



Supplementary Figure 1: Selection of articles for the manuscript through PRISMA.

Supplementary Table 1.

No. of Samples	Total No of Collection Sites	Article Type	Study Duration	DNA Extraction Source	Journal Name	Reference
1	Not Mentioned	Research Article	Not Provided	Meat	<i>Journal Of Bioresource Management</i>	(Rehman <i>et al.</i> , 2015)
1795	225	Research Article	2010-2016	Single Leg	<i>PLOS One</i>	(Ashfaq <i>et al.</i> , 2019)
10792	28	Research Article	2019-2021	Single Leg	<i>PeerJ</i>	(Samreen and Manzoor, 2024)
3	1	Short Communication	Not Provided	gills and caudal region	<i>Mitochondrial DNA</i>	(Karim <i>et al.</i> , 2016)
5	33	Research Article	Not Provided	Left Leg	<i>Pakistan Journal Of Zoology</i>	(Tahir <i>et al.</i> , 2016b)
27	3	Research Article	Not Provided	Caudal fin	<i>Journal Of Bioresource Management</i>	(Kamran <i>et al.</i> , 2020)
10	Not Mentioned	Research Article	2012	Not Provided	<i>Molecular Biology Reports</i>	(Awan <i>et al.</i> , 2013)
		Research Article	Not Provided	Review Article	<i>Pak-Euro Journal Of Medical And Life Sciences</i>	(Hanif <i>et al.</i> , 2023)
872	Not Mentioned	MITO Communication	June-August 2017	Not Provided	<i>Mitochondrial DNA Part B</i>	(Tahir <i>et al.</i> , 2019)
53092	4	Research Article	7 February–13 December 2012, 9 April–3 July 2014, November 2014–February 2015	Leg	<i>PLOS One</i>	(Ashfaq <i>et al.</i> , 2018)
4	1	Research Article	Sep-16	Skin samples	<i>Nucleosides, Nucleotides And Nucleic Acids</i>	(Khan <i>et al.</i> , 2018)
35	7	Research Article	Not Provided	Not Provided	<i>Journal Of King Saud University – Science</i>	(Sajjad <i>et al.</i> , 2023)
407	107	Research Article	2009-2012	Single Leg	<i>Molecular Ecology Resources</i>	(Ashfaq <i>et al.</i> , 2013)
41	8	Research Article	Not Provided	Fins	<i>Diversity</i>	(Hasan <i>et al.</i> , 2021)
342	8	Research Article	May and October in 2020	Whole body except 1cm anal region	<i>Journal Of Oleo Science</i>	(Hussain <i>et al.</i> , 2022)
36	6	Research Article	Not Provided	meat, scales, fins	<i>Pakistan Journal Of Agriculture Sciences</i>	(Khan <i>et al.</i> , 2024b)
59	2	MITO Communication	July-November 2017	Left Leg	<i>Mitochondrial DNA Part B Resources</i>	(Tahir <i>et al.</i> , 2018)
75	3	Research Article	Not Provided	fin tissue	<i>Journal of King Saud University – Science</i>	(Zafar <i>et al.</i> , 2024)
Not Menti	24	Research Article	Not Provided	Larvae	<i>Current Trends in OMICS</i>	(Naz <i>et al.</i> , 2023a)

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	Not Mentioned	Research Article	Not Provided	Review Article	<i>Annals of Animal Science</i>	(Fayaz et al., 2024)
51	3	Research Article	Not Provided	Not Provided	<i>Systematic Entomology</i>	(Memon et al., 2006)
35	52	Research Article	2017-2019	Not Provided	<i>Journal of Animal &amp; Plant Sciences</i>	(Sajid et al., 2021)
30	Not Mentioned	Research Article	2016	Blood	<i>Journal of Bioresource Management</i>	(Ali et al., 2016)
	Not Mentioned	Review	Not Provided	Not Provided	<i>Mitochondrial DNA</i>	(Tahir and Akhtar, 2016a)
6	6	Research Article	Not Provided	gills	<i>LGU Journal of Life Sciences</i>	(Malkani et al., 2022)
21	5	Research Article	August 2018-July 2022	Not Provided	<i>Journal Of Wildlife and Biodiversity</i>	(Ali et al., 2024b)
2736	6	Research Article	July-October 2018-2019	Not Provided	<i>Punjab University Journal of Zoology</i>	(Mehmood et al., 2021)
25	2	Research Article	Not Provided	pectoral fins	<i>International Journal for Multidisciplinary Research (IJFMR)</i>	(Karim et al., 2024)
Not Mentioned	1	Research Article	Not Provided	Muscle	<i>Journal Of Animal &amp; Plant Sciences</i>	(George et al., 2021)
5	2	Research Article	Not Provided	Blood	<i>Brazilian Journal of Biology</i>	(Karamat et al., 2020)
10653	46	Research Article	Summer 2022	Not Provided	<i>PLOS One</i>	(Gul et al., 2024)
10	Not Mentioned	Rapid Communication	Not Provided	Not Provided	<i>Mitochondrial DNA Part B</i>	(Hussain et al., 2020a)
15	1	Research Article	Not Provided	Muscle	<i>Frontiers In Chemical Sciences</i>	(Usman et al., 2024)
12	1	Research Article	Not Provided	(coats, tanned skins) and fur (mufflers) samples	<i>Mitochondrial DNA Part A</i>	(Janjua et al., 2017)
784	5	Research Article	April-November 2019	Not Provided	<i>PLOS One</i>	(Hussain et al., 2024)
Not Mentioned	2	Research Article	Not Provided	Tissues	<i>Journal Of Biological Regulators &amp; Homeostatic Agents</i>	(Ahmad et al., 2019c)
15	5	Research Article	Not Provided	fin tissue	<i>Philippine Journal of Science</i>	(Khan and Naeem, 2024)
Not Mentioned	multiple sites	Short Communication	2017	Not Provided	<i>International Journal of Tropical Insect Science</i>	(Hussain et al., 2020b)
30	Not Mentioned	Research Article	Not Provided	Blood	<i>Acta Entomology and Zoology</i>	(Khanum et al., 2024)
multiple samples	3	Short Communication	Not Provided	whole body	<i>Pakistan Journal of Zoology</i>	(Ahmad et al., 2019a)

85	19	Research Article	2010-2011	Piece of leg	<i>Journal Of Threatened Taxa</i>	(Nazir <i>et al.</i> , 2014)
Not Mentioned	Not Mentioned	Research Article	May-June 2007	nymphs/adult females	<i>Appl. Entomol. Zool.</i>	(Ashfaq <i>et al.</i> , 2010)
multiple samples	multiple sites	Research Article	June 2014-October 2017	Single Leg	<i>International Journal of Bioscience</i>	(Ullah <i>et al.</i> , 2018)
8430	1	Research Article	11th December 2018 to 10th December 2019	one or two legs of adults	<i>Pol. J. Environ. Stud.</i>	(Khan <i>et al.</i> , 2024a)
72	Not Mentioned	Research Article	Mar-14	Piece of muscle	<i>Pakistan Journal of Zoology</i>	(Xiao <i>et al.</i> , 2016)
8721	1	Research Article	June 2017- May 2018	Insect body	<i>Asian J Agric &amp; Biol.</i>	(Ahmed <i>et al.</i> , 2020)
31	2	Research Article	July 2020- Marh 2021	dorsal muscle tissue	<i>Journal Of Materials and Environmental Science</i>	(Khan <i>et al.</i> , 2023)
99	2	Research Article	Selected months from 2009, 2012, 2013, 2014, 2015	Muscle Pieces	<i>Pakistan Journal of Zoology</i>	(Liu <i>et al.</i> , 2017)
1738	1	Research Article	2017-2018	Adult leg	<i>Pakistan Journal of Zoology</i>	(Ahmad <i>et al.</i> , 2019b)
8	3	Research Article	2018	whole body	<i>Acta Entomologica Serbica</i>	(Khan <i>et al.</i> , 2020)
1085	1	Research Article	October-February	Not Provided	<i>Bioscientific Review</i>	(Naz <i>et al.</i> , 2022)
17	8	Mitogenome Announcement	2016	Muscles	<i>Mitochondrial DNA Part B</i>	(Akhtar and Ali, 2016)
365	6	Research Article	March-August 2023	Not Provided	<i>Journal of Advanced Zoology</i>	(Muhammad <i>et al.</i> , 2024)
94	10	Research Article	April 2021-October 2021 and April 2022-October 2022	Leg	<i>Journal of Applied Research in Plant Sciences</i>	(Babar <i>et al.</i> , 2023)
multiple samples	1	Short Communication	Not Provided	Not Provided	<i>Cellular and Molecular Biology</i>	(Amad <i>et al.</i> , 2019)
400	4	Research Article	Not Provided	female body	<i>Annales De La Société Entomologique De France (N.S.)</i> ,	(Badshah <i>et al.</i> , 2017)
86	14	Rapid Communication	2019	fecal, muscle tissue, skin and hair samples	<i>Mitochondrial DNA Part B</i>	(Naseem <i>et al.</i> , 2020)
300	6	Research Article	Not Provided	Legs	<i>Acta Tropica</i>	(Islam <i>et al.</i> , 2018)
25	2	Research Article	2023	Whole body	<i>Frontiers in Veterinary Science</i>	(Ali <i>et al.</i> , 2024a)
10	1	Research Article	Not Provided	Muscle	<i>International Journal of Applied and</i>	(Riaz <i>et al.</i> , 2024)

					<i>Experimental Biology</i>	
multiple samples	1	Research Article	Not Provided	muscle tissues	<i>Current Trends in OMICS (CTO)</i>	(Naz et al., 2023a)
8641	45	Research Article	April 2017-March 2018	Not Provided	<i>Frontiers In Physiology</i>	(Ali et al., 2019)
7	7	Research Article	Summer, Spring 2017	Keel Tissues	<i>Journal Of Bioresource Management</i>	(Rafiqet al., 2020)
multiple samples	1	Research Article	Not Provided	Not Provided	<i>Cellular And Molecular Biology</i>	(Khan et al., 2017)
multiple samples	1	Research Article	2013-2014	Blood (wing/leg vein)	<i>Acta Entomology and Zoology</i>	(Ali et al., 2020)
multiple samples	15	Research Article	September-October	feather follicle	<i>Research Square</i>	(Asif et al., 2023)
22	4	Research Article	Not Provided	muscle tissues	<i>Journal of Animal &amp; Plant Sciences</i>	(Khaliq et al., 2015)
160	6	Research Article	March 2014-November 2015	First Pair of left leg	<i>Mitochondrial DNA Part A</i>	(Naseem and Tahir, 2018)
23	5	Research Article	Not Provided	Patagium and hind legs tissue samples	<i>Brazilian Journal of Biology</i>	(Ahmad et al., 2021)
542	1	Research Article	2022	muscle tissues	<i>Punjab University Journal of Zoology</i>	(Sial et al., 2024)
3	2	MITO Communication	Not Provided	Muscles	<i>Mitochondrial DNA Part B</i>	(Ayesha et al., 2019)
240	10	Research Article	2015-2017	Thorax	<i>Applied Ecology and Environmental Research</i>	(Rizwan et al., 2018)
36	50	Research Article	March 2022-March 2023	Tail tip biopsies, liver and spleen	<i>Journal Of Wildlife and Biodiversity</i>	(Hussain et al., 2025)
60	19	Short Communication	Not Provided	Not Provided	<i>Mitochondrial DNA Part A</i>	(Tahir et al., 2016c)
123	1	Research Article	2018	3rd and 4th legs	<i>Experimental and Applied Acarology</i>	(Zeb et al., 2019)
15	1	Rapid Communication	Not Provided	Not Provided	<i>Mitochondrial DNA Part B</i>	(Naeem et al., 2020)
14	6	Research Article	July 2022 - July 2023	muscle tissues	<i>Journal of Freshwater Ecology</i>	(Anjum et al., 2025)
70	7	Research Article	2023	Blood Samples	<i>Scientific Reports</i>	(Khan et al., 2025)

Supplementary Table 2.

Classification	Congeneric Divergence	Conspecific Divergence	Most Abundant Species	Total Species Identified
Turtles: <i>Lissemys punctata</i>	N/A	N/A	N/A	1
Arachnida: Spiders	2.8-23.2 (mean 8.8%)	0-5.3 (mean 0.8%)	N/A	27 families, 202 species
Arthropods	N/A	N/A	Diptera and Hymenoptera	324 genera, 191 species
Cyprinidae: <i>C. carpio</i> , <i>C. mrigala</i> , and <i>C. idella</i>	mean 8.45%	mean 0.28%	N/A	3
Arachnida, Spiders	17.6-23.7%	0-1.6%	N/A	5
Channa Species: <i>C. punctata</i> , <i>C. marulius</i> and <i>C. striata</i>	0.182-0.195	0.00-0.023	N/A	3
Pakistani collared dove: <i>Streptopelia decaocto</i>	N/A	N/A	N/A	1
Review: family Coccinellidae, ladybird beetles spiders	N/A	N/A	N/A	
	N/A	N/A	Arenidae	12 families, 29 genera and 49 species
Insects	N/A	N/A	Diptera and Hymenoptera	17 Orders, 254 families
<i>Gazella bennettii</i> and <i>Bos taurus</i>	N/A	N/A	<i>Gazella bennettii</i> and <i>Bos taurus</i>	2 Species
<i>Wallagu atto</i>	N/A	0.000-0.038%	Wallagu atto	1
Butterfly	1.7 to 14.3% (mean 8.0%)	0.0 to 1.6% (mean 0.2%)	Nymphalidae	52 Genera, 81 Species
Fishes: Family <i>Mugilidae</i>	N/A	N/A	N/A	7
Pheretimid Earthworm ( <i>Metaphire</i> sp. and <i>Amyntas</i> sp.)	N/A	0.0% to 0.57%	<i>Metaphire (Metaphire posthuma)</i>	86 pheretimid complex
Cyprinidae	N/A	mean 1.84%	N/A	9 genera, 9 species
insect pests of rice crops	N/A	0-2.26%	N/A	11 families, 16 genera, 20 species
Cyprinidae: Genus Labeo: <i>C. catla</i> , <i>L. rohita</i> , <i>L. calbasu</i> , <i>L. gnius</i> , <i>L. bata</i>	N/A	N/A	N/A	5 species
<i>Cactus Moth C. cactorum</i>	N/A	0.012 to 32.18%	N/A	1
Fishes	N/A	N/A	N/A	
stink bug genus Halys (Heteroptera: Pentatomidae)	N/A	0-0.16%	N/A	2, 1 new
Weaving Spider (Araneae: Araneidae)	N/A	N/A	N/A	11, 6 new species
Goat, <i>Capra hircus</i>	N/A	N/A	N/A	2
Review: Services of DNA barcoding in different fields	N/A	N/A	N/A	
Museum-Preserved Fish Specimen	N/A	N/A	N/A	6
Herpetofauna	0-0.04 in frogs	N/A	N/A	11
Spider diversity: 38 morph-species, 22 genera, and 8 families	N/A	N/A	Family Araneidae (37.569%) (n=10)	38

Cyprinidae families: <i>M. cavasius</i> , <i>M. bleekeri</i> , <i>O. cotio</i> , <i>L. rohita</i> , <i>L. culbasu</i> , <i>L. goniis</i> , <i>C. carpio</i> , <i>C. catla</i> , and <i>C. mrigala</i>	N/A	N/A	N/A	9
GASTROPOD <i>Telescopium telescopium</i>	N/A	N/A	N/A	1
flying fox	0.02%	0.00%	N/A	<i>Pteropus giganteus</i>
mango fruit flies: <i>B. dorsalis</i> and <i>B. zonata</i>	0.12%	0.011 and 0.016%	<i>B. dorsalis</i> in Sindh and <i>B. zonata</i> in Punjab	2
genus <i>Duttaphrynus</i> : <i>D. Stomaticus</i> , <i>D. melanostictus</i>	16%	0-1% for one species and 10-11% for the other species	N/A	2
<i>Cirrhinus reba</i>	N/A	N/A	N/A	1
Illegal trade confiscated samples	N/A	N/A	<i>Vulpes vulpes</i> ,	4
Hymenopteran insects	N/A	N/A	Bethylidae sp.	13
Bats, Vespertilionidae, Emballonuridae	N/A	N/A	N/A	Not Mentioned
<i>Channa marulius</i>	0.0000-0.0067%	N/A	N/A	1
sugarcane black bug: <i>Cavelarius excavates</i>	N/A	N/A	N/A	1
ring-necked Korean Pheasant, <i>Phasianus colchicus</i>	N/A	N/A	N/A	
Hemiptera: Dusky Cotton Bug, <i>Oxycarenus hyalinipennis</i>	N/A	N/A	N/A	1
Acridid Grasshoppers	N/A	N/A	genus <i>Gastrimargus</i>	15 Genera, 26 species morphologically , 21 DNA Barcoding
Hemiptera: Pseudococcidae: mealybug <i>P. solenopsis</i>	N/A	N/A	N/A	1
Hemiptera: Heteroptera: <i>Pentatom morpha</i>	mean 11.7%, 4.1-17.1%	mean 0.7%, 0.0-1.1%	N/A	13 species, 12 genera
Hymenoptera	N/A	N/A	<i>Camponotus compressus</i>	50 species morphologically , 8 species molecularly
Perciformes: Sillaginidae, <i>Sillago indica</i>	17.1-22.2%	mean 0.2%	N/A	
Arthropods diversity	N/A	N/A	<i>Tricimba humeralis</i>	82
<i>Pseudorhombus arsius</i> , <i>Psettods erumei</i> and <i>Cynoglossus cynoglossus</i>	N/A	N/A	<i>Psettods erumei</i>	3
Keel Backed <i>Liza</i> Species, <i>Liza affinis</i> , <i>Liza carinata</i> , and <i>Liza klunzingeri</i>	7.93%	0.26%, 0.52%, and 0.47%	N/A	3
Diptera: Tephritidae: Genus <i>Bactrocera</i> , <i>B. zonata</i> , <i>B. Dorsalis</i> , <i>B. cucurbitae</i>	N/A	N/A	<i>Bactrocera zonata</i>	3
HEMIPTERA: PYRRHOCORIDAE: <i>Dysdercus koenigii</i> , <i>Dysdercus cingulatus</i>	N/A	N/A	N/A	2
Fish Diversity	0.63% mean	0.57% mean	<i>Hypophthalmichthys molitrix</i>	22
<i>S. plagiostomus</i> , <i>S. esocinus</i> , <i>S. progastus</i> , <i>S. niger</i>	N/A	N/A	<i>S. plagiostomus</i>	4
Mosquitoes: <i>Aedes aegypti</i> and <i>Culex quinquefasciatus</i>	N/A	N/A	<i>Aedes aegypti</i>	2

Acrididae (Orthoptera: Caelifera)	N/A	N/A	<i>Oxya hyla hyla</i>	7
Peach Fruit fly, <i>Bactrocera zonata</i>	N/A	N/A	N/A	1
Hemiptera: Pseudococcidae: <i>Phenacoccus solenopsis</i>	N/A	N/A	N/A	1
ungulate species: <i>Moschus cupreus</i> , <i>Muntiacus muntjac</i> , <i>Axis procinus</i> , <i>Axis axis</i> , <i>nilope cervicapra</i> , <i>Gazella subgutturosa</i> , <i>Gazella bennettii</i> , <i>Boselaphus tragocamelus</i> , <i>Naemorhedus goral bedfordi</i> , <i>Capra aegagrus blythi</i> , <i>Capra sibirica</i> , <i>Capra falconeri falconeri</i> , <i>Capra falconeri megaceros</i> , <i>Pseudois nayaur nayaur</i> , <i>Ovis ammon polii</i> , <i>Ovis vignei punjabiensis</i> , <i>Ovis vignei blanfordi</i> , <i>Ovis vignei vignei</i> , <i>Sus scrofa cristatus</i>	0.0025-0.229%	0-0.0246%	N/A	19
families Libellulidae and Gomphidae	37.02%	0.82-24.96%	Libellulids family	21 Species and 15 genera
<i>Alectorobius coniceps</i> and <i>Dermacentor</i> species	N/A	N/A	<i>Alectorobius coniceps</i>	2
freshwater catfish: <i>Wallago attu</i>	N/A	N/A	N/A	1
Head Taunsa Barrage Fish Species	0.99%	0.97%	N/A	19
<i>Rhipicephalus microplus</i>	N/A	N/A	<i>R. microplus</i>	17
Aves: Columbidae, Pakistan: <i>C. livia livia</i> , <i>C. eversmanni</i> , <i>S. senegalensis</i> , <i>S. decaocta</i> , <i>S. tranquebarica</i> , <i>S. chinensis</i> , <i>T. phoenicoptera</i>	N/A	N/A	N/A	7
dusky cotton bug, <i>Oxycarenus hyalinipennis</i>	N/A	N/A	N/A	1
pheasants of northern Pakistan	N/A	0.044% to 67.10%	N/A	7
<i>Sturnus roseus</i>	N/A	N/A	N/A	1
Mahseer ( <i>Tor putitora</i> )	N/A	0-0.5%	N/A	1
Family Salticidae and Lycosidae of spiders	N/A	0.62-1.71% for Lycosidae and 0-2.99% for Salticidae	N/A	15
insectivorous bat species, <i>Pipistrellus coromandra</i>	N/A	mean 0.073%	N/A	1
Northern Arabian Sea: Rare Fish Diversity	0.31-4.23%	N/A	N/A	15 species, 13 families, 7 orders
Cyprinids: <i>Labeo rohita</i> , <i>Catla catla</i> and <i>Cirrhinus mrigala</i>	1.23%	0.03%	N/A	3
<i>Apis mellifera</i>	0.26-2.36%	N/A	N/A	1 species, 7 subspecies
Snake Fauna from Cholistan Desert Pakistan	N/A	N/A	<i>Eryx johnii</i> , <i>Platyceps rhodorachis</i>	11
Anopheles mosquitoes	8-22.3%	0-2.5%	<i>An. stephensi</i>	7
<i>Rhipicephalus microplus</i> and <i>Hyalomma anatolicum</i> collected from cattle	N/A	6	N/A	2
<i>Catla catla</i>	0.43	0.38	N/A	1
three orders of fish, 8 species	0.23	N/A	N/A	8
common house crows ( <i>Corvus splendens</i> )	N/A	0.002-0.013	N/A	1