the landscape, have the potential to affect the delicate balance of the aquatic ecosystems, including fish populations. Given the paucity of information on fish diversity in the Krishna river and Dhom dam, especially in the face of increasing anthropogenic pressures, it is imperative to revisit and reassess the ichthyofaunal of this critical waterbody. This study aims to provide a comprehensive overview of the current diversity and distribution of fish species in the Krishna river and Dhom dam, more than a decade after the last substantial survey. By documenting the present status of fish fauna, and making comprehensive comparative analysis with earlier reports, we provide baseline data that will aid in identifying key threats to fish populations and inform conservation efforts in the region.

## Materials and Methods

### Study area and duration of study

The study was conducted to assess the fish diversity of the Krishna river at Wai and Dhom dam over the period two years from January 2021 to December 2022.

### Collection, preservation of fishes and identification

Fish specimens were collected with the help of local fishers using different mesh-sized gill nets and cast nets. Alternatively, fish samples were also procured from local fish markets at Wai. Collected fishes were stored in ice-containing insulated boxes and transported to the laboratory. In the laboratory small-sized fish were preserved in 4% aqueous formalin solution, while larger fish were preserved in 10% aqueous formalin solution. The specimens were stored in airtight plastic bottles to ensure proper preservation. In the laboratory, fish specimens were identified using recent published literature and standard taxonomic keys, including Jayaram (1981), Jayaram (1999), (Talwar & Jhingran, 1991) Jayaram & Jeyachandra Dhas (2000) and Jayaram,(2002). The online database FishBase was utilized for verification and authentication of scientific names (Froese & Pauly, 2024).

## Results

The present study documented the freshwater fish diversity in the Krishna River at Wai and the upstream

Dhom Reservoir, recording 56 species distributed across 9 orders, 14 families, and 35 genera. The fish population in the Krishna River at Wai faces significant threats due to various factors, including excessive exploitation of fish resources, competition and predation from non-native species, and habitat deterioration caused by both organic and inorganic pollution (figure 1). Total number of 56 fish species were recorded in this study period, Cypriniformes was most abundant order with 36 species belonging to four families with cyprinidae dominant family having 23 species. Siluriformes is second dominant order with seven species belonging to it. Perciformes, Anabantiformes and Cichliformes have four, three and two species respectively. Beloniformes, Gobiiformes, Osteoglossiformes and Synbranchiformes have one species each (Table 1). The assessment, based on the IUCN Red List (2025), reveals that the majority of species are classified as Least Concern (LC), indicating their widespread distribution and stable populations. However, several species, such as *Hypselobarbus curmuca* (Curmuca Barb), *Labeo potail* (Deccan Labeo), and *Clarias magur* (Mangur Catfish), are categorized as Endangered (EN), highlighting the urgent need for conservation efforts due to habitat degradation, overfishing, and ecological changes. Additionally, species like *Cyprinus carpio* (Common Carp) and *Hypselobarbus kolus* (Kolus Barb) fall under the Vulnerable (VU) category (Figure 2), signifying a high risk of population decline if threats persist. Among the Cyprinidae family, which constitutes a major portion of the dataset, species such as *Labeo rohita* (Rohu), *Cirrhinus mrigala* (Mrigal), and *Puntius ticto* (Ticto Barb) are classified as Least Concern (LC), reinforcing their adaptability and stable population dynamics. However, the presence of Near Threatened (NT) species like *Hypophthalmichthys molitrix* (Silver Carp) suggests potential risks due to environmental disturbances. The Siluriformes (catfish group) also exhibit varying conservation statuses, with *Mystus malabaricus* (Jerdon's Catfish) and *Ompok bimaculatus* (Butter Catfish) marked as Near Threatened (NT), indicating possible future vulnerability due to habitat alterations and fishing pressures.

Interestingly, within the Cichlidae family, *Oreochromis mossambicus* (Mozambique Tilapia) is identified as Vulnerable (VU), likely due to its invasive nature affecting native ecosystems and competition with other species. In contrast, *Oreochromis niloticus*

(Nile Tilapia) remains Least Concern (LC), reflecting its ecological resilience and adaptability. Similar patterns are observed in the Perciformes and Anabantiformes orders, where species such as *Channa marulius* (Great Snakehead) and *Parambassis ranga* (Indian Glassy Fish) are Least Concern (LC), suggesting minimal immediate threats to their survival.

## Discussion

The study highlights the diversity and conservation status of freshwater fish species across different taxonomic groups, with a particular focus on Cypriniformes, Siluriformes, Perciformes, and other significant orders. Among these, the Cyprinidae family exhibited the highest species richness, a pattern consistent with previous ichthyofaunal surveys in the Western Ghats. The majority of species were classified as Least Concern (LC) under the IUCN Red List (2025), suggesting stable populations in the study area. However, a notable subset of species was found to be globally threatened, with three species classified as Endangered (EN) (*H. curmuca*, *L. potail*, and *S. nukta*) and four species as Vulnerable (VU) (*C. carpio*, *H. kolus*, and *O. mossambicus*, ) (Figure 1). Additionally, three species were Near Threatened (NT) (*H. molitrix*, *M. malabaricus*, and *O. bimaculatus*), while *Puntius amphibious* was categorized as Data Deficient (DD), highlighting the need for further ecological studies.

A comparative analysis with previous studies Silas (1953) Arunachalam et al. (2002) and Kharat et al. (2012) revealed both similarities and deviations in species presence. Several species recorded in earlier studies were confirmed in the present study (*G. mullya*, *P. ticto*, *T. khudree*, *B. barna*), reinforcing their persistence in the ecosystem. However, some species were newly recorded or absent compared to past surveys, indicating possible population fluctuations influenced by habitat modifications, climatic variations, or anthropogenic pressures (Bunn & Arthington, 2002, Arunachalam et al., 2002, Kharat et al., 2012).

The presence of introduced and transplanted species in the study area poses significant threats to native ichthyofauna. *C. carpio* (Common Carp) and *H. molitrix* (Silver Carp), both introduced for aquaculture, have been implicated in habitat alteration and competition with native species.

Freshwater biodiversity is experiencing unprecedented and growing pressure from human

activities such as overfishing, pollution (organic and inorganic), and habitat degradation were observed as key stressors affecting fish populations in the region (Dudgeon et al., 2006). The study underscores the urgent need for site-specific conservation strategies, particularly for threatened and endemic species.

Presence of additional species such as *Labeo calbasu* and *Labeo potail*, which were not recorded in earlier studies, highlighting potential changes in fish assemblages over time. The presence and distribution of species were compared with historical records, revealing that *Garra mullya*, *Pethia ticto*, and *Devario aequipinnatus* showed consistent presence across all studies, reinforcing their stable populations. However, species such as *Hypselobarbus curmuca*, which were absent in Arunachalam et al. (2002), were recorded in this study, indicating a possible resurgence or improved detection methods. *Mystus malabaricus* and *Ompok bimaculatus* were identified as Near Threatened (NT), consistent with previous assessments. These findings align with past research but also underscore the need for enhanced conservation measures, particularly for species that were previously data deficient or unrecorded. Our study suggests that habitat modifications, pollution, and overfishing might be contributing factors to population shifts. *Clarias magur*, categorized as Endangered, was absent in previous surveys, suggesting either a recent decline or historical underreporting. The proliferation of invasive species such as *Oreochromis mossambicus* and *Oreochromis niloticus* (previously unrecorded in certain studies) may pose competition threats to native species (Trewavas, 1983). The highly carnivorous *Clarias gariepinus*; African cat fish which is illegally introduced into freshwaters of India made severe damage to indigenous fish fauna. The union agriculture ministry has ordered killing of these fishes and preventing further culture of these fishes but this order did not have any impact as it lacked any specific guidelines to destroy this fish (Kumar, 2000).

## Conclusions

The ichthyofaunal diversity of the Krishna River at Wai is facing significant threats due to multiple anthropogenic stressors. Intensive exploitation of fish resources, competition and predation by invasive species, and habitat degradation driven by organic and inorganic pollution are major concerns. A substantial portion of the river is impacted by agricultural runoff,

leading to water quality deterioration. Given the global decline in freshwater biodiversity, particularly in ecologically sensitive regions such as the Western Ghats of India, which support a high number of endemic species, urgent conservation interventions are required. The presence of globally threatened species in this region further emphasizes the need for systematic monitoring, habitat restoration, and effective management strategies to ensure the long-term sustainability of native fish populations in the Krishna River system.

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## Author's contributions

SS and VD were involved in designed the study, SS and RM involved in collection and analysis of the results and writing of the manuscript. RM and SS collected laboratory and field data.

## Conflict of Interest

Authors have no conflict of interest to declare.

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**Table 1: List of Freshwater Fish from the Krishna River at Wai and Dhom Reservoir, Including Species Recorded in Previous Studies**

Family/species <sup>a</sup>	Common Name	Threat status (As per IUCN 2017) <sup>b</sup>	Previous study status			Present study
			Silas (1953)	Arunachalam et al. (2002)	Kharat et al. (2012)	
<b>Cyprinidae</b>						
<i>Cirrhinus mrigala</i> (Hamilton, 1822)	Mrigal	LC	-	-	+	+
<i>Cirrhinus reba</i> (Hamilton, 1822)	Reba carp	LC	-	-	+	+
<i>Cyprinus carpio</i> (Linnaeus, 1758)	Common carp	VU	-	-	+	+
<i>Garra mullya</i> (Sykes, 1839)	Sucker fish	LC	+	+	+	+
<i>Hypophthalmichthys molitrix</i> (Valenciennes, 1844)	Silver Carp	NT	-	-	+	+
<i>Hypselobarbus curmuca</i> (Hamilton, 1807)	Curmuca Barb	EN	+	-	+	+
<i>Hypselobarbuscfjerdoni</i> (Day, 1870)	Jerdon's Carp	LC	+	-	+	+
<i>Hypselobarbus kolus</i> (Sykes, 1839)	Kolus Barb	VU	-	-	+	+
<i>Labeo calbasu</i> (Hamilton, 1822)	Orangefin Labeo	LC	-	-		+
<i>Labeo catla</i> (Hamilton, 1822)	Catla	LC	-	-	+	+
<i>Labeo potail</i> (Sykes, 1839)	Deccan Labeo	EN	-	-	+	+
<i>Labeo rohita</i> (Hamilton, 1822)	Rohu	LC	-	-	+	+
<i>Osteobrama vigorsii</i> (Sykes, 1839)	Bheemaosteobrama	LC	-	-	+	+
<i>Puntius amphibius</i> (Valenciennes, 1842)	Scarlet-banded barb	DD	-	-	+	+
<i>Pethia conchoni</i> (Hamilton, 1822)	Rosy barb	LC	-	+	-	+
<i>Puntius sahyadriensis</i> (Silas, 1953)	Khavli barb	LC	-	-	+	+
<i>Systemus sarana</i> (Hamilton, 1822)	olive barb	LC	-	-	+	+
<i>Pethia ticto</i> (Hamilton, 1822)	Ticto barb	LC	+	+	+	+
<i>Rohtee ogilbii</i> (Sykes, 1839)	Rohtee	LC	-	-	+	+
<i>Schismatorhynchus nukta</i> (Sykes, 1839)	Nukta	EN	-	-	+	+
<i>Tor khudree</i> (Sykes, 1839)	Deccan Mahseer	LC	-	+	-	+
<b>Danionidae</b>						
<i>Barilius barna</i> (Hamilton, 1822)	Barna baril	LC	+	-	+	+
<i>Barilius bendelisis</i> (Hamilton, 1807)	Indian Hill Trout	LC	+	-	+	+
<i>Devario malabaricus</i> (Jerdon, 1849)	Malabar danio	LC	-	-	+	+
<i>Devario aequipinnatus</i> (McClelland, 1839)	Giant danio	LC	-	+	+	+
<i>Rasbora daniconius</i> (Hamilton, 1822)	Slender rasbora	LC	-	+	+	+
<i>Salmostoma boopis</i> (Day, 1874)	Boopis razorbelly minnow	LC	-	+	+	+
<i>Salmostoma novacula</i> (Valenciennes, 1840)	Novacula razorbelly minnow	LC	-	-	+	+
<b>Nemacheilidae</b>						
<i>Acanthocobitis mooreh</i> (Sykes, 1839)	Maharashtra zipper loach	LC	-	-	+	+
<i>Indoreonectes evezardi</i> (Day, 1872)		LC	-	+		+
<i>Nemacheilus anguilla</i> (Annandale, 1919)	eel loach	LC			+	+
<i>Nemacheilus rueppelli</i> (Sykes, 1839)	Mongoose loach	LC	-	+	-	+

<i>Schistura denisoni</i> (Day, 1867)	Stone loach	LC	+	-	+	+
<b>Cobitidae</b>						
<i>Lepidocephalichthys thermalis</i> (Valenciennes, 1846)	Common spiny loach	LC	-	+	+	+
<b>Bagridae</b>						
<i>Mystus cavasius</i> (Hamilton 1822)	Gangetic mystus	LC	-	-	+	+
<i>Mystus malabaricus</i> (Jerdon, 1849)	Jerdon's catfish	NT	-	-	+	+
<i>Mystus seengtee</i> (Sykes, 1839)	Seengtee	LC	-	-	+	+
<i>Sperata seenghala</i> (Sykes, 1839)	Giant river catfish	LC	-	-	+	+
<b>Clariidae</b>						
<i>Clarias gariepinus</i> (Burchell, 1822)	North African catfish	LC	-	-	+	+
<b>Siluridae</b>						
<i>Ompok bimaculatus</i> (Bloch, 1794)	Butter catfish	NT	-	-	+	+
<b>Ambassidae</b>						
<i>Chanda nama</i> (Hamilton, 1822)	Elongate glassy perchlet	LC	-	-	+	+
<i>Parambassis lala</i> (Hamilton, 1822)	Highfin glassy perchlet	NT	-	-	-	+
<i>Parambassis ranga</i> (Hamilton, 1822)	Indian glassy fish	LC	-	-	+	+
<b>Channidae</b>						
<i>Channa gachua</i> (Hamilton, 1822)	Dwarf snakehead	LC	-	-	+	+
<i>Channa marulius</i> (Hamilton, 1822)	Great snakehead	LC	-	-	+	+
<i>Channa punctata</i> (Bloch, 1793)	Spotted snakehead	LC	+	-	+	+
<i>Channa marulius</i> (Hamilton, 1822)	great snakehead	LC	-	-	-	+
<b>Cichlidae</b>						
<i>Oreochromis mossambicus</i> (Peters, 1852)	Mozambique tilapia	VU	-	-	+	+
<i>Oreochromis niloticus</i> (Linnaeus, 1758)	Nile tilapia	LC	-	-		+
<b>Belonidae</b>						
<i>Xenentodon cancila</i> (Hamilton, 1822)	Freshwater Garfish	LC	-	-	+	+
<b>Gobiidae</b>						
<i>Glossogobius giuris</i> (Hamilton, 1822)	Tank goby	LC	-	+	+	+
<b>Notopteridae</b>						
<i>Notopterus notopterus</i> (Pallas, 1769)	Bronze featherback	LC	-	-	+	+
<b>Mastacembelidae</b>						
<i>Mastacembelus armatus</i> (Lacepede, 1800)	Zig-zag eel	LC	-	+	+	+

<sup>a</sup>Taxonomic status as per FishBase

<sup>b</sup>IUCN (2017) status., EN - Endangered, NT - Near Threatened, LC - Least Concern, NE - Not Evaluated, DD - Data Deficient.

Key: + = present, - = absent.





Figure 1. Major threats to the fish fauna of Krishna River at Wai. (A). Pollution of the river stretch; (B and C): Heavy harvesting of threatened species and endemic fishes.

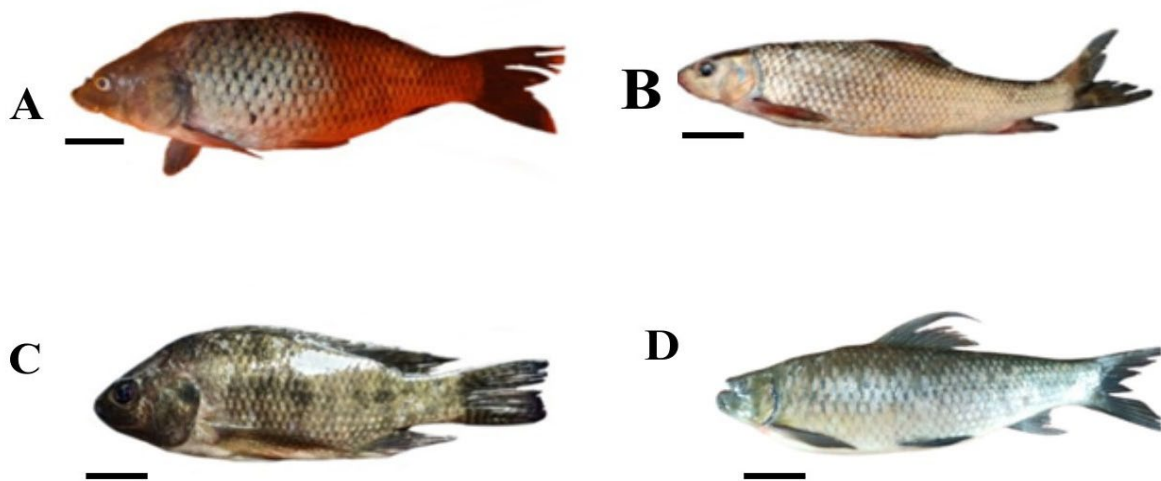


Figure 2. A. *Cyprinus carpio*, B. *Hypselobarbus kolus*, C. *Oreochromis mossambicus*, D. *Schismatorhynchus nukta*

## Quantitative evaluation of proteins, carbohydrates and lipids in the leaves of *Tridax procumbens* L.

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### Abstract

*Tridax procumbens* L. in marathi called kambarmodi, jakhamjudi or tantani is a trailing herb and is reported as a weed in India. (Reyes-Garcia et al., 2006). It has many bioactive molecules valuable in medicines and as food supplements. In this study phytochemical constituents from the crude extract from the leaves of *Tridax procumbens* collected from Vetar hill Dist. Pune have been studied during two consecutive years 2017 and 2018 throughout the year. Many investigators have reported only phytochemical constituents in the leaves of *Tridax procumbens*. The crude extract from the leaves of *T. procumbens* was subjected to methanol extraction. It was phytochemically analyzed for different qualitative and quantitative properties. This investigation revealed proteins, lipids, alkaloids, terpenoids, flavonoids, carbohydrates, glycosides, and phenols in the leaves. In this study we have focused on proteins, carbohydrates and lipids for further utility.

For both years, quantitatively protein contents were found highest as compared to carbohydrates and lipids during winter, monsoon and summer seasons, followed by carbohydrates as moderate and lipids were found lowest during the winters, monsoons and summers. The total annual average of Proteins (P), Carbohydrates (C) and Lipids (L) were found more during 2018 (P 19.10 mg/g, C 16.47 mg/g and L 4.87 mg/g respectively) and recorded less during 2017 (P 16.91 mg/g, C 15.83 mg/g and L 4.85 mg/g respectively). Utility of these findings may offer a clue for their isolation on large scale implementing vast cultivation for the farmers to yield some new edible products like protein chocolates, carbohydrate cakes and lipid syrups, some of which may have medicinal properties.

**Keywords** - Quantitative, phytochemical, seasonal, annual, variation, proteins, carbohydrates and lipids.

## Introduction

Medicinal plants play an essential role in health care and are the major raw materials for both traditional and conventional medicinal preparations; and people choose herbal medicines to conventional medicines (WHO, 2002). *Tridax procumbens* is one of the important medicinal plants. (WHO, 2002). Medicinal plants attracted the attention of scientists due to their effectiveness, lower incidence of side effects, lack of current alternatives, increasing cost of modern medicines and cultural preferences (Heinrich et al., 2000; Tabuti et al., 2003). The quantitative ethnobotanical studies were used to identify the plant uses as food, (Pieroni et al., 2001) medicines for human health care, (Kim et al., 2013) veterinary medicine (Upadhyay et al., 2011) and economic importance due to their low cost (Reyes-Garcia et al., 2006).

Conventionally, *T. procumbens* is used in India as antifungal, insect repellent and anticoagulant medicine. In folk medicine, leaf extracts are used to treat skin infections and in Ayurvedic medicine as a remedy for liver complaints for its hepato-protective nature in addition to use in gastritis and heart burn (Wani et al., 2010). Hence, in this study we have focused on the seasonal and annual evaluation of phytochemical composition of *T. procumbens* leaves.

## Material

### Study Area

Vetal hill in Pune city has an elevation of 2600 ft. Geographically it is 18° 30' to 18° 32' N latitude and 73° 48' to 73° 59' E longitude covering an area of 10.5 square kilometers. It runs North-South with some spurs perpendicular extensions. Vetal hill runs across areas like MIT College, Y.M. College, Bharati Vidyapeeth, SNDT Women's University, ILS Law College, Gokhale Nagar, Symbiosis Society and Chaturshrungi.

**Fig: A and B: Study Area Vetal Hill, Taluka- Haveli, District- Pune, Maharashtra State, india.**

### Authentication

Botanical Survey of India (BSIP) Pune helped us in identification of *T. procumbens*. Subsequently, a sample of the plant with reference number No. BSI/WRC/IDEN.CER. / 2016/135 (A) specimen No.-- PAM 01FHI 1008876 was deposited in the same institute.



**Fig A - Taluka - Haveli, District- Pun**



**Fig B - Vetal Hill collection spot**

### Extraction

Leaves *Tridax procumbens* were washed and sliced into small pieces, shade dried and then using an electrical blender ground into a powder. Five grams of powder was subjected to successive solvent extraction in 100 ml of methanol and kept on shaker for 24 hours to obtain homogenate, filtered by Whatman filter paper-1, and the extracts were stored separately in sterile bottles at 10 °C for phytochemical screening.



Fig. C- Root, stem leaves and inflorescence *T. procumbens*.



Fig. D- Leaves of *T. procumbens* (ventral and dorsal side), hairy, toothed and arrowhead-shaped.



Fig: E and F: Leaf Powder Extraction *T. procumbens*

**Method**

**1) Determination of Total Protein Content *T. procumbens* (Lowry's Method)**

Extraction is carried out with buffers used for the enzyme assay. 500 mg of the sample was taken and ground well with a pestle and mortar in 5-10 ml of the buffer, centrifuged and the supernatant was used for protein estimation.

**a) Procedure for estimation of Protein:**

1. Pipette out 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard into a series of test tubes.
2. Pipette out 0.1 ml and 0.2 ml of the sample extract in two other test tubes.
3. Make up the volume to 1.0 ml in all the test tubes. A tube with 1.0 ml of water serves as the blank.
4. Add 5.0 ml of reagent C to each tube including the blank. Mix well and allow to stand for 10 min.
5. Then 0.5 ml of reagent D was added, it was mixed

well and incubated at room temperature in the dark for 30 min when blue colour is observed.

Take the reading at 660 nm. Draw a standard graph and calculate the amount of protein estimation in the sample.

**2) Determination of Total Carbohydrate Content**

The total carbohydrate content of all the extracts were quantified by using Anthrone method (1952). 100 mg of the *T. procumbens* plant sample was taken into a boiling tube and hydrolyzed by keeping it in a boiling water bath for three hours, with 5.0 ml of 2.5 N HCl and cooled to room temperature. It was neutralized with solid sodium carbonate until the effervescence ceased, made up the volume to 100 ml and centrifuged, collected the supernatant of 0.2 to 1.0 ml was taken for analysis.

Prepare the standards by taking 0.2-1.0 ml of the working standards. 1.0 ml of water serves as a blank,

make up the volume to 1.0 ml in all the tubes with distilled water, then add 4.0 ml of anthrone reagent, heated for eight minutes in a boiling water bath, cool rapidly and read the green to dark green colour at 630 nm.

### 3) Estimation of Total Lipid Content (van Handel 1985)

For the estimation of total lipids, 100 mg per 100 ml of a commercial vegetable oil (Olive oil) in chloroform was used as standard. Aliquots of lipid extracts and standards in test tubes were placed in a water bath at 100° C to evaporate the solvent. 0.2 ml of sulfuric acid was added and heated for 10 min at 100° C. All the samples were made up to 5 ml with vanillin reagent and mixed well. Samples were cooled and kept for approximately 5 min till a reddish color developed. OD was measured at 625 nm within 30 minutes.

## Results

Quantitative estimation of total Protein content of *T. procumbens* leaves was done during summer, winter and monsoon season, 2017 and 2018. *T. procumbens* leaves showed highest concentration of proteins during winter and monsoon season ( $22.18 \pm 0.07$  mg/g and  $19.82 \pm 0.13$  in 2018), followed by winter and monsoon season ( $19.05 \pm 0.98$  mg/g and  $18.25 \pm 0.65$  mg/g in 2017), and the minimum concentration during summer season ( $15.32 \pm 0.19$  mg/g in 2018 and  $13.44 \pm 0.01$  mg/g in 2017).

Phytochemical analysis of *T. procumbens* leaves revealed following results. The prominent results were recorded in summer, monsoon and winter seasons, 2017 and 2018. These results showed highest concentration of carbohydrates during monsoon and winter seasons ( $16.94 \pm 0.21$  mg/g and  $16.54 \pm 0.17$  mg/g in 2018), comparatively less in monsoon and winter seasons ( $16.28 \pm 0.50$  mg/g and  $16.12 \pm 0.23$  mg/g in 2017), and the lowest concentration during summer seasons ( $15.94 \pm 0.25$  mg/g in 2018 and  $15.10 \pm 0.02$  mg/g in 2017).

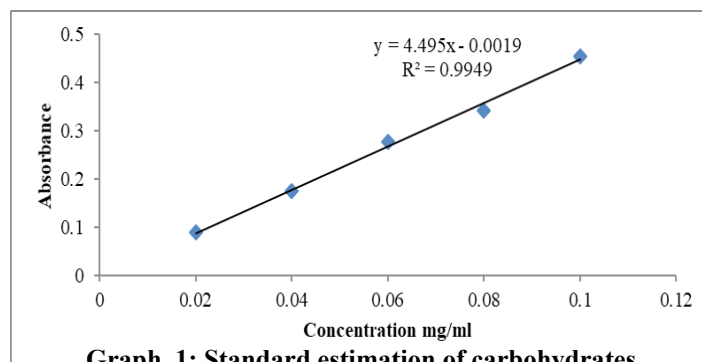
*T. procumbens* leaves recorded highest concentration of lipids during monsoon seasons ( $6.12 \pm 0.19$  mg/g in 2018 and  $5.95 \pm 0.15$  mg/g in 2017), followed by winter seasons ( $5.42 \pm 0.09$  mg/g in 2018 and  $5.02 \pm 0.09$  mg/g in 2017), and the lowest concentration in summer seasons ( $3.60 \pm 0.02$  mg/g in 2017 and  $3.09 \pm 0.23$  mg/g in 2018).

The total annual average of proteins, carbohydrates

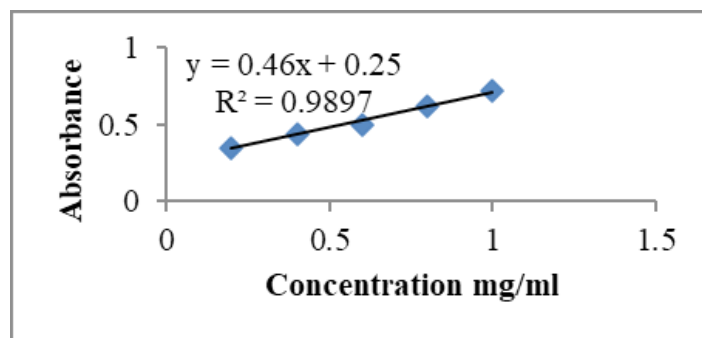
and lipids was more during 2018 (P 19.10 mg/g, C 16.47 mg/g and L 4.87 mg/g respectively) and less during 2017 (P 16.91 mg/g, C 15.83 mg/g and L 4.85 mg/g respectively).

**Graph. 1 and 2: Observations of quantitative phytochemical compositions of *T. procumbens* leaves and stems depicted graphically.**

## Discussion



**Graph. 1: Standard estimation of carbohydrates**



**Graph. 2: Standard estimation of proteins**

Different disciplines like Ethnobotany, Unani medicine, Ayurveda and herbal treatment modalities use *T. procumbens* for treating a variety of diseases. The evaluation of total protein in the leaf extract *Tridax procumbens* revealed higher protein during winter, moderate during monsoon and minimum in summer during both the years 2017 and 2018. This study indicated that increased level of proteins during winter naturally protect the plant from diseases and offer an opportunity to produce edible products. The carbohydrate content is more in monsoon and it gradually decreased in winter and was lowest in summer in both 2017 and 2018. It supports the hypothesis of plant vegetative growth (Jing Zhang et al., 2012) which is maximum in favorable conditions i.e. monsoon periods followed by winter and summer season. The lipid contents were maximum during monsoon period for both years, moderate during winter, while minimum in summer.

We may cultivate *Tridax procumbens* on large scale in our agricultural country for economic benefits through the department of agriculture. Proteins and carbohydrates can be obtained on large scale for the manufacture of different edible foodstuffs with high protein contents in some and higher carbohydrate contents in others.

## Conclusion

Different disciplines like ethnobotany, Unani medicine, Ayurveda and herbal treatment modalities use *T. procumbens* for treating a variety of diseases. These investigations revealed higher concentration of proteins and carbohydrates *Tridax procumbens* in all seasons in both the years 2017 and 2018. The utility of these constituents may be useful in our agricultural country for the economic and all-round progress of our farmers as well as for research in physiology and pharmacological.

## Acknowledgement

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## Conservation of Biodiversity – Review of the Present Status of Remote Sensing Based Mechanisms and Future Applications

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### Abstract

The emergence of advanced mechanisms like remote sensing (RS) systems has added more value to biodiversity conservation analysis. This paper provides a succinct overview of biodiversity conservation and explains the prominence of RS-based mechanism in analyzation of data for conservation of biodiversity. It describes the diverse RS technologies, their types and characteristics for acquisition of imperative data for analysis. This work reviews well-known and advanced technologies like machine learning, computational intelligence, ensemble methods and deep learning adopted by researchers for safeguarding biodiversity. It describes the application of RS and fusion of RS with machine learning (ML) and deep learning (DL) schemes in existing research works for biodiversity protection and explains the significance of these technologies and their advancements for educating the readers regarding their benefits and encouraging further research in this domain with an intent of developing better strategies for achieving biodiversity conservation.

### Keywords

Biodiversity conservation, Remote sensing, Machine learning, Ensemble method, Deep learning

### Introduction

Remote sensing (RS) mechanism has loomed as a prominent tool for mapping and conserving remote and widespread regions (Albalawi & Kumar 2013; Ouellette & Getinet 2016). In the biodiversity protection domain, RS has attracted tremendous interests and is employed in diverse ways (Petrou et al. 2015; Prasad et al. 2015; Alleaume et al. 2018). The RS technology plays an instrumental part in protecting natural features and natural

habitat. The prominent applications of RS technology in conservation of biodiversity include data collection, map generation, protection of forested areas, natural resource region monitoring, change detection, animal conservation, land-cover protection and categorization and environmental conservation (Geller et al. 2017).

This paper provides a detailed review on utilization of RS mechanism and other evolving methods in the analysis of biodiversity conservation research.

## Conservation of Biodiversity

Biodiversity conservation indicates the protection, monitoring, management and upliftment of biodiversity for deriving sustainable benefits for current and future generations (Byjus n.d.). Its vital objectives include preservation of species diversity, sustainable exploitation of ecosystem and species and maintenance of imperative

ecological processes and life-supporting mechanisms. A region with greater amount of species richness possesses a highly stable environment compared to a region with lesser amount of species richness. Currently, biodiversity is being lost owing to over-usage of resources, loss of habitat, pollution, climatic variations, hunting, deforestation, poaching, diseases, invasive external species, etc. (Fletcher et al. 2011). Since biodiversity offers diverse ethical and economic benefits, it is indispensable for conserving the biodiversity.

## Biodiversity Monitoring by Remote Sensing

The United Nations (UN) declared the period 2010-2020 as the decade on biodiversity. However, the goal of minimizing the biodiversity loss was not met. Though the in-situ campaigns are considered as the most reliable way of monitoring different aspects of biodiversity,

**Table 1. Application of RS technology in biodiversity conservation**

Application	Description
Data collection	RS is deemed to be a useful tool for gathering information. For simplifying biodiversity conservation procedure, remote sensors can be employed for acquiring and recording environmental data, which is indispensable for further surveys and analysis.
Map generation	Remote sensors, typically are airborne devices and therefore they are useful in generation of terrestrial ecosystem maps. Further, these maps can be utilized for identifying non-forested and forested areas as well as other physical attributes.
Protection of forested areas	Biodiversity conservation can be achieved through protecting and preventing deforestation in forested zones. As monitoring the dense forest areas from earth's region is tedious, exploitation of RS technologies aids in capturing images at a greater altitude, covering all regions of interest and can be supportive in monitoring forest areas and their conservation.
Natural resource region monitoring	For biodiversity conservation, natural resources must be efficiently utilized. RS technology can be employed for monitoring sites involving resources and regulating activities of over-utilization of resources.
Change detection	As RS has wide coverage, it possesses the potential to recognize environmental variations. Thus, it plays a cardinal part in species and ecosystem services.
Animal conservation	Domestic and wild animals are imperative entities of biodiversity. RS technology aids in monitoring illegal activities like poaching and animal killings.
Land-cover protection and categorization	It assists in monitoring vegetation and its conservation. Moreover, remote sensor information is useful in categorizing plants as per their families.
Environment conservation	Remote sensors aid in environment conservation; air quality monitoring and pollution minimization

they suffer from numerous challenges and have various limitations such as high execution cost, time consuming, and require more effort to execute the process. Remote sensing (RS) is considered as the potential solution for the conventional campaigns. Unlike these campaigns, RS incorporates the data collected from satellites and airborne sensors which accurately collects the data for biodiversity monitoring (Nagendra et al. 2013). The data collected from remote sensing can provide cost efficient and repetitive monitoring of larger regions and provides additional information which is may not be possible using conventional field assessment campaigns.

Several studies have been presented in the existing literary works that use RS data for monitoring (Wang & Gamon 2019; Luque et al. 2018; Reddy 2021). However, most of the existing studies have not incorporated any biodiversity indicator which can be used directly or indirectly to extract any information. Various indicators have been presented in the existing works which results in several incompatible monitoring systems. Furthermore, despite the growing significance of RS data, the relation between the parameters measured by RS and indicators required for monitoring biodiversity is not analyzed in depth. Hence, it becomes essential to use a fundamental set of indicators which can be beneficial to biodiversity using RS.

However, there are certain critical issues which challenges the potential adoption of RS indicators and techniques. Some of the prominent challenges are summarized below (Mairota et al. 2015):

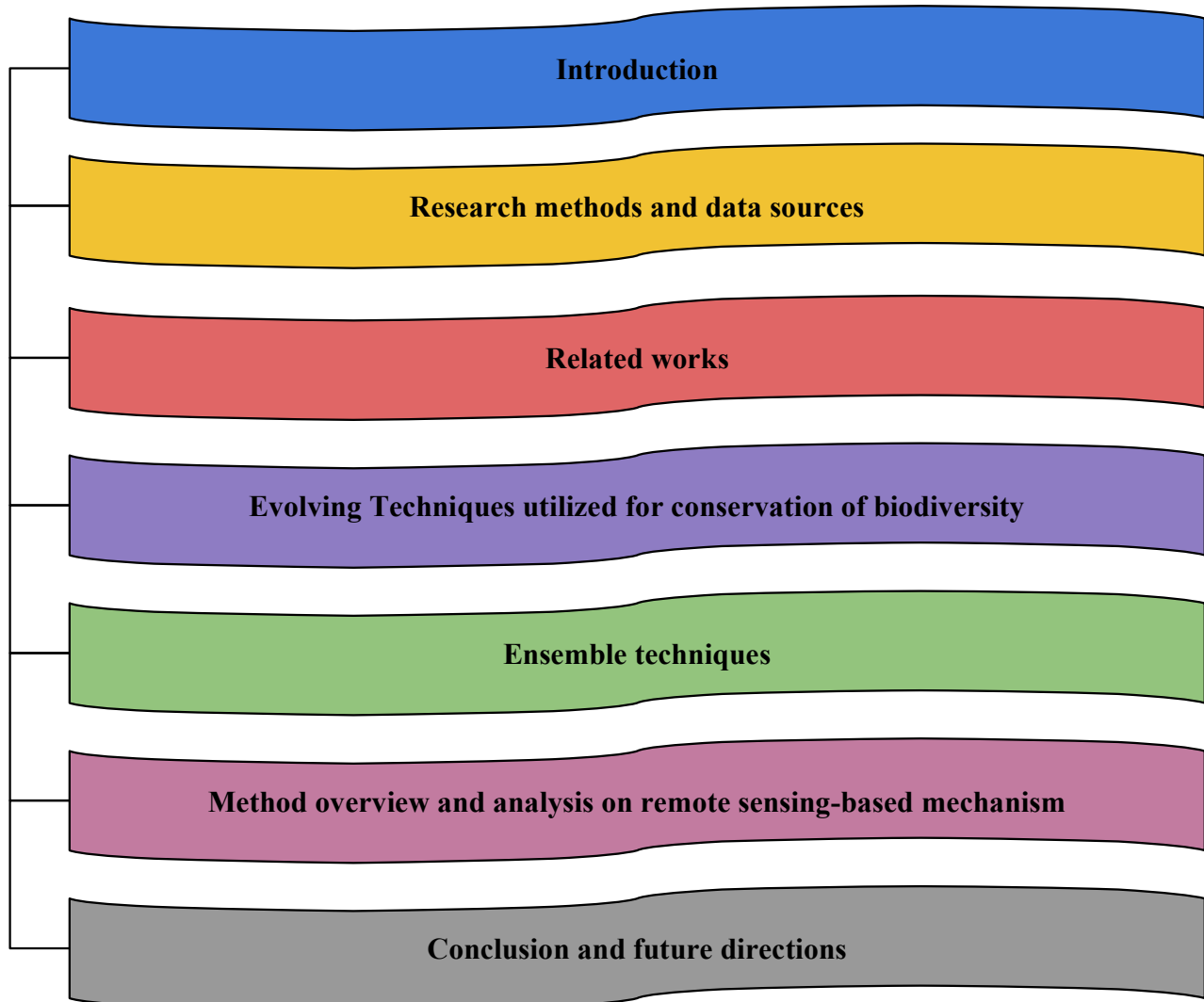
- It is difficult to deal with image corrections and to register image data for detecting changes in biodiversity and for the retrieval of biophysical data along with proper registration of multiple source datasets with multiple dates.
- Incorporating novel and advanced technologies for interpreting images which are currently exploited in the RS area, such as object oriented image analysis (OBIA) and knowledge-driven information mining.
- Defining exact type of habitat and determining its quality can be a complex task.
- Assessing the quality of the habitat and standard of biodiversity from RS is challenging considering the dynamic behavior of RS systems.
- It is highly difficult to accurately map the habitats based on the RS data considering the uncertainties associated with RS systems.
- It requires proficient knowledge related to RS and

ecology along with an appropriate ground truth for monitoring of biodiversity.

- Selecting relevant time and space related applications for RS based monitoring.
- There is a need to analyze the interrelationships between habitat quality, distribution and structure of biodiversity.

It can be inferred from existing works that RS experts must try to bridge the gap between the ecologists and space engineers by precisely defining and interpreting the terms (Rocchini et al. 2016). In addition, the experts should also provide feasibility analysis of relevant satellite observation parameters which can validate the accuracy of RS biodiversity products based on in situ observations or ground proofing. This is one of the prominent requirements which needs to be considered to achieve a proper tradeoff between the technical requirements of RS systems and ecological demands of biodiversity (Rocchini et al. 2016; Skidmore et al. 2021). The above mentioned problems necessitate the need for an empirical analysis of biodiversity conservation and the implementation of remote sensing for biodiversity monitoring.

## Paper Organization



**Fig. 1 Paper Structure**

### Contributions of Survey

The cardinal contributions of this survey include:

- Investigation of emerging techniques employed for conserving biodiversity.

- Exploration of ensemble methods adopted for conserving biodiversity.

- Investigation of remote-based mechanisms for analysis, understanding and future developments.

### Research Methods and Data Sources

The well-known technologies for data acquisition include satellite imagery or remote sensing method (Randin et al. 2020). These technologies aid in acquiring images of study areas considered for investigation. Currently, multispectral imagery has been extensively applied for capturing high resolution analysis images of interest. RS

systems include a heterogeneous array of platforms and sensors. The sensors include active sensors and passive sensors, while platforms range mainly from planet observation satellites, helicopters, planes to drones with rotaries and fixed wings. The popular sensor employed in RS includes an optical imaging mechanism which is capable of capturing information beyond the thermal and infrared wavelengths (visible wavelengths) across the spectrum. Optical sensors include hyperspectral sensors (possess innumerable narrower bands) and multispectral sensors (possess limited amount of bands) (Randin et al. 2020). Passive sensors mainly include thermal systems and optical systems that depend on emitted thermal or reflected sunlight energy and thereby cannot penetrate smoke or clouds. These sensors cannot be utilized during night time. Active sensors mainly include synthetic

aperture radar (SAR) and light detection and ranging (LiDAR) systems (Randin et al. 2020). These sensors can penetrate through smoke and clouds and can be operated at night. Depending on sensor wavelength, the signal can easily penetrate soil, canopy and vegetation. Recently, with the colossal advancements in sensor mechanisms, the mountable active sensor and passive sensor versions have been introduced, which can be easily mounted on drones (UAV), larger planes and satellites. The other sorts of sensors include spaceborne sensors and airborne sensors. Spaceborne sensors make consistent estimations at definite time periods as per the time needed for its revisit to the same location. Airborne platforms can be flown in relation to particular events like fire. They can even be flown under clouds (specifically drones) (Randin et al. 2020). Airborne platforms like drones can capture extremely-high-distance spatial-resolution information. After desired data acquisition via these sensors, the analysis can be conducted using advanced technologies like artificial intelligence or computational intelligence schemes.

## Related Works

In Holloway & Mengersen (2018), the RS mechanism was adopted for biodiversity monitoring. The authors in this work explored the cardinal biophysical procedures underlying the species distribution including land cover variations, disturbances and climate variability. The work also described the prospective synergies between RS communities and ecological modeling for determining the conceptual gaps hindering the application of RS for modeling and monitoring ecological systems. In Holloway & Mengersen (2018), RS and statistical ML schemes for achieving sustainable growth were explored. The authors defined the four cardinal classes of statistical machine learning schemes for investigating RS information namely regression, clustering, dimension diminution and classification. The study further declared that the method selection for analyzing RS data relies on numerous parameters including the amount and nature of training data, inferences needed, and availability of computing power and software and category of estimates. This work also suggested the necessity of advanced approaches and ensemble techniques for performing effective RS analyses. In Silveira et al. (2021), spatio-temporal RS indices were employed for identifying biodiversity conservation hotspots. Here, indices capturing the spatial and inter-annual variability of surface temperature and vegetation

greenness were generated for identifying regions of low, medium and high biodiversity conservation interest. This work indicated that the elevated inter-annual variability posed a huge threat in northeastern parts and southern Argentina regions owing to low spatial variability. This work further declared that in regions with high spatial variability, protection must be strongly considered for maintaining the region's original biodiversity heritage.

Performance of distinct ML methods on categorizing three distinct multispectral and spatial satellite images was described in Rahman (2020a). For categorization, a group of fine and moderate resolution images of Planet, Sentinel-2 and Landsat-8 possessing similar phenological phases were obtained and processed for determining appropriate techniques that offer better performance with regard to land use land cover (LULC) categorization. The classifiers like support vector machine (SVM), random forest (RF) and stacking methods were applied mainly on Planet, Sentinel-2 and Landsat-8 images separately for assessing individual and total class accuracy. Furthermore, two unique training sets were generated for categorizing the imagery. It was identified that the Sentinel-2 performed better compared to all the employed images and SVM outperformed the RF and stacking methods. Results substantiated that the SVM exhibited 0.968 Kappa value and 98.3% overall accuracy. Findings of this work are useful for planners, decision-makers and RS scientists in monitoring briskly changing, diverse and segregated landscape. In Bojamma & Shastry (2021), automated identification of plant species based on ML was described with an intent of biodiversity conservation. This work aimed at resolving the intricacies involved in recognizing distinct plant species through classical techniques for conservation. This work declared that the inclusion of exhaustive and large databases would aid in attaining greater accuracy and identification rates. The study further suggested the necessity for developing identification mechanisms that are capable of functioning even in natural settings. The study Varotsos et al. (2021) discussed the contribution of RS in investigation of ultraviolet radiation effects on biodiversity, ecology and conservation. Findings of this study signified that the optimized fusion of improved RS instrumentation and ground-based system would aid in better investigation of ultraviolet radiation effects and the associated uncertainties. The work Randin et al. (2020) described the applications of RS in forest bionomics and management. This work provided the prominence of RS

in forest surveillance and discussed the distinct sorts of RS platforms and sensors for mapping the variety of forest parameters. This study declared the RS as a promising technology for forest conservation and management around the globe.

Potential for application of ML and artificial intelligence (AI) in managing forests, related services and achieving biodiversity conservation in India was discussed in Shivaprakash et al. (2022). This work discussed the adoption impediments of AI in biodiversity and Indian forestry domains. The study revealed that AI has been less adopted in existing works for forest management and biodiversity conservation. Thus, exploitation of big data, cloud computing, satellite and digital technology would aid in the adoption of AI with regard to forest management and biodiversity conservation. This study declared that challenges like limited accessibility to existing big data or better data unavailability and computation and technical hurdles of employing AI technologies have led to the constrained application of AI technology in biodiversity and Indian forestry domains. Thus, this study suggested the necessity for a) interdisciplinary associations between forest ecologists, forest officials, conservation practitioners, technologists and academicians for facilitating long-run exploitation of AI, b) cost-effective and cheap computational resources, c) ceaseless augmentation of data collection abilities and d) development of fast processing and less intensive algorithms for data examination. The work Diez et al. (2021) investigated and discussed the prominence of DL and RS technology in forest monitoring. It emphasized on studies employing RGB images acquired through RS mechanism and DL method for settling real-world forest research issues. This work stressed on three crucial topics namely forest anomaly identification (insect infestation and forest fires), tree species categorization and individual tree identification from the viewpoint of forest conservation. This study declared the necessity for additional research on identification and categorization of insect infested trees and exploitation of DL schemes in reviewed articles. It further stated that the cause for limited research on these topics was mainly owing to intricacy of individual tree recognition in dense forests and also the relatively limited amount of available samples for infested or sick trees. Thus, it declared that the exploitation of a strong and publicly existing expert annotated dataset could bring about crucial improvements.

The work Amarasingam et al. (2022) described the prominence of drone-based RS mechanism for crop health enhancement and management. The study concentrated on adoption of RS technology for crop disease and pest management, phenotypic measurement, yield estimation, nutritional status assessment and soil moisture evaluation for achieving environmental sustainability and boosting productivity. This work concluded that drone-based crop RS would be an extremely effective approach for ameliorating the quality and yield and leading to environmental, social and economic benefits. The work Murray et al. (2018) discussed the value of satellite RS in assessing ecosystem risks. It declared that unstructured utilization of RS information for evaluating ecosystem dynamics would introduce substantial uncertainty and error. The authors investigated the usefulness of RS data in evaluating the degradation of terrestrial, aquatic and marine ecosystem types. This work provided a valuable framework and desired guidance for integrating RS information into ecosystem threat assessment. This study highlighted that the RS information should possess fine temporal resolutions and spatial resolutions for representing biome dynamics and enabling the detection of brisk changes, closely correlating with suitable in situ ecosystem degradation indicators and facilitating ecosystem monitoring.

## **Evolving Techniques Utilized for the Conservation of Biodiversity**

In Chaves et al. (2020), ML approach was adopted for mapping tree's floristic patterns through exploiting RS data. The authors utilized RF regression scheme for prediction. Through integrating field information of Peruvian national forest, ML and remotely sensed information, a map indicating the forecasted patterns in the community composition of trees was derived. Comparison with previously-reported works illustrated that the presented RF regression scheme exhibited robustness and could be applied to other tropical areas for recognizing suitable regions for conservation. In Rousset et al. (2021), DL methods were employed for land use land cover (LULC) categorization in a subtropical, complex setting. In this work, a specific database was created using SPOT6 satellite information for LULC data analysis. The LULC categorization performance of the presented DL approach was compared with the XGBoost ML classifier. It was identified that the presented DL method performed better than the XGBoost classifier, achieving 63.61%

amelioration in overall accuracy. In Barrett et al. (2014), performance of distinct ML methods like SVM, RF and highly randomized trees was evaluated for distinguishing the grassland categories over two widespread regions of Ireland through employing ancillary spatial, multi-sensor radar and multi-temporal datasets. The detailed assessment conducted in this work demonstrated the effectiveness of SVM, RF and highly randomized trees in categorizing distinct sorts of grasslands. Experiments indicated that the employed ML schemes attained  $\geq 88.7\%$  overall accuracies for single frequency categorizations and 97.9% maximum accuracies for combined frequency categorizations. Findings also highlighted the suitability of ML technology for biodiversity protection.

In Kafy et al. (2021), seasonal surface temperature variation and LULC prediction via RS data was performed using ML schemes. This work adopted Landsat bands and SVM technique for retrieving surface temperature variation and LULC patterns of 2019, 2009 and 1999. Furthermore, it employed neural networks and cellular automata scheme for determining surface temperature variation and LULC patterns for 2039 and 2029. Results manifested that the cellular automata scheme offered better accuracy with 0.82 Kappa value. Experimental LULC analysis results illustrated a substantial augmentation in the suburban built-up regions by 13.59% (2039) and 9.23% (2029) compared to 2019. Experimental summer surface temperature data analysis illustrated that 35.02% and 31.30% region would likely to experience greater than 36° C surface temperature, followed by 29.53% and 1.28% region in winter season for 2039 and 2029. Moreover, the seasonal surface temperature conditions in distinct LULC manifested comparatively elevated temperatures in urban zones. The findings indicated the possibility of larger heat zones owing to augmentation of urban region and diminution of green cover in future. This work further recommended the necessity for natural resource conservation with sustainable and eco-friendly future urban advancement plans through ensuring greater urban-plantation. In Anand et al. (2021), prediction of regional dissemination of a therapeutic plant species (*Rhododendron arboreum*) was achieved using a deep learning (DL) scheme. The employed DL scheme utilized atmospheric and ecological parameters which were resampled statistically and were further employed in establishing a nonlinear and linear relationship for determining the occurrence conditions of the species. Results clarified that the presented DL scheme

outperformed the existing competing method with regard to species occurrence prediction. The work Saha et al. (2022) aimed at detecting deforestation probable regions at Jaldapara protected area and its neighboring zones with an intent of biodiversity preservation. This work adopted five ML algorithms namely RF, decision trees (DT), Naive Bayes (NB), neural networks and support vector machine (SVM) for detection. This study revealed that the middle and northern sections suffered extreme deforestation owing to massive timber trafficking, poaching and human encroachment. It was clearly observed from experimental outputs that the SVM approach outperformed the RF, neural networks, NB and DT schemes through providing 0.90 AUC value. It further stated that deforestation probability region analysis together with proper management policies and daily monitoring would aid in forest sustainability. Moreover, creation of unnatural forest buffer region near the protected forest area would promote the livelihood of forest fringe folks and forest dwellers. Additionally, this work suggested the necessity for enforcing restriction on non-timber product usage for forest fringe folks and forest dwellers and timber trafficking and wild animal trafficking with an intent of biodiversity conservation.

With the intent of biodiversity conservation, the Da Silveira et al. (2021) exploited the RS mechanism and ML scheme. The authors integrated Landsat-8 and WorldView-3 imagery along with ML schemes for mapping appropriate habitats pertinent to the existence of *Millepora alcicornis*, a hydrocoral species in coral reefs of Northeast Brazil's marine protected regions. The ML schemes were employed for determining the species distribution and habitat mapping. This work stated that human activities strongly inhibited coral growth. Findings of this work reinforced the significance of taking safety measures for local biodiversity maintenance. The work Vega Isuhuaylas et al. (2018) discussed Peruvian forest mapping using ML technology for biodiversity conservation. It aimed at determining the best framework for categorizing shrubland and Andes forest using land cover information and Landsat-8 satellite data. The authors employed three distinct ML methods like RF, SVM and K-Nearest Neighbor (KNN) for categorization. Experiments ascertained that the SVM exhibited better classification performance than the RF and KNN. In Choe et al. (2021), a DL scheme was employed for mapping prospective plant species richness along the Korean peninsula. Initially, the richness of South Korean plant

species was estimated through fusing the probability-dependent species distribution framework outputs and plant surveys were employed for validation of proposed prospective species abundance maps. Experiments substantiated that the presented DL scheme showed better estimation potential for species richness compared to the extensively employed RF scheme.

For biodiversity conservation, the work Guirado et al. (2020) estimated the tree cover in drylands through exploiting a DL framework. Findings of this work declared that the adopted DL-based tree cover categorization framework exhibited 23% amelioration in accuracy compared to visual manual interpretation. In DeLancey et al. (2019), DL method was employed for categorizing widespread wetland via RS technology. This work validated a DL-oriented landcover categorization scheme against a classical XGBoost method for mapping an intricate set of landcover types and wetland classes. It was substantiated from experimental observations that the presented DL scheme superseded the XGBoost method with regard to wetland classification through exhibiting 80.2% accuracy and thereby proved its effectiveness for diverse landcover mapping operations. In Kalantar et al. (2020), ML frameworks like regression tree, multivariate regression and SVM were employed for predicting forest fire vulnerability using RS data. This study employed distinct fire predictors acquired from climatic variables, vegetation indices, topographical features and environmental factors. Experimenting with distinct optimization schemes boosted the frameworks' performances providing better prediction accuracy at 0.91, 0.90 and 0.89 for regression tree, multivariate regression and SVM schemes. In Ahmed et al. (2021), ML schemes were adopted chiefly for wetland classification. Analysis was performed through employing the Landsat optical information as input to ML frameworks. It was noticed from experiments that the employed ML schemes effectively categorized the wetlands with less error and high accuracy. In Bland et al. (2015), ML schemes were utilized for forecasting conservation level of data-insufficient species. The work employed geography, threat data and life history of species for predicting their conservation status. The adopted ML schemes were trained using known status of species. The authors utilized distinct ML schemes including KNN, neural networks and SVM for prediction. Experiments substantiated that the ML schemes displayed extremely high species categorization accuracy (92%) and

exhibited greater potential with reference to correct identification of endangered species richness hotspots. The employed ML schemes forecasted 313 out of 493 species which were at the threat of extinction that in turn augmented the calculated proportion of endangered terrestrial species from 22 to 27%. The analysis further stated that the sites forecasted to comprise numerous threatened species were given conservation priorities beforehand, however species specifically in these sites showed relatively greater levels of threat than previously identified. Although the attine results left general mammalian protection priorities typically unaffected, they recommended that threat levels in these mammals were relatively underestimated. The study concluded that unless targeted directly for monitoring, the species categorized as data-inadequate had high possibilities of extinction. Moreover, it declared that through considering information pertinent to data-inadequate species would assist in alleviating gaps in conserving deficiently known biodiversity and biodiversity indicators.

In Ayhan et al. (2020), a DL method was adopted for vegetation recognition. This work employed testing and training databases acquired from distinct geographical regions with distinct image resolutions. The authors in this work applied the vegetation recognition techniques to high-resolution color images containing near-infrared and RGB bands. Experimental observations explicitly confirmed that the DL framework employing the RGB bands exhibited acceptable performance in terms of vegetation detection than the classical DL schemes. The work Smoliński & Radtke (2017) employed ML-based schemes for spatial forecasting of Baltic Sea's fish diversity. The analysis employed six imperative predictors namely sea depth, bottom salinity, seabed sediments, growth season underside temperature and annual average underside current velocity. This work highlighted the capability of ML-based schemes in diminishing the prediction error while modelling fish diversity. In Mayfield et al. (2017), deforestation prediction with an intent of biodiversity conservation was achieved using the ML technology. The authors employed freely accessible databases for creating deforestation threat maps, considering the Madagascar and Mexico study zones. Results clearly revealed the potential of ML schemes with regard to effective deforestation prediction and confirmed the suitability of these schemes for conservation of biodiversity. The work Rehush et al. (2018) utilized both ML and DL

schemes for detecting tree-linked microhabitats (TliMs). This work aimed at assessing the capability of terrestrial close-range laser scanning mechanism for recognizing distinct TliMs such as bark, cavities, mosses, fungi, ivy and bark pockets. The proposed ML scheme employed local geometric attributes for distinguishing the 2D structures like mosses and bark from 3D structures like fungi, ivy and cavities. However, these attributes were incapable of discriminating the concave stem structures (cavities) and convex structures (fungi and ivy). The proposed DL scheme employed rasterized multi-point orthographic projections, suitable for discriminating the stem structures. The ML approach offered 70% overall accuracy whereas the DL scheme exhibited 83% overall accuracy. Findings illustrated that the proposed approach would be promising for forest monitoring tasks.

In Carbonneau et al. (2020), DL techniques were utilized for classifying RGB fluvial environments. The authors employed a RGB image database comprising 5 land-cover categories namely roads, senescent vegetation, green vegetation, dry sediment and water. The conducted experiments manifested that the adopted DL techniques effectively categorized the RGB fluvial environments and outperformed the ML schemes with regard to fluvial scene categorization. In Cao et al. (2018), ML methods were employed for identifying eight distinct mangrove species in China's Qiao island. For mangrove species identification, hyperspectral imaging data was employed as input. Furthermore, waveband selection techniques namely stepwise discriminant investigation, successive projections method and correlation-directed feature selection were applied chiefly for dimensionality diminution and effective waveband selection. Then, distinct ML approaches like SVM, KNN and RF were used for mangrove species categorization. Experimental evaluations ascertained that the SVM classifier provided 93.54% accuracy and superseded the ML approaches in mangrove species recognition. Findings of this work would be promising for forest conservation and management. In Naderpour et al. (2021), a spatial DL-oriented model was built for forecasting forest fire threat in Sydney's northern beach region. The authors selected thirty-six prominent key determinants contributing to fire threat. Further, they spatially mapped this information considering distinct contexts like topography, human-induced, climate, morphology, physical and social viewpoints as input. The developed DL approach followed a stepwise process for executing

several scenarios in order to estimate the fire threat probability with neoteric input contributing variables. It generated fire threat levels depending on vulnerability and susceptibility frameworks. Here, diverse geospatial variables were employed for modeling susceptibility and diverse socio-economic variables were employed for physical and social vulnerability modeling. Finding of this work substantiated the presented DL model's efficacy with regard to fire threat prediction, making it an appropriate and favorable candidate for biodiversity protection.

ML with satellite imagery was exploited in Jackson & Adam (2021) for categorizing jeopardized tree species of submontane forest. The work employed an imbalanced database and evaluated its effect in mapping and detecting tree species under risk in a chosen sub-montane diversified tropical forest by utilizing SVM, RF classifiers and multispectral imagery. The analysis illustrated that among the recognized species, *Zanthoxylum gillettii* and *Syzygium guineense* were most precisely mapped while *Newtonia b Buchananii* was least precisely mapped. The presented methodology exhibited greater potential for forest biodiversity conservation. In Tian et al. (2020), DL scheme was employed for acquiring phenological-spectral features necessary for understanding the *Spartina alterniflora* species, which has led to severe degradation to biodiversity and ecosystem. The phenological features greatly assisted in boosting the DL scheme's performance with regard to *Spartina alterniflora* detection. Furthermore, the regional scale and long-term maps of *Spartina alterniflora* served as a baseline information for the evaluation, conservation and management of diverse ecosystem services like climate change, carbon storage, biodiversity richness and biological invasion. In Mosebo Fernandes et al. (2020), a ML approach for conservation of biodiversity through locating habitat regions for irreproachable species like sage-grouse depending on existing climate conditions and further pinpoint future habitat regions depending on climate projections was described. For analysis, data pertinent to wildlife location, vegetation cover, precipitation, normal temperature and remaining ecosystem-related information was used. Furthermore, ML schemes were crucially applied for locating the current wildlife habitat sites and forecasting appropriate future regions where wildlife would probably migrate to, depending on the impact of climate variation. The conducted experiments explicitly illustrated that the RF approach outperformed

the available competing models through attaining 88.5% specificity, 91.7% sensitivity and 89.7% accuracy. Moreover, it exhibited better potential in modeling species distribution for providing necessary vision into habitat forecasting. The presented methodology revealed that the Utah's sage-grouse habitats would continue to diminish over the succeeding years owing to climate

variation, producing an extremely segregated habitat and inducing a loss of nearly 70% of their existing habitat. Findings of this work highlighted that the protected sites and priority regions of conservation might be considered inadequate for preventing the habitat loss and therefore more effort would be needed for conserving sage-grouse population.

**Table 2. Evolving Techniques used for conservation**

References	Publication year	DL/ML techniques	Evaluation Parameters
Chaves et al. (2020)	2020	ML	RMSE = 0.467
Rousset et al. (2021)	2021	ML	Accuracy = 63.61%
Barrett et al. (2014)	2014	ML	Accuracy = 97.9%
Kafy et al. (2021)	2021	ML	MSE = 0.523, Correlation coefficient (R) = 0.796
Anand et al. (2021)	2021	DL	AUC = 0.917
Saha et al. (2022)	2022	ML	Efficiency = 90.7%, Sensitivity = 88.5%, Specificity = 84.6%
Silveira et al. (2021)	2021	ML	Accuracy = 79%
Vega Isuhuaylas et al. (2018)	2018	ML	AUC = 0.81
Choe et al. (2021)	2021	DL	Accuracy = 98%
Guirado et al. (2020)	2020	DL	Accuracy = 79%
DeLancey et al. (2019)	2019	DL	Accuracy = 80.2%
Kalantar et al. (2020)	2020	ML	AUC = 0.90
Ahmed et al. (2021)	2021	ML	Accuracy = 99%
Bland et al. (2015)	2015	ML	Classification Accuracy = 92%
Ayhan et al. (2020)	2020	DL	Accuracy = 82.98%
Smoliński & Radtke (2017)	2017	ML	RMSE = 0.08, Mean = 1.39, Standard Deviation = 0.66
Mayfield et al. (2017)	2017	ML	AUC = 0.85
Rehush et al. (2018)	2018	ML	Accuracy = 70%
Carbonneau et al. (2020)	2020	DL	F1 Score = 91%
Cao et al. (2018)	2018	ML	Accuracy = 93.54%
Naderpour et al. (2021)	2021	DL	ROC = 95.1%, k coefficient = 94.3%
Jackson & Adam (2021)	2021	DL	F1 Score = 68.56 %
Tian et al. (2020)	2020	DL	Accuracy = 96.22 %
Mosebo Fernandes et al. (2020)	2020	ML	Accuracy = 89.7%

AUC = area under the curve, MSE = Mean Square Error, RMSE = Root Mean Square Error, F1 score = harmonic mean, ROC = Receiver-operating characteristic curve

Table 2 summarizes the application of different ML and DL techniques for monitoring different biodiversity regions. These techniques are highly suitable for predicting the tropical patterns and helps in identifying suitable areas for conservation. A ML based XGBoost classification is used to obtain cartographic information for environmental monitoring Rousset et al. (2021). However, ML techniques are more complex to handle while processing large scale environmental data. In this context, DL models simplify the process. Several research works have implemented DL models in their monitoring process (Anand et al. 2021; Saha et al. 2022; Choe et al. 2021; Guirado et al. 2020; DeLancey et al. 2019; Ayhan et al. 2020; Carbonneau et al. 2020; Naderpour et al. 2021; Jackson & Adam 2021; Tian et al. 2020). The work mentioned in DeLancey et al. (2019) distinguished the effectiveness of deep learning and shallow learning processes. Observations show that, shallow learning cannot provide satisfactory results in terms of image recognition Landover classification, and different remote sensing applications. This is mainly due to the fact that shallow learning cannot analyze contextual information such as complex data patterns and habitats, vegetation, marshes and water bodies etc. However, DL models need more labelled samples for training and more investigation is required for analyzing different wetland or land cover mapping studies. This constitutes as one of the prominent research gaps and needs to be addressed.

## Ensemble Techniques

In Muthoka et al. (2021), ensemble ML techniques were employed for detecting *Opuntia stricta* which poses a threat for biodiversity. This work utilized RS imagery data and ensemble techniques like random forest (RF) and extreme gradient boost (XGBoost) for detection. It was identified that the RF and XGBoost techniques offered 92.4% and 89.2% overall accuracies. Experimental findings manifested the efficacy of RS imagery information and ensemble techniques with regard to detection and mapping *Opuntia stricta* for conserving biodiversity. In Halmy & Gessler (2015), ensemble schemes were adopted for categorizing land-cover in arid regions. The authors employed the Landsat5 mapper data acquired for an Egyptian desert landscape with ancillary parameters for categorizing 13 distinct LULC classes. The employed ensemble schemes classified the land-covers accurately and exhibited greater than 85% overall accuracy and 0.83 Kappa coefficient.

Ensemble modeling framework was employed in Rather et al. (2022) for identifying appropriate regions for restoring habitats of endangered biodiversity. The authors adopted ensemble techniques for mapping the current possible distribution and forecasting the prospective appropriate habitats for species, considering the likely climate variation scenarios. The community data results declared that the considered Himalayan Trillium was positively linked with four shrubs, two tree species and a herbaceous species. The developed ensemble approach provided a nature-dependent panacea in guiding habitat restoration activities in Himalaya and across the world. In Rew et al. (2020), an ensemble approach was employed for estimating the habitat suitability. This work employed environmental and observational data for seven species of birds and four species of amphibians for analysis. It was viewed from results that the presented ensemble approach offered accurate habitat suitability estimation compared to earlier-reported schemes. In Mohamed et al. (2018), RS and ML-based ensemble approach was adopted for monitoring benthic cover and mapping. Initially, videos pertinent to benthic habitats were collected using a towed camera, and were transformed to geo-located habitat images. Further, these habitat images were labeled for grouping the habitats manually. The attributes for classifying these images were automatically extracted using the bag of attributes algorithm. Then, benthic cover classes were automatically detected using a majority voting ensemble of KNN, bagging and SVM classifiers. The accurately classified geo-located images offered ground truth instances for mapping benthic cover using satellite imagery. Testing of presented methodology over Ishigaki island of Japan manifested that the presented ensemble approach offered 89% accuracy in classifying algae, sediments, corals and seagrass species. Additionally, the presented ensemble technique provided 92.7% accuracy in benthic cover mapping using the satellite image. Experimental outputs substantiated the amelioration in automatic monitoring of benthic habitats and mapping accuracies.

The work Stohlgren et al. (2010) adopted an ensemble approach for identifying invasive plant habitats. In this work, five individual ML frameworks namely logistic regression, multivariate regression splines, regression trees, maximum entropy framework and RF schemes were adopted and compared with the presented ensemble approach for a chosen plant species recognition. Analysis was performed using the field information gathered from

**Table 3. Ensemble Techniques used for conservation**

References	Publication year	Evaluation Parameters
Muthoka et al. (2021)	2021	Accuracy = 90.8%
Halmy & Gessler (2015)	2015	Accuracy = 85%
Rather et al. (2022)	2022	High Predictive Accuracy
Rew et al. (2020)	2020	True skill statistic (TSS) score = 0.886
Mohamed et al. (2018)	2018	Accuracy = 92.7%
Stohlgren et al. (2010)	2010	High risk analysis and robustness
Chakraborty et al. (2016)	2016	Accuracy = 70%

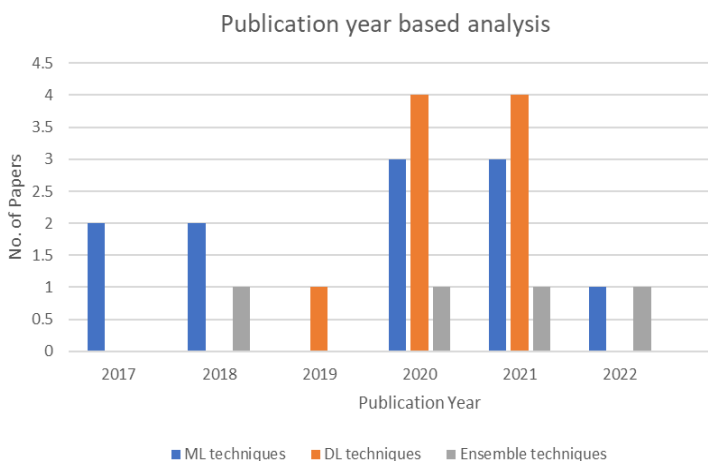
the park members, combined with vegetation, climatic and topographic predictors acquired from satellite information. Experimental outputs ascertained that the presented ensemble approach performed better than the individual ML frameworks and exhibited greater robustness. In Chakraborty et al. (2016), a tree-directed ensemble classification scheme was adopted chiefly for mapping LULC variation in Himalayan region. For handling high temporal and high spatial variability and sophisticated multi-signature categories, this work presented an ensemble classification method. The authors used multi-seasonal data using temporal signatures in diverse spectral sites and heterogeneous environmental variables for recognizing twenty LULC categories, focusing on discriminating geographically predominant forest types. This work employed a knowledge-dependent decision level combination for producing annual fusion maps and RF classifier for creating seasonal maps. Results demonstrated that this approach offered nearly 70% accuracy. This methodology could be extended to

distinct RS databases for further assisting in widespread forest site mapping and forest type monitoring, thereby supporting in developing forest conservation strategies and realistic field inventories.

The works reviewed in Table 2 and Table 3 based on publication year are depicted in Fig. 2. From Fig. 2, it could be observed that in most of the recent works (published in 2020 and 2021) DL techniques have been largely utilized compared to ML and ensemble techniques.

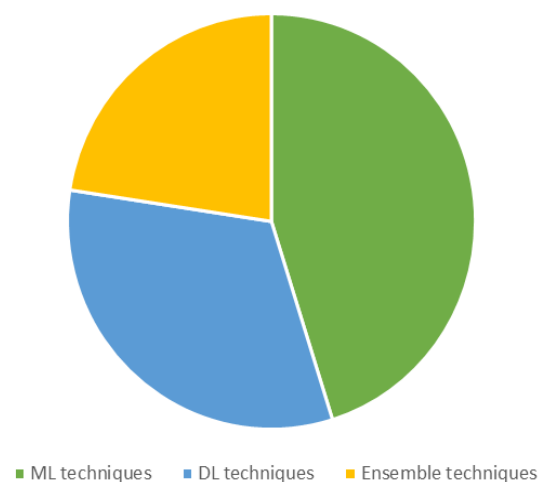
The works reviewed in Table 2 and Table 3 based on techniques employed for analysis are depicted in Fig. 3. The techniques adopted for biodiversity monitoring and conservation in existing works are comprehensively categorized as ML, DL and ensemble methods. From Fig. 3, it could be noted that ML techniques are largely adopted in reviewed works than the DL and ensemble techniques.

The works reviewed in Table 2 and Table 3 based on achieved accuracy percentages are depicted in Fig. 4. From

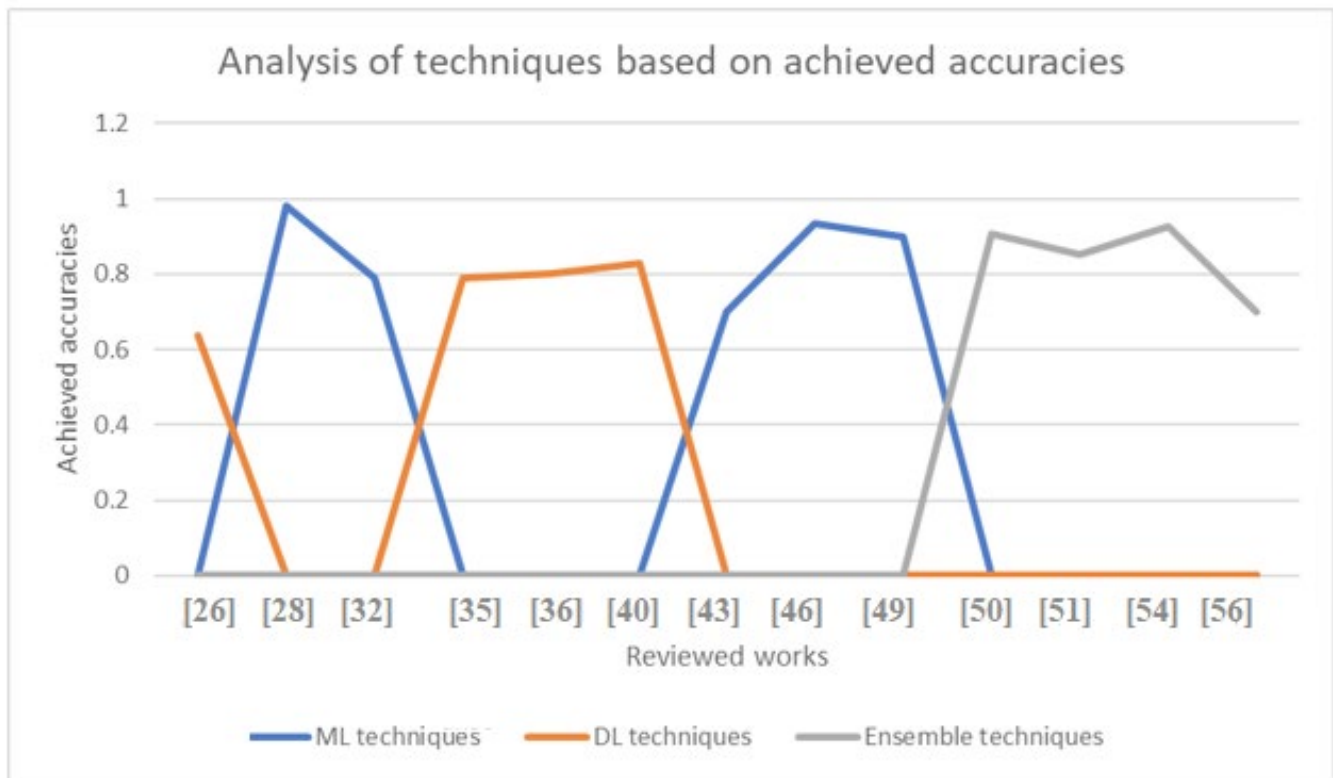


**Fig. 2 Analysis of reviewed works based on publication year.**

**Analysis based on techniques**



**Fig. 3 Analysis of reviewed works based on techniques.**



**Fig. 4 Analysis of reviewed works based on achieved accuracies**

Fig. 4, it could be inferred that among the reviewed studies, the ML technique employed in work Barrett et al. (2014) offered highest accuracy of about 97.9%. The DL method utilized in work Ayhan et al. (2020) provided the highest accuracy of 82.98% and the ensemble technique employed in work Mohamed et al. (2018) provided the highest accuracy of 92.7%.

## Method Overview and Analysis on Remote-sensing based Mechanism

In Rahman et al. (2020b), RS-based mapping of carbon to nitrogen ratio (CNR) of senescent leaf using ML schemes was presented. The ML schemes were employed for validating the predictability of employing the Landsat8 and Landsat TM5 for mapping temporal and spatial distribution of CNR of leaves in Bangladesh's Sundarbans Forest. Findings clarified that the remote sensing information, namely Landsat TM5 effectively mapped the CNR of leaves with acceptable accuracy. In Traganos et al. (2018), RS based mechanism was employed for widespread, temporal and high spatial mapping and surveillance of seagrasses and optically shallow sites. The employed RS based mechanism aimed at providing the cardinal seasonal to annual baseline mapping, surveillance of seagrass habitats on a widespread basis for identifying threat regions, assisting management planning, coastal conservation and finally climate variation

mitigation. In Wang et al. (2020), RS-based mechanism was integrated with landscape characteristics for soil salinity estimation. This work derived an overall 35 indices such as terrain attributes, salinity spectral, and vegetation spectral along with RS classifiers and correlated them with soil salinity. Findings clarified that the farmland reclamation influenced the soil salinity distribution. In Ortega Adarme et al. (2020), RS imagery was employed for detecting deforestation in Cerrado and Amazon biomes of Brazil via DL-directed strategies. Experimentation was conducted using two Landsat 8 images captured at distinct dates. Results ascertained that the fusion of RS-based mechanism with DL technology exhibited greater effectiveness with regard to deforestation detection and reduced the time spent in the visual investigation of deforested regions.

In Arruda et al. (2021), a RS-based mechanism was integrated with the DL scheme for identifying burned regions in the Brazilian Cerrado biome. This work reported that 67% and 31% of the overall burned region occurred in native vegetation and farming lands. Experiments substantiated that the adopted approach offered 97% overall accuracy in terms of burned region prediction. Moreover, the research findings revealed that the presented approach could be applied successfully to the RS domain using huge RS databases and requiring low labor demand and time. The work Nguyen et al. (2021) combined the RS

technology with ML technique for evaluating land usage variation and environmental monitoring. The authors employed Sentinel-2 images and RF method for map generation. Results revealed that the employed RS and ML technology fusion exhibited acceptable efficiency with regard to evaluation of land usage variation and environmental monitoring. In Tuanmu et al. (2010), a RS mechanism was utilized for detecting understory vegetation. This work employed phenology features derived from the RS approach for spatial distribution mapping of bamboo. Owing to its extensibility, flexibility and generality, this approach constituted an amelioration with regard to remote identification of vegetation, confirming its efficacy for mapping distinct understory species in heterogeneous geographic settings. Findings of this study declared that both the wildlife ecosystem management and biodiversity conservation would be benefitted by the detailed understory vegetation data via the application of presented approach.

In Pontoglio et al. (2021), RS technology was integrated with ML approach for identifying automated features in fluvial settings. The analysis was executed through acquisition of several photogrammetric images. Further, an algorithm was developed for identifying the water table. The presented approach successfully identified distinct patterns such as vegetation, gravel bars, water and ground categories in specific geomatics and hydraulic conditions and provided 11% amelioration in accuracy and 13.5% amelioration in F1-score mean values. This work also suggested the suitability of applying this approach in diverse fluvial scenarios for monitoring distinct fluvial regions and preventing catastrophic incidents induced by climate change. In Ye et al. (2019), DL with RS-based mechanism was applied mainly for detecting landslides. In this work, hyperspectral RS data was used for landslide recognition. Initially, the spatial-spectral attributes of the landslide were extracted. Further, the high-level attributes were inserted into the DL classification framework for landslide identification. It was clearly found from experimental outputs that the RS-DL technology fusion helped in precise landslide detection with about 97.91% precision when compared to customary hyperspectral image categorization techniques. In Meng et al. (2021), the RS mechanism was integrated with ML approach for mapping *Kobresia pygmaea*, a meadow grassland community. In this work, ML techniques were integrated with topographic indices and satellite images for categorizing the grassland community. Findings indicated that for the considered field regions, the alpine vegetation communities illustrated an acceptable spatial heterogeneity.

Moreover, the employed ML approach with texture attributes, topographic indices and vegetation indices displayed superior performance achieving 83.92% overall accuracy and 0.80 Kappa coefficient. Results manifested the feasibility of ML and RS mechanism with regard to categorization of vegetation community.

In Wagner et al. (2020), RS technology combined with DL method was applied chiefly for examining spatial distribution and regional mapping of canopy palms of Amazonian forest. The regional mapping was executed for investigating spatial dissemination of palms and their relation to edaphic and human disturbance conditions. It was found that this approach offered 95.5% overall mapping accuracy and 0.7 F1-score. The palm distribution analysis revealed that the abundance of palms was naturally controlled by soil water level, avoiding both waterlogged and flooded regions near dry areas and rivers. Moreover, their distribution indicated a considerable pristine landscape which is severely endangered owing to illegal cutting and deforestation. Furthermore, this work declared that the new species distribution information would aid in documenting the species distribution and understanding species spatial diversity and distribution. In Doyle et al. (2021), RS and ML technologies were employed for identifying patterns in land-cover, land-use variation and the augmenting loss of wetlands and forests. In this work, a RF classifier was adopted chiefly for mapping land-cover and land-use across the complete Landsat record. Furthermore, the seasonal and multiyear composites were utilized for acquiring good coverage and discriminating distinct land-cover and land-use types. The employed RS and ML technological fusion exhibited acceptable performance with regard to interpretation of crucial trends in land-cover, land-use variation and the expanding loss of wetlands and forests. In Abeysinghe et al. (2019), RS and ML technology was employed for mapping an invasive plant species known as *Phragmites australis*. The RS technology was adopted for acquiring invasive plant images. Further, pixel-based schemes (SVM, neural networks and classical parametric optimum likelihood classifier) and object-based schemes (SVM and KNN) were utilized for detecting *Phragmites* in Estuary. It was identified that the pixel-oriented NN offered 94.80% overall detection accuracy. Moreover, this work provided an approach for identifying invasive plant species with greater accuracy in a limited region with just a constrained amount of samples. In Knopp et al. (2020), RS mechanism was integrated with DL technology for burned region segmentation. The authors employed a U-Net

structure-based neural network and shortwave infrared, near infrared and visual spectral bands for analysis. Results indicated that this methodology offered 0.94 Kappa coefficient and 98% overall accuracy. In Huerta et al. (2021), DL method and RS-based mechanism was applied chiefly for mapping metropolitan green spaces with the cardinal goal of ameliorating the urban management, necessary for natural resource conservation. The authors utilized a U-Net structure for analysis. Experimental outputs indicated that the adopted DL methodology precisely mapped the metropolitan green spaces and exhibited 0.94 Kappa value, 0.80 recall, 0.57 dice coefficient and 0.97 overall accuracy. In Miyoshi et al. (2020), a RS with DL methodology was described for recognizing a tree species specifically in the extremely-dense areas. For analysis, a Brazilian biome's semi-deciduous forest images were utilized. This work then employed a DL architecture for recognizing a tree species. The employed architecture considered the probability of each image pixel to be analogous with an original tree-species, as this was imperative for providing accurate outputs in an extremely-dense scene. Experiments confirmed that the presented methodology offered notable performance with reference to identification and geolocation of trees in RS-dependent hyperspectral images, offering 0.945 recall, 0.973 precision and 0.959 F-measure values. Experimental outputs and findings demonstrated the DL architecture's effectiveness in terms of forest monitoring and accurate recognition of single-trees. The work Morin et al. (2021) applied RS-ML technological fusion for mapping deforestation, forest degradation and LULC changes in Armenia's Dilijan national reserve. The authors employed high resolution imagery for measurement and validation of forest density and related variations. Analysis of study outputs clarified that the majority of degradation and deforestation occurred before 2000 with a considerably constant period until 2006 when both degradation and deforestation started to augment again. From the LULC map analysis it was identified that the chief reasons for forest cover variations were linked to agriculture, human settlements and transformation to other sorts of vegetation. This study served as an imperative decision-making tool for developing sustainable forest policies and management.

In Wu & Liang (2018), a RS-dependent biodiversity index was exploited for forecasting animal species abundance. This work introduced a neoteric biodiversity index through fusing diverse highly correlated RS metrics. It was identified that the evapotranspiration metrics highly influenced the richness of species at global levels. Moreover,

the RS metric in texture group exhibited higher dependency with species abundance at regional level. The temperature and radiance metric showed a greater effect on bird richness distribution, compared to their effects on distributions of mammals and amphibians. The results attained in this work demonstrated the efficacy of RS mechanism with regard to biodiversity conservation analysis.

## Conclusion

This paper provided an overview of RS technology and its crucial role in biodiversity conservation, discussing its distinct components and their features for desired data acquisition. The study investigated previously reported research works on the adoption of RS mechanisms for ecosystem monitoring and biodiversity protection, exploring popular technologies like ML, computational intelligence, DL and ensemble methods employed in existing studies for conservation. Furthermore, this review article investigated the utilization of integrated frameworks exploiting hybrid mechanisms involving RS and AI technologies in existing research studies to determine their efficacy towards biodiversity conservation. The review concluded that the development and adoption of appropriate hybrid frameworks through integrating RS with AI technologies would be significantly more effective for biodiversity conservation analysis than their individual exploitation. This review has highlighted various RS and ML techniques presently preferable for different types of biodiversity analysis: for burnt forest analysis, RS combined with DL techniques like U-Net excels in segmenting burnt regions with high precision; ocean beds for corals benefit from RS combined with ML techniques such as RF and SVM for habitat mapping and species distribution; tree species identification is best achieved using DL models, particularly CNNs, even in dense forests; species richness can be predicted using both ML and DL techniques, with DL models being particularly useful for large-scale and complex data patterns; sick trees can be detected using RS combined with ML algorithms like SVM and RF, effective for identifying anomalies such as insect infestations and diseases; LULC classification benefits from DL models providing high accuracy in environmental monitoring; wetland classification is effectively performed using ML algorithms like RF and SVM; forest fire prediction is best achieved using ML models, including regression trees and SVM, which predict susceptibility based on various environmental factors; invasive species detection is effectively performed using ensemble ML techniques like

RF and XGBoost; and lastly, habitat suitability estimation benefits from ensemble ML models, providing accurate estimations by combining multiple ML algorithms. These summaries will aid researchers in selecting the appropriate technology for their specific biodiversity analysis needs. Moreover, the utilization of evolving technologies like ML, DL, and computational intelligence would significantly aid policymakers and ecosystem conservation authorities in sustainable environmental resource management, as well as wild habitat and species conservation. Additionally, the implementation of effective environment or ecosystem management policies and the enforcement of rules for natural resource usage would assist in ameliorating environmental health and in conserving unblemished natural resources.

## Declaration

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## Rescue and re-wilding of Lesser Adjutant Stork *Leptoptilos javanicus* after ringing and tagging at Solapur, Maharashtra, India: A Case Report

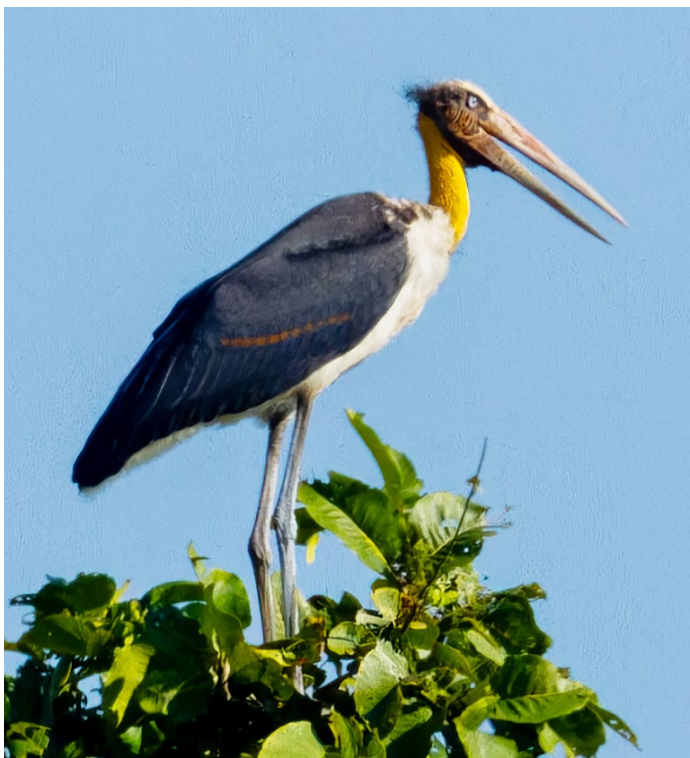
Satish Pande\*, Bharat Cheda#, Rahul Vanjari#, Rahul Lonkar\*, Rohitkumar Gangurde\*\*, Ajit Shinde\*\*, Tushar Chavan\*\*, Mahadev Mohite\*\* and N.R.Praveen\*\*

(Ela Foundation, Pune; Nature Conservation Circle, Solapur; Maharashtra Forest Department)

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An exhausted Lesser Adjutant Stork *Leptoptilos javanicus* was found on the road at the IIT College Chowk on 29<sup>th</sup> April 2025 in Solapur. It was not flying and therefore was rescued by the members of Nature Conservation Circle, Solapur. Examination after rescue revealed that there was no external injury. It was weak and dehydrated as indicated by dry mucous membranes and eyes. The stork was admitted to TTC Solapur of Maharashtra Forest Department (MFD). It was kept in a separate enclosure and there was no disturbance. TTC of Ela Foundation at Pingori, Taluka Purandar, Pune, of MFD and Ela Foundation was requested for treatment advice. The stork was offered fresh clean water and fresh fish (Rohu *Labeo rohita*, Catla *Catla catla*, Eel *Mastacembelus armatus*, and Tilapia *Oreochromis mossambicus*) in a shallow pond in the housing aviary, which it consumed voluntarily. It was monitored 24x7 with a CCTV camera. The stork gained strength and started flying and was seen perching on the high horizontal artificial perch in the tall aviary where it was housed.

This stork is distributed in South and SE Asia with patchy distribution in India<sup>1,2</sup>. It is listed as Vulnerable in IUCN Red Data Book since 2020<sup>3</sup>. Hence, this rescue is an important conservation measure.

Permission (dated 8/5/2025) was granted by N. R. Praveen, IFS, CCF-T, Pune, to TTC of Ela Foundation and MFD, Pingori, to ring and tag this rescued and treated Lesser Adjutant Stork prior to rewilding to monitor the stork in the future. The procedure was successfully carried out at TTC Solapur on 15<sup>th</sup> May 2025, by ornithologists and experts from Ela Foundation [Dr. Satish Pande (Hon. WLW), Rahul Lonkar, Shrikant Kulkarni and Vivek Kelkar] in collaboration with Solapur ACF (Ajit Shinde) and RFO (Rohitkumar



**Experts after ringing and tagging of Lesser Adjutant Stork in the aviary of forest department, Solapur**

Gangurde), veterinary doctor of TTC Solapur, Forest Department Maharashtra and Nature Conservation Circle, Solapur led by Bharat Cheda, Hon. WLW. The stork was found to be alert, strong and able to fly with strong wing beats. Hence, a decision was taken to release the stork near a water body on the next day.

**The details of tagging are as follows:**

- **Wing Tag** – Red, B 17-03 (right and left wings); upper and under wing-U shaped
- **Ring** – Yellow, Right tibia, No. 360; (Contact numbers mentioned)
- **Small overlay tag** – Orange No. 21 and 22

**The details of morphometry are as follows:**

- **Biometry:** Biomass: 4770 gm; Stretched wing: 1090 mm; Wing chord: 640 mm; Beak: 255 mm; Tarsus: 300 mm; Middle toe: 122 mm; Middle talon: 167 mm; Body temperature: 35 deg C;

**Laboratory tests:** (Done at Dr. Bharat Mulay's Pathology Laboratory, Solapur)

- **Fecal (stool) examination:** No parasites. No blood. White splash and central GIT pellet.
- **Haematology:** Hb 17.5 g/d
- **Serology:** CRP 1.75 mg/L
- **Biochemistry:** BSL:210.8 mg/dL; TChol: 160 mg/dl; TG: 54 mg/dl; HDL Chol: 71.4 mg/dl; S. Amylase: 2282IU/L.
- **Liver Function Tests:** TBl: 0.4 mg/dl; DBl: 0.3 mg/dl; In Bl: 0.1 mg/dl; SGOT: 592 IU/L; SGPT: 57.9 IU/L; S. ALP: 99 U/L; TPr: 3.2 g/dl; Alb: 1.2 g/dl; Glob: 2 g/dl; A:G ratio: 0.6
- **Renal (Kidney) Function Tests:** B Urea: 15.6 mg/dl; S Cr: 0.4 mg/dl; S. Uric acid: 16.7 mg/dl; S Ca:

9.7 mg/dl; S P: 4 mg/dl; S Na: 153 mmol/L; S Cl: 102 mmol/L.

Above parameters indicated mild stress induced pancreatitis after sun stroke, which is expected to regress after rewilding. Stork was active, alert flying, eating fish (Tilapia, Catla, Rohu, Eel), drinking water and defecating. The stork was successfully released on the banks of Hipparga Lake, Solapur, and flew to freedom in the presence of ACF and RFO, Solapur and the flight was recorded on both still and video digital cameras. The stork was not sighted again. It is requested to the birding community to inform the author SP if it is sighted again (Phone: 9822193707).

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