International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2025; 9(1): 647-650
www.biochemjournal.com
Received: 25-11-2024
Accepted: 28-12-2024

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A report of Leith's Softshell Turtle (*Nilssonia leithii*) from Pench tiger reserve Maharashtra

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DOI: https://doi.org/10.33545/26174693.2025.v9.i1h.3581

Abstract
Leith's Softshell Turtle (*Nilssonia leithii*), a medium to large-sized freshwater species native to India, is classified as Vulnerable by the IUCN due to habitat destruction, pollution, and poaching. This species plays a key ecological role in riverine ecosystems by regulating aquatic prey populations and contributing to sediment turnover and nutrient cycling. During a crocodile survey in Pench Tiger Reserve, Maharashtra, a putrefied turtle carcass was recovered and identified as *Nilssonia leithii* using molecular techniques. DNA extraction and PCR amplification produced a 440 bp amplicon, which was sequenced and matched to *Nilssonia leithii* through nBLAST analysis. The presence of this species in Pench Tiger Reserve (PTR) highlights the reserve's role in conserving not only terrestrial wildlife but also freshwater biodiversity. *Nilssonia leithii* faces significant threats from pollution, dam construction, and habitat degradation, which have led to its decline across its historical range, particularly in large river systems like the Krishna and Godavari. The discovery of this species in the relatively undisturbed aquatic ecosystems of PTR underscores the importance of protected areas in preserving vulnerable species. Conservation strategies must prioritize not only large mammals but also freshwater species, ensuring the survival of *Nilssonia leithii* and maintaining ecosystem health for future generations.

Keywords: Leith's Softshell Turtle, *Nilssonia leithii*, Pench Tiger reserve, Maharashtra

Introduction

Leith's Softshell Turtle (*Nilssonia leithii*) is a medium to large-sized freshwater turtle native to India. This species is part of the Trionychidae family, which is known for its leathery, soft carapace, a key distinguishing feature. It is primarily found in large river systems, slow-moving streams, lakes, and ponds with sandy banks in the southern peninsular India. Kerala [1, 2], Andhra Pradesh [3], Karnataka [4], Maharashtra [4], Orissa and Tamil Nadu [5] have reported the species. The distribution is majorly limited to south-east Asia [6]. Despite its relatively wide historical distribution, Leith's Softshell Turtle has become rare due to several anthropogenic factors and is classified as *Vulnerable* by the International Union for Conservation of Nature (IUCN) [7]. Pench Tiger Reserve lies in the southern reaches of the Satpura Hills, straddling the Maharashtra-Madhya Pradesh border. The Pench River flows through the core area of the reserve, supporting a wide range of aquatic biodiversity, and it provides an important habitat for aquatic species like turtles, crocodiles, and fish. While primarily a tiger reserve, Pench's aquatic ecosystems, such as its streams, ponds, and rivers, are vital for the survival of many species, including *Nilssonia leithii*. The turtle has an oval, flattened body with a leathery, smooth carapace. Its colour ranges from olive green to brown, which provided excellent camouflage against the muddy riverbank. The average size of carapace length is about 40-50 cm indicating a fully mature adult. The elongated snout and webbed feet were characteristic of this species, aiding in identification. Leith's Softshell Turtles play a crucial role in the ecosystem by maintaining the balance of aquatic life. They are omnivores, feeding on a variety of aquatic organisms, including small fish, invertebrates, and plant matter, which helps regulate population dynamics in the riverine ecosystem.

The primary threat to *Nilssonia leithii* is habitat destruction. Water bodies in and around the reserve are subject to agricultural runoff, deforestation, and unregulated fishing, which reduce the quality of the habitat [7]. Moreover, the construction of dams and water diversion projects can significantly alter the river systems that the turtle depends on.

Though hunting is illegal in Pench Tiger Reserve, poaching for turtle meat, skin, and for the pet trade remains a threat in some parts of India. This species, in particular, is sometimes targeted due to its larger size and economic value. Chemicals from nearby agricultural practices, especially pesticides, can infiltrate the rivers, affecting not only the turtles but also their food sources. Increasing tourism and human activity in protected areas can disturb the natural behaviour of wildlife, especially shy species like *Nilssonia leithii* [8].

Materials and Methods

During the crocodile survey undertaken at Pench Tiger Reserve, Maharashtra; a putrefied carcass of a turtle was recovered near a water body. The identification of the carcass was not possible based on the features of the carapace alone. The carcass was submitted to Wildlife Research & Training Centre, Gorewada, Nagpur for the identification. The carcass was cleaned with tap water to get rid of the dirt and soil. The measurements of the carcass were taken and the carapace was examined closely (Fig 1 and 2.). The tissue was incised carefully and subjected to continuous washing under tap water to remove mud and soil. The DNA was isolated using the manufacturers' advice using Blood and Tissue DNA kit (Mfg. Qiagen). The species identification was undertaken by the primers under reference [9]. PCR conditions were: initial denaturation at 95 °C for 10 min, followed by 35 cycles each of denaturation at 95 °C for 45 seconds, PCR conditions were: an initial denaturation at 95° for 10 min, followed by 35 cycles each of denaturation at 95 °C for 45 seconds, annealing at 51 °C for 1 min, and extension at 72 °C for 2 min. The extension step at the 35th cycle was held for 10 min. The PCR Products were tested on agarose gel electrophoresis on 1 % agarose gel. The Tris Acetate EDTA (TAE buffer) was utilised in the electrophoresis.

Results

An amplicon of nearly 440 bp was obtained after PCR (Fig. 3) and the amplicon was purified using QIAquick PCR Purification Kit (Mfg. Qiagen) and submitted for the sequencing to an outsourced firm (Eurofins Pvt Ltd., Bangalore, India). The sequencing was carried out using forward and reverse to ensure the consistency of the sequence. A consensus sequence was constructed after correcting the errors and subjected to nBLAST analysis. The sequence was found to be similar to *Nilssonia leithii* (Fig.4).

Discussion

Nilssonia leithii plays an important role in maintaining the health of freshwater ecosystems. As an omnivorous species, it feeds on a variety of aquatic organisms, including invertebrates, small fish, and plant matter. This makes it a keystone species in regulating prey populations, which helps maintain a balanced food web in river systems. By foraging on the bottom of rivers and lakes, *Nilssonia leithii* helps in sediment turnover and nutrient cycling, promoting healthier aquatic habitats. Its consumption of aquatic plants may aid in the dispersal of seeds, contributing to plant biodiversity in its habitat. The turtle serves as both predator and prey. It controls populations of smaller aquatic species, while also being a food source for larger predators, including crocodiles and birds of prey. The health and presence of species like *Nilssonia leithii* are often indicators of clean and stable freshwater systems, as they are highly sensitive to pollution and habitat degradation.

Several studies have noted a sharp decline in the number of *Nilssonia leithii* sightings across its historical range. Over the past few decades, the turtle has become difficult to spot in many areas where it once thrived. Large-scale dam projects on rivers like the Krishna and Godavari have altered natural river flow, resulting in habitat loss for freshwater species [10]. The construction of the Nagarjuna Sagar Dam on the Krishna River, for instance, led to a significant reduction in the available habitat for *Nilssonia leithii*. Increased pollution from industrial waste, agricultural runoff, and urban effluents has made many rivers unsuitable for the turtle's survival. Pollution can impact both the turtle directly and the prey species it depends on. In Maharashtra, *Nilssonia leithii* was historically found in the larger river systems such as the Godavari, Bhima, and Tapi rivers. However, over the years, the species has become increasingly rare in these regions [5].

The rivers and reservoirs within Pench Tiger Reserve provide an ideal environment for the species due to the relatively lower levels of human-induced pollution and habitat disturbance compared to other parts of Maharashtra.

While Pench is primarily known for its tiger population, it is home to a rich variety of wildlife, including reptiles like *Nilssonia leithii*. The discovery of this species in Pench emphasizes the reserve's critical role not just in terrestrial wildlife conservation but also in preserving freshwater biodiversity. The presence of *Nilssonia leithii* in Pench enhances the reserve's ecological diversity. Freshwater ecosystems, such as the Pench River and its tributaries, are vital for supporting this biodiversity, and the turtle's presence signals a relatively undisturbed aquatic ecosystem, at least in certain parts of the reserve.

Fig 1: Carapace of the dead turtle recovered from Pench Tiger Reserve

Fig 2: Carapace of the dead turtle recovered from Pench Tiger Reserve with scale

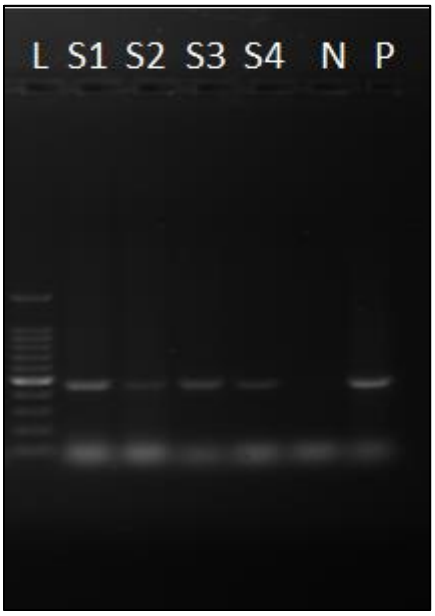

Fig 3: Agarose Gel Electrophoresis (1% gel) indicating a product of 440 bp. Lanes S1 to S4 samples from the turtle. N: Negative Control. P: Positive Control (Known turtle sample)

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Score            Expect   Identities        Gaps        Strand
750 bits(406)    0.0      430/442(97%)      0/442(0%)   Plus/Plus

Query  1    ACAAATATCATTCTGAGGGGCTACAGTAATCACAAATCTACTATCAGCCATCCCATACAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  309  ACAAATATCATTCTGAGGGGCTACAGTAATCACAAATCTACTATCAGCCGTCCCATACAT  368

Query  61   TGGCACCACCATAGTACAATGAATCTGAGGAGGATTCTCCGTAGATAATGCCACCCTAAC  120
            |||||||||||||||||||||||||||||| || ||||||||||||||||||||||||||
Sbjct  369  TGGCACCACCATAGTACAATGAATCTGAGGGGGCTTCTCCGTAGATAATGCCACCCTAAC  428

Query  121  ACGATTCTTTACTCTACACTTCCTACTACCCTTTATAATCCTAGGTCTCACCATAATTCA  180
            ||||||||||||  ||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  429  ACGATTCTTTACCTTACACTTCCTACTACCCTTTATAATTCTAGGTCTCACCATAATTCA  488

Query  181  CCTAATATTTTTACATGAAACAGGATCAAACAACCCAACAGGATTAAACTCAAACACCGA  240
            |||||||||||||||||||||||||||||| |||||||||||||||||||||||||||||
Sbjct  489  CCTAATATTTTTACATGAAACAGGATCAAATAACCCAACAGGATTAAACTCAAACACCGA  548

Query  241  TAAAATCCCATTCCACCCTTACTTCTCATATAAAGACCTAATAGGTCTCATAACAACACT  300
            |||||||||||||||||||||||||||||| |||||||||||||||||||||||||||||
Sbjct  549  TAAAATCCCATTCCACCCTTACTTCTCATACAAAGACCTAATAGGTCTCATAACAACACT  608

Query  301  AACCCTCCTCTTATTTATCACCATATTCTACCCTAACCTATTAGGAGACCCAGACAACTT  360
            |||||| |||||||||||||||||||||||||| ||||||||||||||||||||||||||
Sbjct  609  AACCCTTCTCTTATTTATCACCATATTCTACCCCAACCTATTAGGAGACCCAGACAACTT  668

Query  361  CACACCAGCTAACCCACTGTCCACACCACCCCATATTAAACCAGAATGATACTTCCTATT  420
            ||||||||| |||||||| |||||||||||||||||||||||||||||||||||||||||
Sbjct  669  CACACCAGCCAACCCACTATCCACACCACCCCATATTAAACCAGAATGATACTTCCTATT  728

Query  421  TGCTTACGCAATCCTACGATCA  442
            ||||||||||||||||||||||
Sbjct  729  TGCTTACGCAATCCTACGATCA  750
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Fig 4: Sequence alignment after nBLAST search

Conclusion

The sighting of *Nilssonia leithii* in Pench Tiger Reserve, Maharashtra is a reminder of the species' historical significance and ecological importance in India. Given the sharp decline in its population due to habitat destruction and pollution, the discovery highlights the role protected areas play in preserving vulnerable species. For Maharashtra, this is a call to enhance conservation efforts, not just for large mammals but for the entire spectrum of biodiversity, including freshwater ecosystems. This rare sighting provides a valuable opportunity to focus on conservation strategies that can help safeguard the remaining populations of *Nilssonia leithii* and ensure its survival for future generations.

Acknowledgements

The authors acknowledge the kind approval of the Principal Chief Conservator of Forest (Wildlife) & Chief Wildlife Warden, Maharashtra State, for carrying out the research work. Forest Development Corporation of Maharashtra Ltd., Nagpur and Office of the Divisional Manager, Gorewada Project, Nagpur, for the facilities provided for the research.

Field Director, Pench Tiger Reserve, Maharashtra for the permission and access to the samples. Director, Wildlife Research & Training Centre to facilitate the research work at WRTC, Gorewada, Nagpur.

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