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## Contents

### Articles

- 21      **Clustering and Differentiation of glr-3 Gene Function and Its Homologous Proteins**  
Yue Ma   Tiantian Guo   Yihe Wang   Xinna Li   Jingyu Zhang
- 28      **Histological Study of the Male Internal Genital Tract of Dwarf Caiman (*Paleosuchus palpebrosus* Cuvier, 1807)**  
Teresinha Inês Assumpção   Adrielly Julien Silva Lopes   Lilja Fromme   André Luís   Quagliatto Santos
- 33      **The Growth Parameters and Mortalities of Five Species of *Synodonits* in the Lower River Benue at Makurdi**  
Akombo, Pauline Mbakaan   Atile, John Iornyiman   Shima, Judith Nguvan
- 44      **Habitat Suitability Index Modelling for Bluebull (*Boselaphus tragocamelus*) in Pench Tiger Reserve, M.P. India**  
Abdul Haleem   Orus Ilyas

### Review

- 1      **A Review of the Engineering Role of Burrowing Animals: Implication of Chinese Pangolin as an Ecosystem Engineer**  
Song Sun   Hongliang Dou   Shichao Wei   Yani Fang   Zexu Long   Jiao Wang   Fuyu An   Jinqian Xu  
Tingting Xue   Huangjie Qiu   Yan Hua   Guangshun Jiang

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## REVIEW

# A Review of the Engineering Role of Burrowing Animals: Implication of Chinese Pangolin as an Ecosystem Engineer

Song Sun<sup>1,2</sup> Hongliang Dou<sup>2#</sup> Shichao Wei<sup>2</sup> Yani Fang<sup>3</sup> Zexu Long<sup>1</sup> Jiao Wang<sup>2</sup> Fuyu An<sup>2</sup> Jinqian Xu<sup>2</sup> Tingting Xue<sup>1,2</sup> Huangjie Qiu<sup>2</sup> Yan Hua<sup>2\*</sup> Guangshun Jiang<sup>1\*</sup>

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## ABSTRACT

Ecosystem engineers are organisms that alter the distribution of resources in the environment by creating, modifying, maintaining and/or destroying the habitat. They can affect the structure and function of the whole ecosystem furthermore. Burrowing engineers are an important group in ecosystem engineers as they play a critical role in soil translocation and habitat creation in various types of environment. However, few researchers have systematically summarized and analyzed the studies of burrowing engineers. We reviewing the existing ecological studies of burrowing engineer about their interaction with habitat through five directions: (1) soil turnover; (2) changing soil physicochemical properties; (3) changing plant community structure; (4) providing limited resources for commensal animals; and/or (5) affecting animal communities. The Chinese pangolin (*Manis pentadactyla*) is a typical example of burrowing mammals, in part (5), we focus on the interspecific relationships among burrow commensal species of Chinese pangolin. The engineering effects vary with environmental gradient, literature indicates that burrowing engineer play a stronger role in habitat transformation in the tropical and subtropical areas. The most common experiment method is comparative measurements (include different spatial and temporal scale), manipulative experiment is relatively few. We found that most of the engineering effects had positive feedback to the local ecosystem, increased plant abundance and resilience, increased biodiversity and consequently improved ecosystem functioning. With the global background of dramatic climate change and biodiversity loss in recent decades, we recommend future studies should improving knowledge of long-term engineering effects on population scale and landscape scale, exploring ecological cascades through trophic and engineering pathways, to better understand the attribute of the burrowing behavior of engineers to restore ecosystems and habitat creation. The review is presented as an aid to systematically expound the engineering effect of burrowing animals in the ecosystem, and provided new ideas and advice for planning and implementing conservation management.



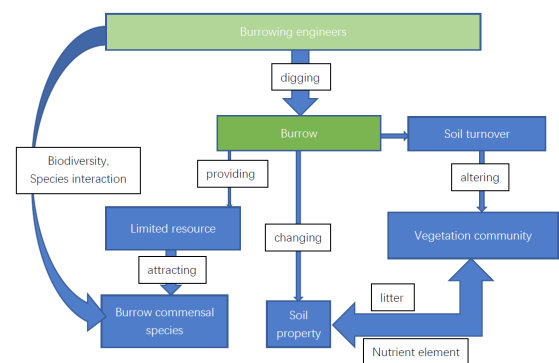
## 1. Introduction

Ecosystem engineers can influence resource availability for other species by modifying, maintaining or creating habitats <sup>[1]</sup>. It has been more than 20 years since the concept of ecosystem engineers was defined <sup>[2]</sup>. During this time, researchers have found strong evidence that ecosystem engineers play a pivotal role in the habitat. Contemporary studies about ecosystem engineers focus on their impacts on soil physicochemical property and vegetation community structure and, increasingly, on their potential as agents of ecosystem restoration.

Research on ecosystem engineering covers many species and themes. Coggan (2017) collected 214 articles covering the interactions of 121 engineering species across four taxa (mammals, reptiles, birds, invertebrates). The most observed types of ecosystem engineers in mammals are the burrowing ones, and they can have large impacts on the other species, environments, and ecological processes <sup>[3]</sup>. Burrowing activity is an important form of natural disturbance in many types of ecosystems, existing evidence suggests that the burrows of these engineers has profound effects on shaping the abiotic environment, leading to the integrity of the biotic community <sup>[4,5]</sup>. By creating discrete patches of disturbance, burrowing engineers can increase abiotic heterogeneity at the landscape level, generating novel microhabitats, as described in previous studies on the burrow function of aardvarks (*Orycteropus afer*), warthogs (*Phacochoerus aethiopicus*) and Cape porcupines (*Hystrix africaeaustralis*) <sup>[6,7,8]</sup>. However, the ecology research of other burrowing animal still lacking these insights, meanwhile, there are not enough review papers to summarize the studies of burrowing engineers and provide suggestions for current hot issues and future research directions.

To explore the roles played by burrowing engineers in their habitat, we searched the key terms such as “Chinese pangolin”, “burrowing engineers”, “soil disturbance”, “biodiversity”, “biogeomorphology”,

and “soil turnover”, and filtered these papers with the limit of “burrowing animal” to identify papers about burrowing engineers. By using the database compilation method, 117 papers were documented and 65 papers were selected as represents of engineer species (Table 1). We found that since the concept of ecosystem engineer was put forward, the number of studies related to burrowing engineers has been on the rise, indicating that the ecological function of burrowing engineers has been paid more and more attention by the researchers (Figure 2). We classified the known ecological functions of burrowing engineers in the literature into five directions: (1) soil turnover; (2) changing soil physicochemical properties; (3) changing plant community structure; (4) providing limited resource about open microsites, shelters, thermal refuges and food for commensal animals; and (5) affecting animal communities (Figure 1).



**Figure 1.** Research framework of burrowing engineers.

Burrowing engineers digging burrows for foraging or dwelling, removing big amounts of sediment from deep layer to surface in this process, generated soil turnover (1), changed soil physicochemical property, too (2). On the one hand, soil turnover firstly altering the vegetation community structure, due to the change of physicochemical properties of burrow soil, the vegetation community is continuously affected (3); On the another

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hand, burrow providing limited resource such as shelter, thermal refugia, food resource, mating site ... (4). Other species are attracted by abundant resource from burrowing and forming a burrow commensal population,

and their interaction affected ecosystem biodiversity (5). In addition, the soil physicochemical property is also affected by plant litter decomposition (2).

Research contents: Summarize the content of the

**Table 1.** The studies of burrowing engineers

Species	Research contents	Enviroment	Positive/ negative	Reference
( <i>Myrmica rubra</i> ) / ( <i>asius niger</i> )	B, E	grassland	Positive/ negative	[133]
Aardvark ( <i>Orycteropus afer</i> )	B, C	grassland, savannah, arid scrubland	negative	[21]
African ice rat ( <i>Otomys sloggetti robertsi</i> )	A, D	alpine meadow	Positive	[134]
Arctic foxes ( <i>Vulpes lagopus</i> )	B	grassland, woodland	Positive	[135]
Badgers ( <i>Meles meles</i> )	B, C	shrub-steppe/annual grasslands	Positive	[86]
Badgers ( <i>Meles meles</i> ) and foxes ( <i>Vulpes vulpes</i> )	B, C	European temperate forest	Positive	[64]
Banner-tailed kangaroo rat ( <i>Dipodomys spectabilis</i> )	B	creosote bush shrubland	neutral	[136]
Bare-nosed wombat ( <i>Vombatus ursinus</i> )/cattle ( <i>Bos taurus</i> )	A	floodplains and terraces	Positive	[13]
Bear ( <i>Ursidae</i> )	A, B	Alpine	neutral	[137]
Beaver ( <i>Castor canadensis</i> )	E	Wetlands	Positive	[138]
Black-tailed prairie dog ( <i>Cynomys ludovic-ianus</i> )	D, E	grassland(arid)	Positive	[139]
Burrow-dwelling tortoises ( <i>Gopherus polyphemus</i> )	D	coastal dune ecosystem	Positive	[93]
Burrowing bettong ( <i>Bettongia lesueur</i> )	B, C	woodland	Positive/ negative	[50]
Burrowing crab ( <i>Helice tientsinensis</i> )	A, B	intertidal salt marsh	Positive	[24]
burrowing seabirds	B, C, E	secondary forest	Positive	[140]
Camel spider ( <i>Arachnida, Solifugae</i> )/Black-tailed prairie dogs ( <i>Cynomys ludovicianus</i> )	C, E	grassland(arid)	Positive	[141]
Cape ground squirrels ( <i>Xerus inauris</i> )	C, E	Namib Desert grasslands	Positive/ negative	[28]
Cape porcupines ( <i>Hystrix africaeaustralis</i> )	A, D	semi-arid environment	Positive	[4]
Common vole ( <i>Microtus arvalis</i> )/European mole ( <i>Talpa europaea</i> )/ earthworm ( <i>Lumbricus terrestris</i> )	B	floodplain	Positive	[142]
Earthworm ( <i>Lumbricus terrestris</i> )/salamander ( <i>P. glutinosus</i> )	D, E	forest is mixed deciduous	Positive	[143]
Eastern barred bandicoots ( <i>Perameles gunnii</i> )	A	woodland and grassland	neutral	[18]
Eastern bettong ( <i>Bettongia gaimardi</i> )	A, B	grassy woodland	Positive	[144]
Eastern bettong ( <i>Bettongia gaimardi</i> )/European rabbit ( <i>Oryctolagus cuniculus</i> )	B, C, D	grassland/woodland/forest	neutral	[145]
Eastern bettongs ( <i>Bettongia gaimardi</i> )/short-beaked echidnas ( <i>Tachyglossus aculeatus</i> )	A, B	forest	Positive	[25]
Eurasian badger ( <i>Meles meles</i> L.)	A	woodland	neutral	[14]
European bee-eater ( <i>Merops apiaster</i> )	A, E	arid desert environments	Positive/ negative	[16]
European ground squirrel ( <i>Spermophilus citellus</i> ) /spider ( <i>Araneae</i> )/ harvestman ( <i>Opiliones</i> )	E	Grassland	Positive	[146]
European rabbit ( <i>Oryctolagus cuniculus</i> )	C, E	Grasslands	Positive	[33]
Fat sand rats ( <i>Psammomys obesus</i> )	A.C	desert/shrub(arid)	negative	[127]
Fossorial rodent ( <i>Parotomys brantsii</i> )	B, C	Desert	Positive	[55]
Giant armadillo ( <i>Priodontes maximus</i> )	C, D, E	dry forest plains	Positive	[108]
Giant kangaroo rat ( <i>Dipodomysingens</i> )	C	semiarid annual rangeland	negative	[129]
Gopher Tortoise ( <i>Gopherus polyphemus</i> )	A	pine forest, scrub	neutral	[27]

Species	Research contents	Enviroment	Positive/ negative	Reference
Harvester ants ( <i>Messor barbarus</i> )	B, C	Mediterranean grassland	Positive	[35]
House mouse ( <i>Mus musculus</i> )	A	limestone island	negative	[12]
Indian crested porcupine ( <i>Hystrix indica</i> )/harvester ants ( <i>Messor spp.</i> )	C, E	desert	Positive	[147]
Kangaroo rats ( <i>Dipodomys spectabilis</i> )	B, C	arid grassland	Positive	[148]
Lamprey larvae ( <i>Eudontomyzon sp.</i> )	B, E	river substrates	Positive	[149]
Liberian mongoose ( <i>Liberiictis kuhni</i> )	C	lowland rain forest	Positive	[150]
Malleefowl ( <i>Leipoa ocellata</i> )/bumowing bettong ( <i>Bettongia lesueur</i> )	B, C	semi-arid woodland	Positive	[151]
Marsh crabs ( <i>Sesarma reticulatum</i> )	A	salt marshes	negative	[126]
Mole-rats ( <i>Bathyergidae</i> )	B, C	grassland	Positive	[152]
Mycophagous woylie ( <i>Bettongia penicillata ogilbyi</i> )/omnivorous quenda ( <i>Isodon fusciventer</i> )	C, E	mesic forest	Positive	[31]
Nine-banded armadillos ( <i>Dasypus novemcinctus</i> )	A	forest	neutral	[11]
Pikas ( <i>Ochotona curzoniae</i> )	B	alpine grasslands	neutral	[26]
Plains vizcachas ( <i>Lagostomus maximus</i> )	B	shrub	Positive	[153]
Plateau zokors ( <i>Myospalax fontanierii</i> )	B, C, E	alpine meadow ecosystems	Positive	[154]
Pocket gopher ( <i>Thomomys talpoides</i> )	B, C	grassland	neutral	[155]
Prairie dogs ( <i>Cynomys gunnisoni</i> )/ kangaroo rats ( <i>Dipodomys spectabilis</i> )	B, C, E	Desert grassland	Positive	[156]
Prairie dogs ( <i>Cynomys ludovicianus</i> )	B	Semiarid Grasslands	Positive	[157]
Pygmy rabbit ( <i>Brachylagus idahoensis</i> )	B, C	high-elevation sagebrush steppe	Positive	[158]
Rabbit ( <i>Oryctolagus cuniculus L.</i> )	B, C	Australian semiarid woodland	negative	[125]
Red fox ( <i>Vulpes vulpes Linnaeus</i> )	B, C	grasslands, wetlands, alkaline marshes.	Positive	[62]
Relic bilby ( <i>Macrotis lagotis</i> )	B, C, D	woodland	Positive	[48]
Richardson's ground squirrel ( <i>Urocitellus richardsonii</i> )	B, C	open grassland	Positive	[159]
Short-beaked echidna ( <i>Tachyglossus aculeatus</i> )	A, C	semi-arid woodlands	Positive	[160]
Sooty shearwaters ( <i>Puffinus griseus</i> )	A, B	woods	neutral	[161]
Southern brown bandicoot ( <i>Isodon obesulus</i> )	B, C	forest	Positive	[38]
Tatra marmots ( <i>Marmota marmota latirostris</i> )	B, C	alpine meadow	Positive	[32]
Termites/ants/earthworms	B, C, E		Positive	[162]
Trapdoor ( <i>Mygalomorphae</i> ) spiders/Pygmy bluetongue lizards ( <i>Tiliqua adelaidensis</i> )	E	semi-arid native grassland	Positive	[163]
Tuco-tuco ( <i>Creilomys fnlnmnz</i> )	B, C	natural coastal grassland	Positive	[34]
Water voles ( <i>Arvicola amphibious</i> )	C	wet grassland	neutral	[164]
Wedge-tailed Shearwaters ( <i>Puffinus pacificus</i> )	C	limestone island	negative	[165]
Woylie ( <i>Bettongia penicillata</i> )	A	woodland	Positive	[40]

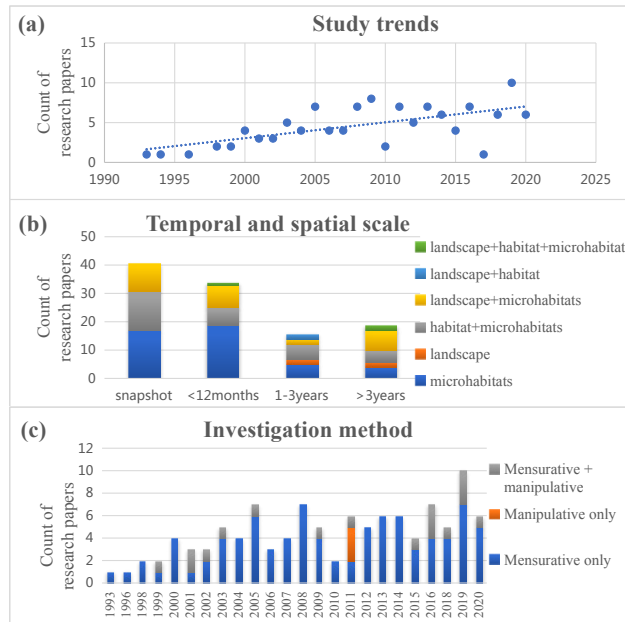
selected articles and divide them into five directions as is described above.

Due to the continuously loss of global biodiversity, burrowing engineers will play an increasingly important role in biodiversity conservation, because of their unique attribute to increase the heterogeneity of habitats and provide limited resource for other biological communities. The function of engineers will promote interspecific

connections and increase environmental capacity. For the conservation of burrowing engineers, we should recognize that the rejuvenation of their population may also benefit other species to achieve double the result with half the effort. Conversely, the extinction of engineers is likely to cause a chain of ecological losses. In this article, we provide a better understanding of the interaction among burrowing engineers with local habitats and also discussed



the composition and interspecific relationship of Chinese pangolin commensal species.



**Figure 2.** Trends in the methods used to study burrowing engineering. (a) The counts of research papers published on burrowing engineers, (b) Temporal and spatial scale of experiments performed and (c) the investigation method of field observations

## 2. Engineering Role of Burrowing Animals in Ecosystems

The unifying idea of the studies we identified is that burrowing activity is an important form of natural disturbance in many ecosystems as it increases habitat heterogeneity and improves the living conditions of some species<sup>[4,5]</sup>. We briefly discuss the ecological function of burrowing engineers from five directions, introduce the major findings and flaws in these research fields and propose some suggestions for further research.

We found that the dominating experiment method of burrowing engineers research is comparative measurement, rather than manipulative experiment (Figure 1). These studies explored the ecological function of burrowing engineers by comparing soil properties, plant community structure between disturbed and undisturbed areas. Pringle once said that manipulative studies have greater utility in identifying the driving mechanisms behind the impacts of engineers than measurement studies because they require the elimination of competing hypotheses by controlling sources of variation<sup>[9]</sup>. The probable reason may be the manipulative experiment is difficult to implement and the environmental interference is difficult to eliminate.

On the contrary, the difficulty in experimental design and operation of comparative measurement is lower, and the statistical results of data are more accurate.

### 2.1 Soil Turnover by Engineers

Burrowing activity causes the massive amounts of soil been turnover, directly modifies the habitat geography.. The burrowing activity usually serves two purposes: dwelling and foraging, or both (as for the pangolin). The degree of soil turnover of burrowing animals mainly depends on their body size and morphological traits. In some regions, burrowing animals are thought to be the dominant geomorphic agents, displacing more sediment through their burrowing than all other abiotic processes combined<sup>[10]</sup>. Many researchers have measured the volume/quality of removed soil to quantify this activity, which has major effects on ecosystems. The morphological volume of burrow calculated by two methods: One is measure burrow tunnel size (think of them approximately as regular three-dimensional structures, such as cylinders and cones<sup>[11,12]</sup>, the another one is measure soil mound size<sup>[13,14]</sup>. The mass is converted from volume  $\times$  density. Then, by multiplying the average soil transport volume of a single burrow by the excavation frequency or the density of the burrow, the soil transport volume of the target population can be roughly estimated and the ability of the engineer to physically modify the surface topography can be quantified<sup>[15,16,17,18]</sup>. For instance, the European bee-eater (*Merops apiaster*) was conservatively estimated to move 8.71 L of sand during the construction of nests, which equals to 12.94 kg of sand. Sixty-seven nests were dug during the 3 study years, amounting to approximately 583 L or 867 kg. In addition, because all the nests were on a cliff, four large pieces collapsed from the cliff during the study period, amounting to 3064 L or 4554 kg<sup>[16]</sup>. In the semiarid regions of Western Australia, rush-tailed bettongs (*Bettongia penicillata*) and southern brown bandicoots (*Isodon obesulus*) can turnover 4.8 and 3.9 tonnes of soil annually, respectively<sup>[15,17]</sup>.

At present, the burrow volume measurement methods used in relevant soil turnover studies can only be used for rough estimation, but cannot obtain accurate volume values. When this value is applied to the physical modification of the geography of the quantitative population, a large deviation will occur. Some researchers have tried to measure the burrow volume more accurately through mathematical modeling<sup>[19]</sup>. Therefore, how to simplify this method and successfully apply it to related researches is a difficult problem that needs to be solved at present. In addition, the soil removal by engineers should be combined with its population size, and the

biogeographical function of burrowing engineers should be discussed at the landscape level.

## 2.2 Burrowing Engineers Changes Soil Physicochemical Properties

Bioturbation of engineers contributes to soil mainly via mechanical turnover by excavating deep soil to the surface and burying the original surface organic matter underground. Researchers detected soil samples to obtain physicochemical property data, such as moisture, temperature, compaction, hydrophobicity, ammonium nitrogen, conductivity, distribution of litter and the present all kinds of trace elements in the soil. In this part, we mainly discuss the experiment methods and the result of burrowing engineer changes soil physicochemical properties.

### 2.2.1 Comparative Measurement for Soil Physicochemical Properties

In order to explore the engineering effect on soil physicochemical properties, researchers usually compared soil property between disturbed and undisturbed surfaces. Mallen-Cooper et al (2019) quantitatively synthesised the findings of 149 published studies that compared disturbed and undisturbed soil surfaces, included 64 engineer species, but not all burrowing engineers<sup>[20]</sup>. We discuss the application of comparative measurement methods in this kind of research on different spatial and temporal scales, divide the spatial scale into three categories: microsite (less than 5 m or 5 m<sup>2</sup>), habitat (up to 1 km or 100 ha) and landscape scale (more than 1 km or 100 ha); divide the temporal scale into four categories: snapshot, <12 months, 1-3 years and >3 years (Figure 1).

At the microhabitat scale, researchers commonly detected soil samples from different microsites at each burrow (inside, the entrance of burrow, excavated mounds, undisturbed sites, etc.) to compare engineering effect on soil properties<sup>[21,22,23,24]</sup>. In addition, some researchers furtherly add artificial burrows as a control group to highlight the unique changes in the soil properties caused by animal digging. For example, Qiu et al (2019) selected three sample plots, artificial conburrow-convex microtopography, natural conburrow-convex microtopography generated by crab burrows and natural flat microtopography with few or no crab burrows, and then compared the differences in the soil carbon and nitrogen content indices<sup>[24]</sup>.

At the habitat and landscape scales, bioturbation is affected by different environmental factors, including plant type, precipitation (different degrees of drying), altitude, etc., they are selected as concomitant variables to quantify

the interaction between environment and burrowing engineers<sup>[21,25,26,27,28]</sup>. Davies et al (2019) distinguished three plant types in Tasmanian temperate woodlands, and they detected soil fertility and structure to explore the different effects of burrowing engineers on soil. These effects on soil fertility and structure were strongest in habitats with dry and poor soil<sup>[25]</sup>. Burbidge et al (2007) detected the difference in soil physicochemical properties between the burrow mounds and the undisturbed areas at the landscape scale, and they found the soil penetrability typically at mounds far greater than surrounding soil that often has a hard pan<sup>[29]</sup>.

More studies have used methods that measure and compare soil physicochemical properties at multiple spatial scales between disturbed and undisturbed areas. For instance, comparative measurement of soil properties in combination with microhabitats scale at the landscape scale<sup>[19,30,31,32,33]</sup>, and combination with microhabitats scale at the habitat scale<sup>[14,24,29,34,35]</sup>, and combination with habitat scale at the landscape scale, but few studies were used this method<sup>[36,37]</sup>. In general, the studies based on the combination of multiple spatial scales have become more popular. Compared with a single-scale measurement, the multi-scale experimental methods can reveal the influence of burrowing engineers on soil properties more comprehensively and deeply.

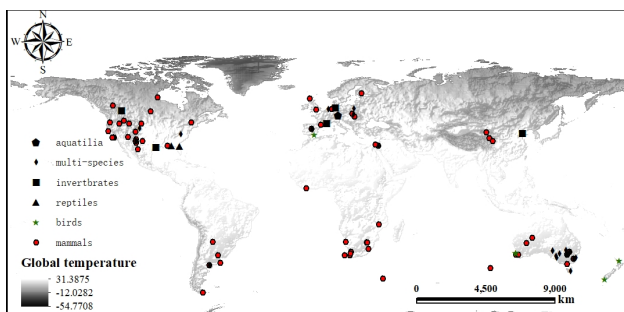
The studies about engineering effect on soil property usually compare and measure property at temporal scale. There is a typical study, in Australia, the southern brown bandicoot (*Isodon obesulus*; Peramelidae) leaves foraging pits after eating, and fresh diggings typically contain a higher moisture content and lower hydrophobicity than undisturbed soil. One month later, the soil fertility is increased, so researchers speculated the reason may be the foraging pits accumulated more organic materials than undisturbed areas<sup>[38]</sup>. However, the soil physicochemical properties don't regularly change linearly with time. For example, the soil permeability in fresh foraging burrows of Chinese pangolin will increase because the hardened topsoil is destroyed. With the accumulation of organic matter and the formation of a new waterproof layer, the soil permeability begins to decline. However, the accumulation of organic matter increased soil fertility, which was beneficial to the development of soil organisms and plant colonization (unpublished data). The burrows created by engineers are characterized by legacy effects because of their persistence. In fact, the burrows of autogenic engineers like pangolins and armadillo (*Oryzomys azer*) often persist long after the organism's death (Hastings et al, 2007). Consequently, the understanding of the soil property transformation in an entire burrow cycle (from dig to collapse) is necessary. However, the literature shows the

most studies only compare and measure soil properties in different areas at the snapshot scale (Figure 1). Long-term monitoring of changes in soil physicochemical properties is still lacking. One method is to compare soil properties of new and old burrows at the same time<sup>[21, 38]</sup>, meanwhile, the environment character and disturbance level of burrows in different ages are supposed to be similar.

### 2.2.2 Engineering Effect on Soil Property is Vary with Environment Gradient

Considerable studies about the engineering effect on soil property caused by burrowing engineers have concentrated on arid and semiarid environments (Figure 3)<sup>[4, 39, 40, 41, 42, 43, 44]</sup>. More evidence has demonstrated that engineering effect is stronger in arid and semiarid regions than in mesic and semi-mesic regions<sup>[25, 38]</sup>. This may be due to increased water infiltration (moist soil excavated by engineers) in xeric environments which may make a substantial difference in plant survival and growth, as soil nutrients are more easily absorbed when in solution<sup>[45]</sup>. In contrast, the high initial soil moisture in mesic environments leaves little opportunity for moisture to be further increased. Another potential reason for this difference in bioturbation is that in arid and poor soil habitats, large amounts of nutrients are lost from top layer soil (heat and weathering, etc.), while subsoil is relatively fertile and rich in certain trace elements<sup>[46]</sup>.

We can reasonably speculate that pangolins would increase soil moisture, consistent with similar disturbances made by aardvark (*Orycteropus afer*), echidna (*Tachyglossus aculeatus*) in other systems<sup>[41, 47]</sup>, and decrease soil compaction, similar to the effects of bilby (*Macrotis lagotis*), echidna (*Tachyglossidae*) and brush-tailed bettong diggings (*Bettongia penicillata*)<sup>[21, 41, 47, 48]</sup>. Meanwhile, Chinese pangolins are mainly distributed in mesic and semi-mesic regions, and much less is known about the role of burrowing species play in mesic environments.



**Figure 3.** Global distribution of burrowing engineers research. Points on the map represent the type of engineering species by shape and colors. The proportion of all kinds of burrowing engineers.

## 2.3 Burrowing Engineers Change Plant Community Structure

Engineers play an important role in the local ecosystem and affect plant community structure with two ways: by directly foraging for individual plants and vegetation communities (trophic effects) and by unique bioturbation (burrowing), which can affect soil properties and indirectly influence vegetation. Changes in soil properties as a direct result of bioturbation by burrowing engineers and generate distinct patterns of plant community composition and diversity, which are enhanced by the indirect effects of engineered soil properties on the productivity, biomass and growth rates of plant species<sup>[49]</sup>. Previous studies have explored the roles of burrowing engineers in affecting plants structure by measuring and comparing seedbanks and plant structures in the presence and absence of burrow, respectively.

### 2.3.1 Burrows and Mounds Change the Vegetation Seedbank

Burrowing engineers affect seed banks, mainly reflected in seed aggregation and seed germination<sup>[50, 51]</sup>. Valentine et al considered the reason for the preferable seed aggregation effect in disturbed pits because i) the undulating surface heterogeneity (caused by diggings) reduces seed removal by wind or seed predators, ii) the diggings collapsed and slightly buried seeds and hence protected them<sup>[38]</sup>. These hypotheses are also expressed in other related studies<sup>[21, 31, 35]</sup>. These studies tested their hypotheses by gathering seeds in soil from disturbed and undisturbed areas to highlight the otherness between sampling points. For example, more seeds are accumulated in mounds constructed by kangaroo rats (*Dipodomys spectabilis*)<sup>[52]</sup>, bilby (*Macrotis lagotis*)<sup>[53]</sup> and bettong (*Bettongia lesueur*)<sup>[54]</sup> than in undug adjacent soils.

The mechanical turnover of soil by burrowing engineers can increase soil moisture and bury organic matter, bringing it in close contact with soil microorganisms and thereby altering soil microbial activity and litter decomposition<sup>[41, 43, 55, 56]</sup>. Engineered soil enhances seed germination under laboratory conditions, too<sup>[38, 57]</sup>. Some researchers seeded five endemic species in artificial foraging pits (imitating the natural foraging pit, as the physicochemical properties of soil are basically similar), spoiled heaps and the undug surface and then the number of each germinated seed at each site during the next 18 weeks are recorded. They found that the presence of artificial pits contributed to greater seedling recruitment for



three of the plant species tested and seed germination at artificial foraging pits was generally higher than at other sites <sup>[38]</sup>.

The soil property changed by burrowing engineers may depress germination of some species over others, too. Particularly in drier habitats, bioturbation has brought about more significant changes in soil physicochemical properties. The content of some trace elements will change greatly. Canals et al. (2003) has reported 10-fold higher nitrate concentrations on pocket gopher mounds in an annual California grassland, excessive nitrate inhibits seed germination <sup>[58,59,60]</sup>.

### 2.3.2 Burrow Changing the Vegetation Community Structure

Burrowing activity is a process that manufactures pioneer microhabitats, adding habitat heterogeneity and creating a small area of open habitat (soil heap). Novel plant communities invade and colonize in this open area of the habitat, which leads to secondary succession of the ecosystem. The combination of a consistent soil disturbance and an altered soil nutrient concentration promotes the growth or hinders the recruitment of some plant species, an important driver of plant assemblage succession <sup>[61]</sup>.

To reveal the effect of fox burrowing behavior on plant community structure, researchers conducted a plant sample survey (50 × 50 cm plots on the surface of the fox burrow and in the adjacent dry grassland) and recorded the percentage cover of all vascular plant species and the thickness and percentage cover of the litter layer in each plot <sup>[62]</sup>. They found i) a high proportion of nutrient-demanding species on fox burrows; ii) that the total species richness was lower on the burrows than in grasslands; and iii) that the total species richness was also lower in cleared areas (surrounded by cropland) than in complex landscapes (surrounded by more than 20% dry grasslands and a low proportion of arable lands within a radius of 200 m). These findings suggested that open microsites provided by the soil heap supported the encroachment of vanguard species due to the decreased level of competition <sup>[63]</sup>, increased or changed nutrient availability <sup>[64,65]</sup> and reduced the amount of litter <sup>[66,67]</sup>.

During the succession, Paschke et al (2000) considered the slower-growing perennials become dominant when nutrient uptake by early-seral species can no longer support their rapid aboveground growth <sup>[68]</sup>. Godó et al (2018) also recognized this point because after persistent observations, they found that tussock-forming grass species were particularly successful in recolonization due to their robust physiognomy and higher competitive

ability <sup>[62,69]</sup>. Based on this phenomenon, they believed that despite the recent disturbance causing a temporal local encroachment of noxious species, patches of disturbed surfaces can be overgrown by specialist species <sup>[70]</sup>. The pangolin burrow consists of two parts: the burrow tunnel and the soil mound outside the burrow (see Figure 4).



**Figure 4.** The appearance of the pangolin burrow, including the tunnel and the mound outside the burrow: (a) and (b) are new burrows that are less than one year old, (c) and (d) are old burrow that are more than one year old.

The soil excavated from deep layer covers the surface outside the burrow mouth and turns the area into bare ground, and mound covers an area from 0.12 square metres to more than 2.98 square metres (unpublished data). We believe that pangolin bioturbation affects plant community succession because the spoil heap buries the old plant community and creates an open microhabitat for the establishment of new vegetation species. In our field investigation of the Chinese pangolin, the variation trend of the plant community structure surrounding the burrow consistent with Paschke's consideration by observing and comparing different burrows with different ages. We made a 2×2 m herb quadrant and a 5×5 m shrub quadrant centred on the burrow and randomly selected two undisturbed areas within a radius of 15 m around the burrow to produce same quadrants. We found that the difference of plant community structure on the

fresh mound and the undisturbed area was the greatest. The older the burrow was, the smaller the difference was. When the burrow collapsed and the mound disappeared due to trampling and rain erosion, the plant community on the mound also evolved to the highest level, similar to the plant community structure in an undisturbed area (unpublished data).

The survey area of pangolin is mainly concentrated in humid mountainous and hilly areas. The bioturbation caused by pangolins may have a transient effect on plant community structure, with shrubs and trees already growing on most of the abandoned old burrows and mounds, which may be due to the abundant rainfall and fertile soil (rapid decomposition of litter) in this area, promoting the growth and breeding of plants. In drylands, soil disturbance by burrowing engineers has shown stronger effects on plant community structure <sup>[21,63,71,72,73]</sup>. We speculate that this phenomenon mainly occurs because drier regions exhibit more landscape unicity than wetter regions, which reveals the vital ecological function of burrowing engineers. Because engineers excavated deep soil rich in water and nutrients out of the ground to form mound, vanguard species are first planted due to their rapid growth and high nutrient requirements. Meanwhile, the naturally low rate of litter decomposition and the plant recruitment characteristics of arid and semiarid environments limit plant growth in undisturbed areas <sup>[74,75]</sup>. Research on the leaf litter decomposition rate, seed bank recruitment and success, and germination should be long-term to detect continuous changes in vegetation structure. Meanwhile, engineers can be herbivores, and the net effect of engineer foraging can have significant impacts on plant community structure <sup>[44,76]</sup>. However, there is a lack of relevant studies, and more attention should be paid to these engineers in future research.

## 2.4 Burrow Providing Limited Resource for Commensal Species

The burrows created by burrowing engineers has the characters as sturdiness, concealment, structural complexity, persistence et al. We summarized the types of burrow resources in relevant literature, including: spawning site, shelter, thermal refugia, foraging grounds. In addition, we have observed pangolin burrows as mating sites and natural toilets for other animals, but lack of strong evidence to support this, so we will not discuss it here.

### 2.4.1 Commensal Species Depend on the Burrow Microhabitat to Complete Their Life Cycle

Changes in plant communities usually cascade to animal communities, such as invertebrates that rely strictly on certain environmental conditions to complete

their life cycle. For example, the decline of open, such as semi-natural grasslands and heathlands in Europe, has caused a general decline in biodiversity, especially for butterflies <sup>[77]</sup>. The digging activity could be effective in reducing grass encroachment and restoring pioneer microhabitats. These usually generate warm, open and sparsely vegetated microsites <sup>[78,79]</sup>. The low grass cover increased occupancy of the favoured host plant *Pyrgus malvae* indirectly supports effective microhabitat selection by females during oviposition, which strongly determines larval survival. In addition, the importance of mounds created by the European mole (*Talpa europaea*) as an oviposition habitat for the small copper (*Lycaena phlaeas*) within Central European mesotrophic grasslands was reported <sup>[80]</sup>. The author found that even though *L. phlaeas* is considered a generalist species inhabiting a wide range of open habitats, a high proportion of eggs was found on molehills where the vegetation structure clearly differed from the surrounding vegetation. The oviposition sites of the small copper were preferentially located at open vegetation structures with a higher proportion of bare ground, a lower cover of herbs and a less dense and low-growing vegetation created by the European mole.

Within mesotrophic grasslands, where bare ground is usually rare, mound-building ecosystem engineers act as important substitutes for missing soil disturbance by diversifying the vegetation structure <sup>[81]</sup> and creating small patches of bare soil that are used for oviposition. Several studies have highlighted the importance of small-scale soil disturbances for the conservation of rare and endangered species <sup>[82,83,84,85]</sup>.

### 2.4.2 Burrows Providing Shelter and Thermal Refugia for Commensal Animals

Burrowing species are often considered to be important ecosystem engineers, as burrow constructions increase environmental heterogeneity <sup>[57,86]</sup> and provide shelter and thermal refuge for themselves or other species <sup>[5,7,87]</sup>. Animals can hide in burrows from fire <sup>[88]</sup>, heavy rain, predators or extreme thermal conditions. Pangolins usually locate their dwelling burrows in secluded places near the top of mountains. The winding passageway of the burrow ensures that the nest room in the burrow will not be flooded in the event of heavy rain <sup>[89,90]</sup>. In Taiwan, Sun et al. (2018) found that when pangolins stay inside a burrow, the hole is usually blocked with mud, leaving only a small gap at the top of the wall to allow air to circulate <sup>[91]</sup>. The researchers speculate that this is a way for pangolins to protect themselves from predators, such as the reticulated python (*Python reticulatus*), which preys on Sunda pangolin (*Manis javanica*) <sup>[92]</sup>, while they are inside



the burrow. Other animals also use pangolin burrows as temporary shelters because of their invisibility and safety. To date, we have monitored more than 37 vertebrates as burrow commensals of the Chinese pangolin; the most frequent users are small mice (unpublished data).

Burrowing engineers can create microhabitats with a more stable temperature and humidity compared to ambient conditions. Gopher tortoises (*Gopherus polyphemus*) are ecosystem engineers that excavate large, deep burrows throughout the coastal plain of the southeastern US<sup>[27]</sup>. Pike and Mitchell (2013) recorded tortoise body temperatures, operative environmental temperatures (operative environmental temperatures are the temperatures available to an ectotherm in thermal equilibrium with its environment) and burrow temperatures and found that the temperature fluctuations inside burrows were minimal<sup>[93]</sup>. Bao et al (2013) reported that the air temperature inside the Chinese pangolin burrow was stable, with only a slight fluctuation; in contrast, the air temperature outside the burrow fluctuated dramatically<sup>[89]</sup>.

Recent studies suggest that rising ambient temperatures associated with the overall trend of global warming may make it more difficult for ectotherms to avoid overheating, especially in tropical dry environments<sup>[94,95,96]</sup>. According to figures from 1981 to 1990, the global average temperature was 0.48 °C higher than the temperature 100 years ago. From the beginning of the 20th century to the present, the average temperature of the Earth's surface has increased by approximately 1.1 °F (0.6 °C), and since 2000, the high temperature record has often been broken throughout the world. To avoid extreme (which can lead to death of ectotherms) environmental temperatures and maintain body temperatures within favorable ranges, animals usually changing their diurnal rhythm or search for thermal refuge<sup>[97,98]</sup>. For example, Walde et al once reported horned larks (*Eremophila alpestris*) using burrows constructed by desert tortoise (*Gopherus agassizii*) in the Mojave Desert during summer as a refuge from the hot above-ground temperatures<sup>[79]</sup>. Consequently, the function of burrowing engineers that dig burrows and provide thermal refuge for other species is receiving increasing attention from researchers, especially in dry tropical regions where seasonal and diurnal temperatures fluctuate greatly.

### 2.4.3 Burrows Providing Food Resources for Commensal Species

One important ecosystem function of burrowing engineers is they can alter the distribution of food resources in the environment<sup>[1,2,99]</sup>, further influence the distribution and diversity of species<sup>[100,101]</sup>. First, bioturbation increasing soil fertility, aggregating seeds and raising the seeds germination, directly increasing plant productivity in the burrow

microhabitat<sup>[101]</sup>. More plant biomass means burrows afford more food resources and attract herbivorous and omnivorous fauna. Additionally, among the species attracted by plant food and thermal refuge, invertebrates account for a huge proportion of the diverse and numerous species, usually regarded as common inhabitants of mammal burrows<sup>[6,8,102]</sup>. For example, Kinlaw documented 302 invertebrate species in the burrows of gopher tortoise<sup>[7]</sup>. Hancox documented 81 insect species, including eight tick species, in badger (*Meles meles*) burrows<sup>[103]</sup>, and over 250 invertebrate species were documented to use gerbil (*Gerbillinae*) burrows<sup>[6]</sup>. Invertebrates are also as food resource provided to other commensal creatures, such as birds, reptiles and amphibians. Galvez-Bravo et al reported that the most common vertebrate taxonomic groups that using rabbit burrows are reptiles and amphibians<sup>[104]</sup>. The similar viewpoint had been suggested in the study of the prairie dog (*Cynomys ludovicianus*)<sup>[105]</sup> and pocket gopher (*Thomomys spp*)<sup>[106]</sup>.

Finally, small mice and birds are frequent burrow users, where they forage for seeds or invertebrates. The frequent appearance of these predators also makes burrows become another feeding grounds for more advanced predators, such as medium and large carnivores<sup>[7,8,107]</sup>. However, these advanced predators may make relatively little use of the burrow, and the burrow is only a spot where they have more prey in their range (personal observation). Most advanced predators have not been monitored using burrows over a long period of time. They enter burrow, search, and leave burrows<sup>[108]</sup>.

## 2.5 Interaction of Burrow Commensal Species

### 2.5.1 Burrow Commensal Species Composition and Investigation Methods

Burrow commensal species are animals which was attracted to burrow microhabitats to use burrow resources, and with burrows as the hub, these species form a close bond with each other<sup>[5,8,73,102,109,110]</sup>. The diversity and richness of burrow commensal animals vary with diggers, investigation methods and monitoring time. The investigation methods vary with animal body size and purpose. Camera traps have been used to monitor medium and large mammals and birds, and it's becoming more common. The methods of monitoring small mammals include live trapping, surveying for signs, tracking, and direct observation; the presence of reptiles and amphibians in burrows is determined by active searches, and they are usually detected at a higher rate in such studies<sup>[102,110]</sup>.

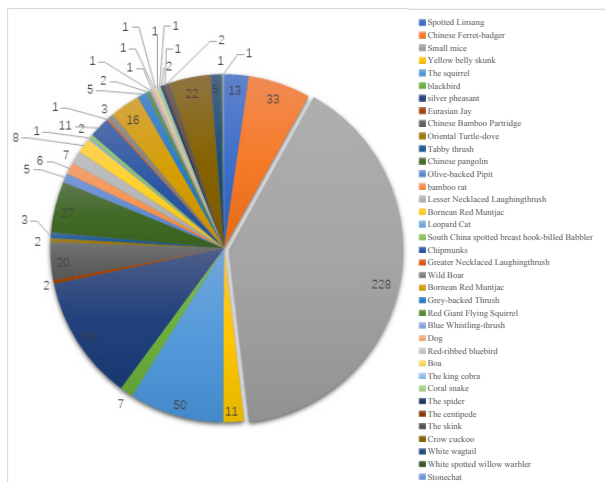
For example, more than 350 species have been documented using gopher tortoise (*Gopherus polyphemus*) burrow systems, of which more than 50 species are vertebrates, including 9 species of amphibians and 25

species of reptiles. The investigation methods included questionnaires, biological traps, and direct observation, the investigation period more than a decade<sup>[102]</sup>. 57 species have been documented using giant armadillo burrows; 24 species were considered to use either the sand mound or the burrow itself, and 2 reptile species (Tegu *Tupinambis teguixin* and lizard *Ameiva sp.*) were identified. No amphibians have been identified. In this study, camera trap is the only investigation method, the investigation period between July 2010 and September 2012<sup>[5]</sup>.

Amphibians, reptiles and invertebrates are poikilotherms, and their ability to regulate body temperature is much lower than that of endotherms. It is a biological instinct to use the appropriate environment to assist thermoregulation. We speculate that poikilotherms need thermal refuges as much as birds and mammals, especially in arid and semiarid lands. One reason for the low proportion of amphibians and reptiles in animal monitoring is the over-reliance on camera traps. These cameras are more sensitive to the presence of warmer mammals and birds but less sensitive to the presence of cooler amphibians and reptiles. Another reason may be the short monitoring time and small monitoring area.

### 2.5.2 Preliminary Study of Burrow Commensal Species Interaction of Chinese Pangolin

Since the survey in 2020, 37 burrow commensal species of Chinese pangolin have been recorded (Figure 5).

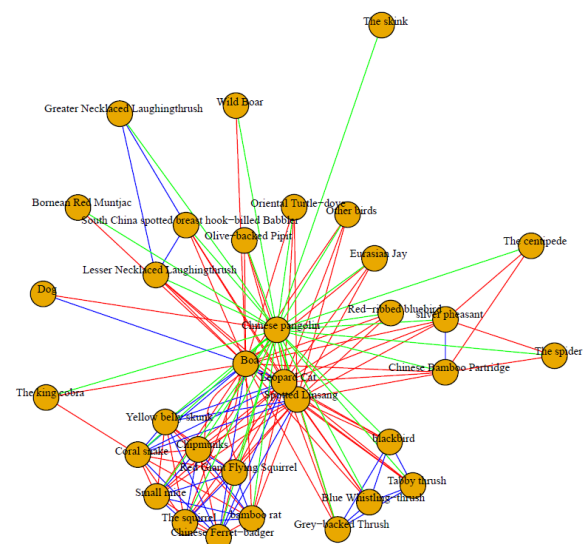


**Figure 5.** The frequency distribution diagram of Chinese pangolin burrow commensal species.

A total of 14 species of mammals, 17 species of birds, 4 species of reptiles and 2 species of invertebrates were recorded in pangolin burrows. These animals enter the pangolin burrow area directly or forage, mate, comb feathers, etc. Among them, small mice were the most used species in the burrow, with a total of 228 small mice are

recorded, accounting for 40%. Followed by silver pheasant (*Lophura nycthemera*; 66), The squirrel (*Sciuridae*; 50) and Chinese Ferret-badger (*Melogale moschata*; 33). Reptiles and invertebrates are less well documented, probably because they are generally smaller and have lower body temperatures than thermostatic species, making them difficult to capture effectively with infrared cameras. Therefore, the utilization rate of ectotherms in the burrow may have been underestimated. At present, an image motion trigger camera has been used to make up for the deficiency.

Pangolin burrows are hot spots of animal activity in the ecosystem. The abundance of burrow resources drives the utilization of burrow commensal species, and complex interspecies interactions are formed during the utilization process. As shown in Figure 5, Chinese pangolin, boa (*Python bivittatus*), spotted linsang (*Prionodon pardicolor*), leopard cat (*Prionailurus bengalensis*), and small mice are the central species in the community of burrow commensal species. Among them, pangolin provides burrow resources for the species, is the basis for the formation of commensal communities, but there is still a risk of being predation by boas and dogs. Boas, spotted linsang, Chinese ferret-badger and leopard cat (*Prionailurus bengalensis*) are predators, which control the biomass of the ecosystem through the top-down effect. Predation is the most frequent interspecific interaction among commensal community, followed by competition with other carnivores. As the most frequent burrow users, small mice themselves also become the indirect burrow resources contributor, attracting other predators. Together with other low-trophic species, they affect community structure through bottom-up effect. The competitive relationship mainly exists among species of the same trophic level (or similar species), which have a high degree of niche overlap and similar demands for habitat, food and other resources.

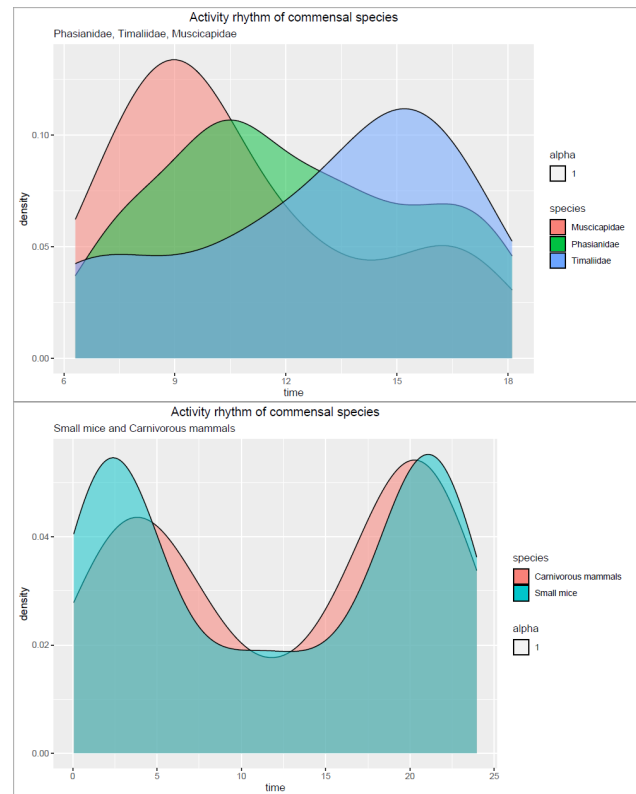
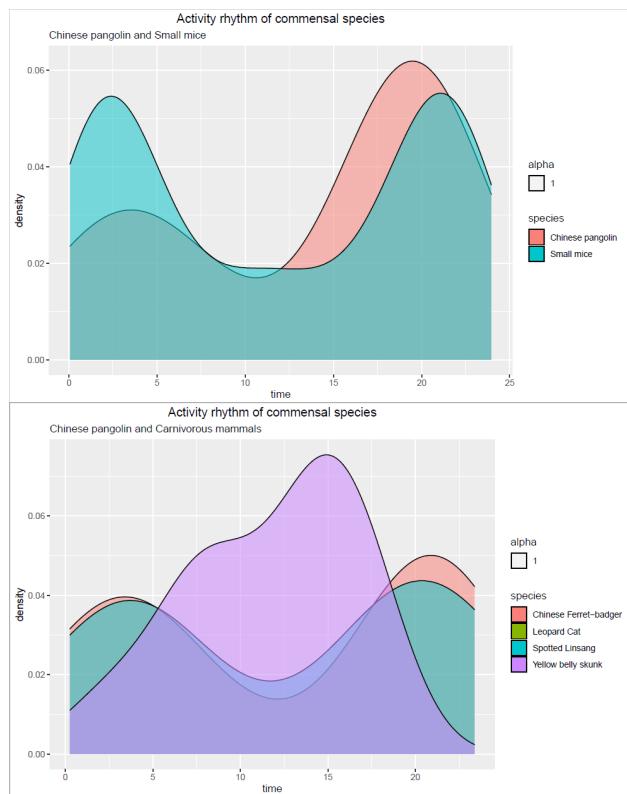


**Figure 6.** The Chinese pangolin burrow commensal animal population network diagram.

Figure note 1: the green line said there is a commensal relationship between two species (pangolin provide burrow resources) red line said two species predator-prey relationships between the blue line shows the competitive relationship between the two species.

Figure note 2: other birds are crow cuckoo (*Centropus sinensis*), white wagtail (*Motacilla alba*), white spotted willow warbler (*Phylloscopus davisoni*) and stonechat (*Saxicola torquata*).

The activity rhythm map of burrow commensal species showed that the activity peaks of pangolin, small mice, spotted linsang, Chinese ferret badger and leopard cat had a high degree of overlap, and concentrated at night. Yellow belly skunk using their burrows mostly in the daytime, which is inconsistent with their biological habits. The specific reasons need to be further studied. The activity peaks of Phasianidae, Timaliidae and Muscipidae were basically staggering, which indicated the differentiation of ecological niche to some extent. The activity peaks of carnivorous mammals and small mice basically coincide, and small mice are the common prey targets of the four carnivorous species, which may reflect the rule that low-trophic species influence high-trophic species through bottom-up effect.



**Figure 7.** The activity rhythm of Chinese pangolin burrow commensal community

### 2.5.3 Necessity of Ecological Research on Chinese Pangolins

The Chinese pangolin, once widely distributed in East Asia, northern Southeast Asia, and parts of South Asia, has a range that exceeds three million square kilometres [111]. They once occupied a large area in the south of the Yangtze River in China, but it is hardly to encounter them in most parts of this range in the last three decades. Chinese pangolins are best known for their trophic role as termite predators. Termite is one of the five major insect pests worldwide and damages a variety of trees, water conservancies, and built dams. According to a previous report, 3 kg of pangolin can eat approximately 300~400 g of termites and can protect 17 hectares of forest from termite damage [112]. Therefore, pangolin serve an important role in controlling the termite population in the natural ecosystem. However, previous studies always neglected the fact that Chinese pangolins are ecosystem engineers with the function of modifying surrounding habitats.

The ecological research on Chinese pangolins is very limited, especially regarding the ecological function of their burrows [89,113,114,115]. There is no qualitative or quantitative knowledge of the ecological role of

pangolin burrows in the local ecosystem, so it is far from sufficient to speculate based on the relevant studies of other burrowing engineers. Considering the extensive distribution area and enormous burrows of Chinese pangolins before the 1980s, we think pangolins must be an important regulator of the ecological balance in the ecosystems<sup>[111,116,117,118,119,120]</sup>. On the one hand, pangolin is a narrow-eating mammal that feeds mainly on termites, and it plays an indispensable role as a termite damage controller in the ecosystem. Furthermore, many species of termites (such as *Coptotermes formosanus*, *Odontotermes formosanus* and *Macrotermes barneyi*) live in nest, with their nests changing topography and landforms and altering the distribution of resources in the environment. These species are also to be ecosystem engineers according to Jones and Lawton's proposed definition<sup>[1,2]</sup>. That is, their predation behavior affects the ability of another kind of engineer to transform the ecosystem. On the other hand, burrows provide shelter, thermal refuge and food resources for burrow commensal species, the negative impact on the survival of commensal species of pangolins, which have declined dramatically in recent decades and even become extinct regionwide, is still unknown. Several studies have shown the decline in biomass, species richness, and abundance of vertebrate species in areas where burrowing engineers have been eradicated<sup>[121,122,123]</sup>. For instance, the extinction of prairie dogs has caused the near-extinction of black-footed ferrets (*Mustela nigripes*) and the drastic decline of mountain plovers (*Charadrius montanus*)<sup>[124]</sup>. Taking into account pangolins' ability to physically modify their habitats, their profound impact on commensal species, and their unique position in the food web, we conclude that pangolins play a key role in their ecosystem. Therefore, it is necessary that continuous study of pangolin ecology for a long time.

### 3. Negative Feedback of Engineering Effects on the Ecosystem

Not all the physical modification made by burrowing engineers to the habitat are positive, some studies have mentioned the negative effect for the habitat caused by the digging behavior of organisms. However, there has been relatively little research on the negative effects of burrowing engineering, so we are here to briefly discuss them. Firstly, excessive digging activity tends to destroy the ground surface, increase bare land area, and accelerate surface erosion<sup>[12,125,126,127]</sup>. Overdigging is often associated with a high-density population of engineers. From this point of view, the effect of engineers' digging activity on the ecosystem can't be purely qualitative. Moderate

excavation is beneficial to soil renewal, while excessive excavation is destructive, which is also in line with the ecological hypothesis that moderate disturbance increases biodiversity. Therefore, it is very necessary to study at population scale and landscape scale. In addition, the bare ground will reduce vegetation cover<sup>[125,128,129,130]</sup> and lead to the invasion of alien species<sup>[131]</sup>. And the change of soil physicochemical properties will also inhibit the colonization of some species<sup>[132]</sup>. The negative feedback of engineering effects on ecosystems can also be seen in animal communities, for example, the digging behavior of cape ground squirrels (*Xerus inauris*) changed vegetation community structure and further decreased habitat quality of beetle, this ultimately leads to a decline in beetle richness<sup>[43]</sup>.

### 4. Suggestions for Future Direction and the Way Forward

The loss of biodiversity is currently one of the most important issues in the world. In this review, we briefly list some positive effects of burrowing engineers on biodiversity, such as increasing geomorphological heterogeneity, promoting plant community renewal and providing resources for many groups<sup>[7,63]</sup>. The ecological impact of these burrowing engineers is more intense in environments otherwise unfavourable for most species considered<sup>[104]</sup>. Indirect conservation by protecting an important species and using its irreplaceable ecological role to benefit other organisms is a much easier and more practical approach than aimless extensive conservation. Consequently, management actions to preserve endangered burrowing engineer populations worldwide are necessary to maintain their effects on vegetation and animal communities.

To better use burrowing engineer species for habitat remoulding functions in ecosystems and equip us for the future challenges of ecological and conservation science, more detailed research is needed on:

- (1) Burrowing engineer ecological cascades through trophic and engineering pathways and their mixed effects on ecosystems.
- (2) Categorizing burrowing engineers according to the action and scope of their engineering effects (e.g., comprehensive assessment of impact duration, the number of species affected, body size, population density, etc.).
- (3) The spatiotemporal relationships of burrow commensal species and the driving mechanisms.
- (4) Emerging studies of engineer reduction and reintroduction.
- (5) The entire periodic effects of burrows on soil,



vegetation, and commensal species (i.e., the process from bioturbation being started to burrows being assimilated into the environment).

## 5. Conclusions

We summarized the habitat modification function of burrowing engineers from five directions, emphasized the critical role that the rejuvenation of engineer species can play in mitigating the ongoing loss of biodiversity. It's providing a strong theoretical support for the necessity of the protection work of burrowing engineers. We also summarized the experimental design methods of burrowing engineer studies, the dominating experiment method is comparative measurement. Researchers are comparing soil properties, plant community structure between disturbed and undisturbed areas at different spatial and temporal scales. This research method is easy to reveal part of the ecological role of burrowing engineers, but it is not conducive to explore the deeper potential impact of engineers on the habitat. More diversified experimental design (e.g. manipulative experiments, replication across multiple sites) and more scientific and accurate data analysis (e.g. mathematical model) are necessary. At present, the most urgent task is to apply the theory of ecosystem engineer to the work of biological protection, and bring into play the ecological role of burrowing engineer in modifying the habitat and regulating the community structure of commensal species. Long term research of engineering impact on ecosystem at population scale is essential, the research on the ecological role of burrowing engineers in humid and subhumid areas also needs to be strengthened. This article aims to identify some implications to better understand the interaction between the burrowing engineer and local habitat. It is our sincere hope that this review will contribute to the conservation of the Chinese pangolin and many other burrowing engineer species.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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## ARTICLE

# Clustering and Differentiation of *glr-3* Gene Function and Its Homologous Proteins

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### ABSTRACT

In order to adapt to the low temperature environment, organisms transmit excitement to the central system through the thermal sensing system, which is a classic reflex reaction. The cold receptor GLR-3 perceives cold and produces cold avoidance behavior through peripheral sensory neurons ASER. In order to further understand the gene encoding of the cold sensing *glr-3* gene and the evolution of its homologous gene group function and protein function, the nucleotide sequence and amino acid sequence of the *glr-3* gene and its homologous gene in 24 species were obtained and compared. By clustering with the GRIK2 gene sequence of *Rana chensinensis*, the bioinformatics method was used to predict and sequence analyze the change of gene, evolution rate, physical and chemical properties of protein, glycosylation sites, phosphorylation sites, secondary structure and tertiary structure of protein. The analysis results show that the *glr-3* gene and its homologous gene have obvious positive selection effect. The protein prediction analysis showed that the *glr-3* gene and its homologous genes encoded proteins in these 25 species were hydrophilic proteins, and the proportion of side chains of aliphatic amino acids was high. The transmembrane helix was widespread and there were more N-glycosylation sites and O-glycosylation sites. The protein phosphorylation sites encoded were serine, threonine and tyrosine phosphorylation sites. Secondary structure prediction showed that the secondary structure units of the encoded protein were  $\alpha$ -helix,  $\beta$ -turn, random coil and extended chain, and the proportion of  $\alpha$ -helix was the largest. This study provides useful information on the evolution and function of the cold sensing gene *glr-3* and its homologous genes.

## 1. Introduction

As a kind of stressor, the low temperature environment can easily induce the body to produce cold stress, which directly or indirectly affects the physiological state and behavior of the animal, and even causes the death of the animal<sup>[1-3]</sup>. During the stress response, the changes of the animal body are very complicated<sup>[2,4]</sup>. Therefore, to

maintain optimal function in a cold environment, animals must detect the temperature of their body and the environment, and make appropriate responses<sup>[5]</sup>. The information of environmental cold is expressed and transmitted by cold-sensitive ion channels in the peripheral sensory nerve endings of the skin. Neurons respond to cold stimuli, and the animal body will produce the corresponding cold escape mechanism<sup>[5,6]</sup>. When an animal stays in a cold stress

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environment for a long time, the neuroendocrine system will respond to the cold stimulus. When the physiological and hormone levels are balanced, the animal can adapt to this low temperature environment, and the animal body will overcome the stressor. Obtained cold adaptation [2,5,7,8].

In order to survive, organisms have evolved sophisticated heat-sensing systems to detect low temperatures and respond accordingly [9], but as of August 2019, only one cold receptor TRPM8 (transient receptor potential cation channel subfamily M) has been discovered. member 8), TRPM8 plays a central role in detecting somatosensory environmental low temperature [10], can be activated by low temperature and coolant menthol [11-13], its ability to sense cold can be fine-tuned in various species, In order to adapt well to the environmental temperature and better participate in energy metabolism [14]. The kainic acid glutamate receptor homolog GLR-3 was only identified as a cold receptor on August 29, 2019. GLR-3 senses cold in peripheral sensory neurons ASER to trigger the cold escape mechanism [15,16], its homolog GluK2 (glutamate ionotropic receptor kainate type subunit 2) can functionally replace GLR-3 in the body for cold sensation [17]. By selecting glr-3 genes and their homologs from 25 species Gene, and the protein sequence that the gene encodes. Use bioinformatics methods to conduct comparative analysis to explore whether the gene has undergone adaptive evolution among different species, and provide useful information for the glr-3 gene and its homologous genes, as well as their evolution and function.

## 2. Materials and Methods

### 2.1 Acquisition and Evolution Rate Calculation of glr-3 Gene and Its Homologous Gene Sequences in 25 Species

The gene sequences and protein sequences of 24 different species were obtained from the GenBank database of NCBI (Table 1) on the official website. In addition, the GRIK2 gene sequence of *Rana dybowskii* was obtained by polymerase chain reaction (PCR), and its protein sequence was obtained on emboss \_ transeq. The ratio of dN / dS was calculated by pamlX-CodeML, namely,  $\omega$  value, to detect the evolution rate of glr-3 gene and its homologous genes.

### 2.2 Construction of Phylogenetic Tree of glr-3 Gene and Its Homologous Genes

The ML tree was constructed by evolutionary analysis software MEGAX. ModelFinder and MrBayes in Phylo-Suite-Pylogeny were used for model selection and Bayesian inference tree construction.

**Table 1.** GeneID and GenBank accession numbers of species

Species	GenBank accession numbers	GeneID	homologous gene
<i>Homo sapiens</i>	NM_001166247	2898	GRIK2
<i>Pan troglodytes</i>	XM_001142208	462899	GRIK2
<i>Macaca mulatta</i>	XM_015136995	695660	GRIK2
<i>Canis lupus familiaris</i>	XM_038684247	481938	GRIK2
<i>Bos taurus</i>	NM_001193063	615226	GRIK2
<i>Mus musculus</i>	NM_001111268	14806	Grik2
<i>Rattus norvegicus</i>	NM_019309	54257	Grik2
<i>Gallus gallus</i>	XM_015284534	428628	GRIK2
<i>Xenopus tropicalis</i>	XM_031902289	100495093	grik2
<i>Danio rerio</i>	XM_021466798	556013	grik2
<i>Drosophila melanogaster</i>	NM_142668	42473	KaiR1D
<i>Anopheles gambiae str. PEST</i>	XM_003437056	4576020	AgaP_AGAP000801
<i>Caenorhabditis elegans</i>	NM_059616	172449	glr-3
<i>Sus scrofa</i>	XM_021073336	100516526	GRIK2
<i>Equus caballus</i>	XM_001503914	100066235	GRIK2
<i>Felis catus</i>	XM_019831025	101089440	GRIK2
<i>Ailuropoda melanoleuca</i>	XM_034670902	100466021	GRIK2
<i>Ictalurus punctatus</i>	XM_017479112	108271497	grik2
<i>Dermochelys coriacea</i>	XM_038395673	119853121	GRIK2
<i>Balaenoptera musculus</i>	XM_036871504	118905139	GRIK2
<i>Cygnus atratus</i>	XM_035561788	118255532	GRIK2
<i>Zootoca vivipara</i>	XM_035109324	118082226	GRIK2
<i>Artibeus jamaicensis</i>	XM_037135552	119042005	GRIK2
<i>Manis pentadactyla</i>	XM_036890423	118915022	GRIK2

### 2.3 Prediction of glr-3 Gene, glr-3 Homologous Gene Encoding Protein Properties

ProtParam was used to predict the physicochemical properties of glr-3 gene and its homologous gene encoded protein. ProtScale was used to analyze the hydrophilicity and hydrophobicity of the encoded protein. TM-HMMServer2.0 was used to analyze the transmembrane topological structure of the encoded protein. Prediction-Servers was used to analyze the glycosylation sites of the encoded protein.

Use SOPMA to predict and analyze the secondary structure of the protein; use Swiss-Model. Predict the tertiary structure of proteins.

## 3. Results and Analysis

### 3.1 Phylogenetic Analysis and Evolution Rate of glr-3 Gene and Its Homologous Genes

In order to compare the phylogenetic relationships of

glr-3 gene and its homologous genes in different species. In order to compare the phylogenetic relationship of glr-3 genes and their homologous genes in different species, phylogenetic trees were constructed for 25 species obtained. The construction methods were Maximum Likelihood (ML) method (Figure 1) and Bayesian inference method. The results show that the two phylogenetic trees are divided into two branches, the ML tree diagram shows that mammals are on the same branch, and the Bayesian inference tree diagram shows that *Mus musculus* and *Rattus norvegicus* are separated On the branch where the mammal is. The evolution rate analysis of the glr-3 genes of 25 species showed that the  $\omega$  value of *Mus musculus* and *Rattus norvegicus* was 2.40, and the  $\omega$  value of the remaining 12 mammals was 1.37, which is obviously compared to the other 12 mammals, Grik2 Genes make more favorable selection in *Mus musculus* and *Rattus norvegicus*; the  $\omega$  value of 25 species is 1.28, which has obvious positive selection effect.

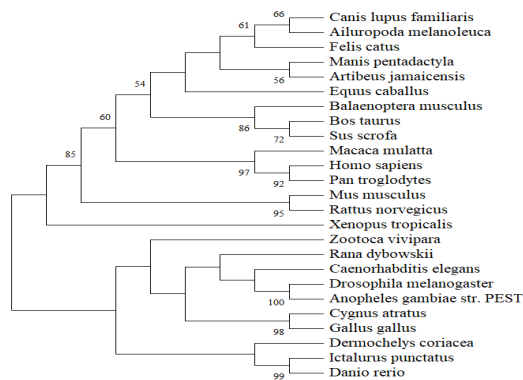


Figure 1. The evolutionary tree

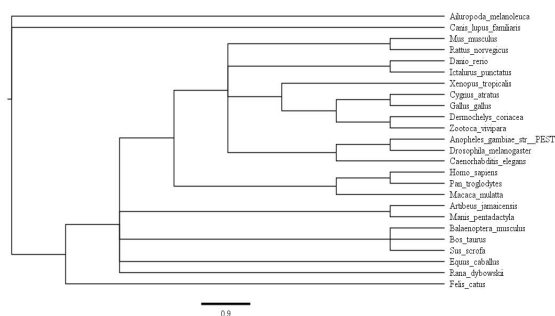


Figure 2. The evolutionary tree

### 3.2 Functional Protein Analysis of glr-3 Gene and Its Homologous Genes

The protein sequences encoded by glr-3 gene and its homologous genes in 25 species were obtained and analyzed. The physical and chemical properties of the encoded protein were predicted by online analysis software. The results showed that the protein sequence length

was 432-915 AA, and the average protein length was 860 AA. The isoelectric point is between 6.22 and 9.75, and the average isoelectric point is 7.80. The instability index was between 36.73 and 47.75, and the instability index of coded proteins in 11 species was lower than the threshold, which was predicted to be stable proteins. The instability index of coded proteins in 14 species was higher than the threshold, which was predicted to be unstable proteins. The total average hydrophobicity was between -0.283 and -0.062, which were hydrophilic proteins. The predicted values of fat coefficient ranged from 80.53 to 97.48, and the proportion of side chains composed of aliphatic amino acids in proteins was higher, which reflected the strong thermal stability of proteins controlling these genes<sup>[18]</sup>.

Table 2. 25 Species glr-3 gene and its homologous gene expression protein physical and chemical properties analysis

Species	AAs	PI	Instability index	GRAVY	Aliphatic index
<i>Homo sapiens</i>	892	6.91	39.36	-0.077	90.72
<i>Pan troglodytes</i>	908	8.05	39.99	-0.120	89.23
<i>Macaca mulatta</i>	908	8.05	39.99	-0.120	89.23
<i>Canis lupus familiaris</i>	908	8.06	40.10	-0.125	89.23
<i>Mus musculus</i>	908	7.83	40.29	-0.113	89.65
<i>Bos taurus</i>	908	8.05	40.10	-0.126	89.12
<i>Rattus norvegicus</i>	908	8.04	40.56	-0.108	89.65
<i>Gallus gallus</i>	908	7.8	40.47	-0.112	89.12
<i>Xenopus tropicalis</i>	913	8.02	39.28	-0.118	87.57
<i>Danio rerio</i>	908	7.29	40.62	-0.138	88.9
<i>Drosophila melanogaster</i>	853	7.59	37.81	-0.085	94.20
<i>Anopheles gambiae str. PEST</i>	888	6.22	40.8	-0.193	85.56
<i>Rana dybowskii</i>	432	9.75	47.75	-0.482	80.53
<i>Caenorhabditis elegans</i>	836	6.83	37.54	-0.062	97.48
<i>Sus scrofa</i>	908	8.05	39.66	-0.125	89.23
<i>Equus caballus</i>	908	8.05	40.10	-0.126	89.23
<i>Felis catus</i>	583	8.00	36.73	-0.091	93.48
<i>Ailuropoda melanoleuca</i>	908	8.05	40.10	-0.126	89.23
<i>Ictalurus punctatus</i>	915	7.86	42.98	-0.125	89.60
<i>Dermochelys coriacea</i>	908	7.80	40.06	-0.111	89.02
<i>Balaenoptera musculus</i>	895	8.35	41.80	-0.185	86.72
<i>Cygnus atratus</i>	859	7.20	39.82	-0.160	87.29
<i>Zootoca vivipara</i>	733	8.13	39.27	-0.283	84.58
<i>Artibeus jamaicensis</i>	908	8.05	40.10	-0.126	89.23
<i>Manis pentadactyla</i>	887	6.94	39.83	-0.162	87.40

\*AAs: number of amino acids; PI: isoelectric point; GRAVY: Grand average of hydropathicity



Membrane proteins play an important role in biological activity, including cell communication, ion transport, transport, signal transduction, and functions as a "sensory organ" of cells. Transmembrane proteins are usually divided into three regions, which are distributed on both sides of the membrane. The hydrophilic part and the hydrophobic part that cross the membrane and form a stable helical structure exist<sup>[19,20]</sup>. Prediction and analysis of transmembrane regions of encoded proteins (Table 3), except for *Rana dybowskii*, there are transmembrane spirals in 24 species. *Homo sapiens*, *Pan troglodytes*, *Macaca mulatta* and other 14 species have 3 transmembrane spirals and their positions are the same.

**Table 3.** *glr-3* gene and its homologous gene expression protein in 25 species

Species	Number of trans-mem-brane spirals	Position		
		transmembrane region	extra mem-brane	intramembrane
<i>Homo sapiens</i>	3	563~582 639~661 822~844	1~562 662~821	583~638 845~892
<i>Pan troglodytes</i>	3	563~582 639~661 822~844	1~562 662~821	583~638 845-908
<i>Macaca mulatta</i>	3	563~582 639~661 822~844	1~562 662~821	583~638 845-908
<i>Canis lupus familiaris</i>	3	563~582 639~661 822~844	1~562 662~821	583~638 845-908
<i>Mus musculus</i>	3	563~582 639~661 822~844	1~562 662~821	583~638 845-908
<i>Bos taurus</i>	3	563~582 639~661 822~844	1~562 662~821	583~638 845-908
<i>Rattus norvegicus</i>	3	563~582 639~661 822~844	1~562 662~821	583~638 845-908
<i>Gallus gallus</i>	3	563~582 639~661 822~844	1~562 662~821	583~638 845-908
<i>Xenopus tropicalis</i>	3	568~587 644~666 827~849	1~567 667~826	588~643 850~913
<i>Danio rerio</i>	4	563~582 639~661 822~844	33~562 662~821	1~12 583~638 845~908
<i>Drosophila melanogaster</i>	3	548~567 622~644 814~836	1~547 645~813	568~621 837~853
<i>Anopheles gambiae str. PEST</i>	3	516~535 592~614 784~806	1~515 615~783	536~591 807~888
<i>Equus caballus</i>	3	563~582 639~661 822~844	1~562 662~821	583~638 845~908

Species	Number of trans-mem-brane spirals	Position		
		transmembrane region	extra mem-brane	intramembrane
<i>Sus scrofa</i>	3	563~582 639~661 822~844	1~562 662~821	583~638 845~908
<i>Felis catus</i>	1	563~582	1~562	583
<i>Rana dybowskii</i>	0	/	1~432	/
<i>Ailuropoda melanoleuca</i>	3	563~582 639~661 822~844	1~562 662~821	583~638 845~908
<i>Ictalurus punctatus</i>	3	570~589 646~668 829~851	1~569 669~828	590~645 852~915
<i>Dermochelys coriacea</i>	3	563~582 639~661 822~844	1~562 662~821	583~638 845~908
<i>Balaenoptera musculus</i>	2	624~646 807~829	1~623 830~895	647~806
<i>Cygnus atratus</i>	3	514~533 590~612 773~795	1~513 613~772	534~589 796~859
<i>Zootoca vivipara</i>	1	638~660	1~637	661~733
<i>Artibeus jamaicensis</i>	3	563~582 639~661 822~844	1~562 662~821	583~638 845~908
<i>Manis pentadactyla</i>	3	542~561 618~640 801~823	1~541 641~800	562~617 824~887
<i>Caenorhabditis elegans</i>	3	525~544 601~623 781~803	1~524 624~780	545~600 804~836

Glycosylation is one of the methods of protein post-translational modification. It plays an important role in changing the conformation and stability of proteins. It participates in many processes of protein transcription and translation, immune response and transportation. Mutations in glycosylation sites may change gene function and play a key role<sup>[21]</sup>. Analysis of glycosylation sites of the encoded proteins of *glr-3* gene and its homologous genes (Table 4) shows that there are more glycosylation sites in 25 species, and N-glycosylation sites are more than O-Glycosylation site<sup>[22]</sup>. *Rana dybowskii* has the most O-glycosylation sites at 19; *Rana dybowskii* and *Caenorhabditis elegans* have 0 and 2 N-glycosylation sites, and the remaining 23 Species N-glycosylation sites are between 4-7.

**Table 4.** Analysis of glycosylation sites of *glr-3* genes and their homologous genes in 25 species

Species	Number of O-glycosylation	Number of N-glycosylation	Position of N-glycosylation
<i>Homo sapiens</i>	4	6	67, 73, 275, 378, 423, 546
<i>Pan troglodytes</i>	3	6	67, 73, 275, 378, 423, 546
<i>Macaca mulatta</i>	3	6	67, 73, 275, 378, 423, 546

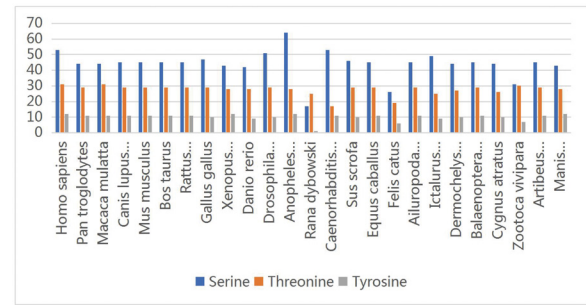


Species	Number of O-glycosylation	Number of N-glycosylation	Position of N-glycosylation
<i>Canis lupus familiaris</i>	3	6	67, 73, 275, 378, 423, 546
<i>Mus musculus</i>	4	6	67, 73, 275, 378, 423, 546
<i>Bos taurus</i>	3	6	67, 73, 275, 378, 423, 546
<i>Rattus norvegicus</i>	4	6	67, 73, 275, 378, 423, 546
<i>Gallus gallus</i>	2	6	67, 73, 275, 412, 423, 546
<i>Xenopus tropicalis</i>	5	7	72, 78, 280, 383, 417, 428, 551
<i>Danio rerio</i>	7	7	67, 73, 275, 378, 412, 423, 546
<i>Drosophila melanogaster</i>	8	4	262, 293, 389, 397
<i>Anopheles gambiae str. PEST</i>	12	4	229, 359, 365, 383
<i>Rana dybowskii</i>	19	0	/
<i>Sus scrofa</i>	3	6	67, 73, 275, 378, 423, 546
<i>Equus caballus</i>	3	6	67, 73, 275, 378, 423, 546
<i>Felis catus</i>	2	4	67, 73, 275, 423
<i>Ailuropoda melanoleuca</i>	3	6	67, 73, 275, 378, 423, 546
<i>Ictalurus punctatus</i>	6	6	74, 80, 385, 430, 437, 553
<i>Dermochelys coriacea</i>	2	6	67, 73, 275, 378, 423, 546
<i>Balaenoptera musculus</i>	7	5	67, 73, 275, 378, 423
<i>Cygnus atratus</i>	2	7	18, 24, 226, 363, 374, 381, 497
<i>Zootoca vivipara</i>	3	6	70, 76, 278, 381, 426, 714
<i>Artibeus jamaicensis</i>	3	6	67, 73, 275, 378, 423, 546
<i>Manis pentadactyla</i>	4	7	46, 52, 254, 357, 391, 402, 525
<i>Caenorhabditis elegans</i>	5	2	257, 356

Protein phosphorylation is one of the common post-translational modifications of proteins in biology. It is an important mechanism in the regulation of signal transduction in cells and participates in cell transduction and maintenance of protein spatial stability. Protein phosphorylation mainly includes serine, threonine and tyrosine phosphorylation<sup>[18,23]</sup>. As shown in Figure 3, 25 species *glr-3* gene and its homologous gene coding proteins contain 3 phosphorylation sites, serine, threonine and tyrosine phosphorylation sites, serine phosphorylation sites are the most, tyrosine phosphorylation sites are the least. It is predicted that the recognition and binding of these encoded proteins with receptor signals are related.

Polypeptide chains form irregular folding along one-dimensional direction by hydrogen bonds. These fragments form the secondary structural units of proteins. The common three secondary structural units are  $\alpha$  helix,  $\beta$  folding, irregular curl and  $\beta$  rotation<sup>[24,25]</sup>. SOPMA was used to predict the secondary structure of proteins. The secondary structure units of the encoded proteins were  $\alpha$ -helix, ran-

dom coil, extended chain and  $\beta$ -turn, and the proportion showed a decreasing trend.

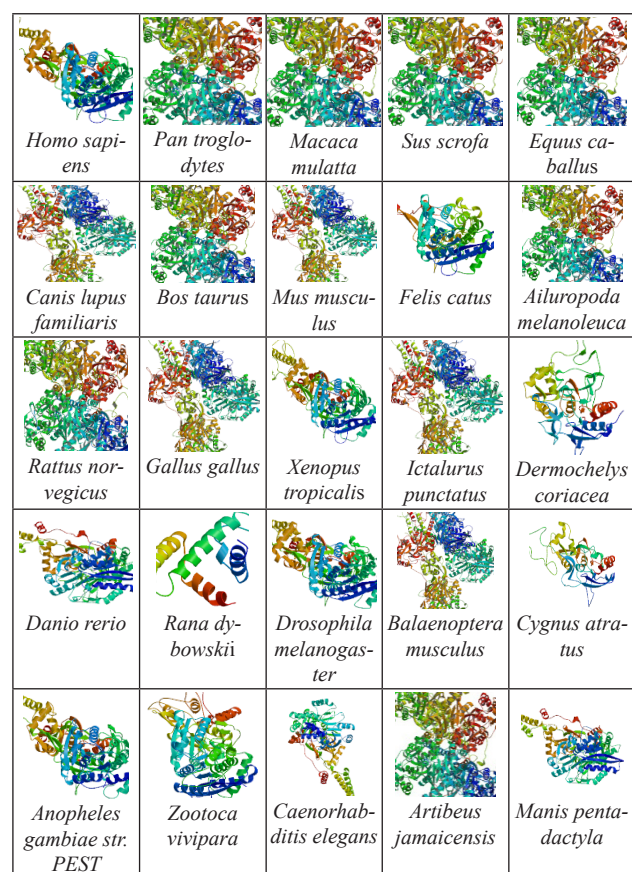


**Figure 3.** Predictive analysis of phosphorylation modification sites of *glr-3* genes and their homologous genes in 25 species

**Table 5.** Prediction of secondary structure of *glr-3* gene and its homologous gene expression protein in 25 species

Species	Alpha helix (%)	Beta turn (%)	Random coil (%)	Extended strand (%)
<i>Homo sapiens</i>	41.37	5.27	35.87	17.49
<i>Pan troglodytes</i>	41.08	5.62	37	16.3
<i>Macaca mulatta</i>	41.08	5.62	37	16.3
<i>Canis lupus familiaris</i>	42.62	5.51	35.9	15.97
<i>Mus musculus</i>	42.29	5.62	36.01	16.8
<i>Bos taurus</i>	41.3	5.95	37	15.75
<i>Rattus norvegicus</i>	41.74	5.51	36.67	16.08
<i>Gallus gallus</i>	42.84	5.18	35.79	16.19
<i>Xenopus tropicalis</i>	40.64	5.7	36.69	16.98
<i>Danio rerio</i>	43.39	5.4	35.57	15.64
<i>Drosophila melanogaster</i>	42.02	5.48	35.48	17.02
<i>Anopheles gambiae str. PEST</i>	40.99	5.41	37.95	15.65
<i>Rana dybowskii</i>	34.49	11.81	33.1	20.6
<i>Sus scrofa</i>	40.75	5.73	37.11	16.41
<i>Equus caballus</i>	42.29	5.51	35.90	16.30
<i>Felis catus</i>	37.39	4.80	38.25	19.55
<i>Ailuropoda melanoleuca</i>	42.29	5.51	35.90	16.30
<i>Ictalurus punctatus</i>	43.17	5.46	35.74	15.63
<i>Dermochelys coriacea</i>	40.97	5.62	37.11	16.30
<i>Balaenoptera musculus</i>	41.01	5.25	37.21	16.54
<i>Cygnus atratus</i>	42.14	6.29	35.86	15.72
<i>Zootoca vivipara</i>	36.43	5.46	39.15	18.96
<i>Artibeus jamaicensis</i>	42.90	5.51	35.90	16.30
<i>Manis pentadactyla</i>	40.81	5.75	36.64	16.80
<i>Caenorhabditis elegans</i>	42.11	4.9	34.33	18.66

Using Swiss-Model to predict the tertiary structure of proteins (Figure 4), the prediction results show that the tertiary structure of 8 mammals, including gorillas (*Pan troglodytes*), macaques (*Macaca mulatta*), and dogs (*Canis lupus familiaris*) are similar. The tertiary structure of human (*Homo sapiens*), zebrafish (*Danio rerio*) and Chinese pangolin (*Manis pentadactyla*) are similar.



**Figure 4.** Prediction of tertiary structure of *glr-3* gene and its homologous gene expression protein in 25 species

#### 4. Discussion

The regulation of temperature by organisms plays an important role in the normal conduct of life activities. In response to cold stimuli, organisms undergo the process of escape, adaptation, selection and evolution of genes and functional proteins.

The evolutionary selection of *glr-3* gene in different species reflects the gain and loss of this gene<sup>[26]</sup>. The construction of ML tree and Bayesian tree reflects the phylogeny of *glr-3* gene and its homologous genes. The results of the rate show that  $\omega > 1$  obviously has an obvious positive selection effect, which may be related to the stress of the organism's nervous system to cold stimulation<sup>[10]</sup>.

The analysis results show that the *glr-3* gene and its homologous gene encoding protein of the research species are all hydrophilic proteins, and the side chain composed of aliphatic amino acids accounts for a higher proportion, indicating that the protein controlling this type of gene has strong thermal stability<sup>[18]</sup>. The encoded protein has obvious glycosylation sites, which is predicted to enhance the stability of the protein by changing the spatial structure of the protein<sup>[27,28]</sup>. The encoded proteins all contain 3 phosphorylation sites, serine, threonine and tyrosine. The phosphorylation sites of amino acids, the most serine phosphorylation sites in the sequence, the least tyrosine phosphorylation sites, it is speculated that this type of protein is widely involved in cell transcription and regulation, signal recognition<sup>[29]</sup>, secondary structural unit of the encoded protein. There are  $\alpha$ -helices, random coils, extended strands and  $\beta$ -turns.  $\alpha$ -helices account for the largest proportion, maintaining the stability of the protein spatial structure. The secondary structure of the protein is also related to the coding region of the mRNA sequence, and the coding protein tends to be encoded by the stem region of mRNA<sup>[30]</sup>. The purpose of this study is to explore the variation of *glr-3* genes and their homologous genes in different species. The evolutionary rate and functional analysis of the encoded protein have certain research significance.

#### 5. Conclusions

Through this study, we have reached the following conclusions: *glr-3* gene and its homologous genes have obvious positive selection effects; through protein prediction analysis, it is shown that the *glr-3* genes and their homologous genes of these 25 species all encode proteins. It is a hydrophilic protein with a high proportion of side chains composed of aliphatic amino acids, transmembrane helices are common, and there are more N-glycosylation sites and O-glycosylation sites, and the encoded protein phosphorylation sites. There are phosphorylation sites for serine, threonine and tyrosine; the secondary structure prediction shows that the secondary structure unit of the encoded protein has  $\alpha$ -helix,  $\beta$ -turn, random coil and extended chain, of which  $\alpha$ -helix accounts for the proportion. Both are the largest. This study provides useful information on the evolution and function of the *glr-3* gene and its homologous genes.

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## ARTICLE

# Histological Study of the Male Internal Genital Tract of Dwarf Caiman (*Paleosuchus palpebrosus* Cuvier, 1807)

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### ABSTRACT

Brazil has six species of crocodiles belonging to the subfamily Caimaninae, one of them being *Paleosuchus palpebrosus* (Cuvier, 1807) (dwarf caiman). It is considered one of the smallest crocodilian species, and is sensitive to environmental changes. The anatomical and histological descriptions of the reproductive tract of reptiles are scarce, so it is important to study the morphology of the genital tract of caimans for a better understanding of the reproduction of these species. We performed the histological analysis of the testis, epididymis, and deferent duct of the dwarf caiman *Paleosuchus palpebrosus*. Structures of the genital tract were collected from euthanized animals. Testes, epididymis, and deferent ducts were separated and prepared according to routine histological techniques for morphological characterization. The testis is covered by a capsule of fibromuscular connective tissue. Spermatogonia are observed as spherical cells with round nuclei and loose chromatin, and primary spermatocytes show dark cytoplasm, round nuclei, and pale chromatin. Spermatids are seen as cylindrical or filamentous cells with nuclei that may be highly condensed, ranging from round to oval. The epididymis is observed as a coiled duct lined by a tall cuboidal epithelium with stereocilia; the deferent duct shows ciliated pseudostratified cylindrical epithelium. The adrenal gland is found in the dorsomedial portion of the testis. The histological structure of the reproductive tract of *Paleosuchus palpebrosus* is similar to that of other reptiles' species.

## 1. Introduction

Brazil has six species of crocodiles belonging to the subfamily Caimaninae: *Caiman latirostris* (Daudin, 1802) (broad-snouted caiman), *Caiman crocodilus* (Linnaeus, 1758) (spectacled caiman), *Caiman yacare* (Daudin, 1802) (swampland alligator), *Paleosuchus palpebrosus* (Cuvier, 1807) (dwarf caiman), *Paleosuchus trigonatus* (Schneider,

1801) (smooth-fronted caiman), and *Melanosuchus niger* (Spix, 1825) (black caiman) <sup>[1]</sup>. *Paleosuchus palpebrosus* is considered one of the smallest crocodilian species, reaching between 100 to 150 cm in length <sup>[2]</sup>. It is found both in clearwater or blackwater rivers, showing preference for cold water, and it is known to live in burrows whose entries are located below water level. The species is sensitive to environmental changes, and it is difficult to

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be maintained and bred in captivity<sup>[3]</sup>.

Crocodylian reproduction, both in natural and captivity conditions, has been receiving attention because of animal management. Environmental factors, such as temperature and water level interfere with gonad activity. For captive animals, improving quality and quantity of food is essential in the stabilization of reproductive periods<sup>[4]</sup>.

Anatomical, morphology and histological descriptions of the male reproductive system of reptiles in general are scarce, but this knowledge is very important to determine the role of these tissues in the production of functional sperm<sup>[5]</sup>. Gribbins<sup>[6]</sup> provided the first complete histological analysis of spermatogenesis and the germ cell development within a species of the order Crocodylia and relate these observations to the accumulating data that suggest that temperate reptiles retain a temporal germ cell development pattern similar to anamniotes. These authors also state that alligator's primitive reptilian characters and its important phylogenetic placement between dinosaurs and birds makes it ideal for comparative, developmental, and evolutionary studies on the vertebrate reproductive system. Nixon<sup>[5]</sup> showed in your research that the general structural feature of the male crocodile excurrent duct system is a reflection of its common ancestry with Archosaurs, as they share closer similarities with those of birds than other clades within the reptilia or mammalia class.

Cabrera<sup>[7]</sup>, in the histological description of the male genital apparatus of *C. crocodilus*, reported the presence of testicles, epididymis and adrenal gland coated by capsule of dense fibromuscular tissue, the adrenal gland located on the dorsomedial surface of the testes, and the testes with great spermatogenic activity. In reptiles, testes show seminiferous tubules lined by germinative cells associated with Sertoli cells and the ductal system consists of the rete testis, epididymis and deferens duct<sup>[8,9]</sup>.

The duct system comprises comprising ductus efferent, epididymis and ductus deferens. The epithelium delineating is dominated by non-ciliated and ciliated cells structured into a simple columnar lining of the ductus efferent through to the high pseudostratified columnar epithelium of the epididymis and ductus deferens. The morphology of these ducts suggests their involvement in seminal fluid production and/or its modification, which likely contributes to the nourishment, protection and/or storage of sperm<sup>[5,10]</sup>.

The reptiles do not possess accessory sexual glands, but the adrenal gland is similar to mammals located suprarenal and shows a close relationship with the gonads<sup>[11]</sup>, and is found closely attached to the epididymis<sup>[8,9]</sup>. The adrenal gland is formed by two portions: a steroidogenic tissue and a chromaffin tissue, and its distribution in turtles and

crocodilians is abundantly intermingled, but the hormones produced are almost the same between the vertebrate classes, the chromaffin tissue produces adrenaline and noradrenaline and the steroidogenic tissue produces most of the steroid hormones present in mammals<sup>[11,12]</sup>. The adrenal gland plays a pivotal role in regulates metabolism, maintains normal electrolyte balance, in the response to predictable stressors of animal daily and seasonal life such reproduction, development and migration<sup>[11,12]</sup>.

Because of the few reports on the morphology of the genital tract of caimans, it is important to study these organs for greater understanding of the reproduction of those species.

Therefore, the objective of this study was to evaluate the histological characteristics of the testes, epididymis, and deferent ducts of the *Paleosuchus palpebrosus*.

## 2. Material and Methods

The research used two crocodiles of the species *Paleosuchus palpebrosus* kept in captivity in the Laboratory for Teaching and Research in Wild Animals at FAMEV/UFU. After immobilization, the animals were euthanized with administration of 2.5% sodium thiopental (5 ml) followed by intravenous injection of 19.0% potassium chloride (10 ml), according to the recommendations of the Brazilian Guide of Good Practices for Euthanasia in Animals of Federal Council of Veterinary Medicine<sup>[13]</sup>. Samples were collected from the portions of the genital tract (testicles, epididymis and vas deferens). The study was authorized by license SISBIO number 13159-1 and protocol CEUA/UFU number 112/2014.

During necropsy of animals, the testes together with the epididymis and part of the vas deferens were quickly separated from the rest of the reproductive tract, placed in plastic bags, and kept in ice at 5°C during the processing. Samples of these structures were collected for histological analysis. Fragments of tissue of about 1 cm<sup>3</sup> were fixed in Bouin's solution and submitted to prefixation for approximately 20 minutes. Then the samples were immersed in 10 % buffered formalin for 48 hours. The material through infiltration was dehydrated in increasing concentrations of ethyl alcohol (starting in 70% alcohol and ending in absolute alcohol) and subsequently cleared in Xylol. Subsequently, the samples were infiltrated with liquid paraffin heated between 56°C-60°C and then embedded in paraffin blocks. Finally, 5 µm thick sections were cut from the blocks, placed on glass slides and stained with hematoxylin-eosin. The slides were digitalized on a Scanscope AT slide scanner (Leica Biosystems®) and evaluated by the software Aperio ImageScope®.

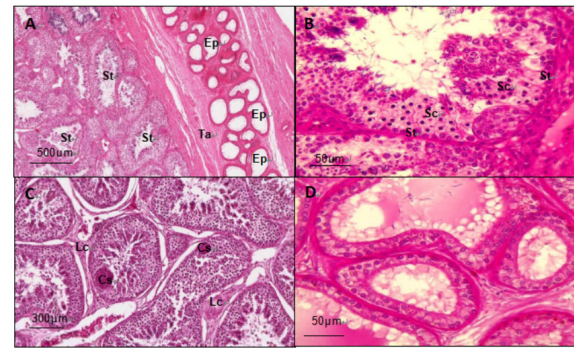
### 3. Results

The testes of *Paleosuchus palpebrosus* are elongated ovoid surrounded by a layer of adipose tissue, are covered by a capsule of dense fibromuscular tissue well vascularised, equivalent to the tunica albuginea (Figure 1A), which also covers the epididymis and the adrenal gland. Trabeculae of connective tissue completely or partially divide the testis in testicular lobules, which include the seminiferous tubules. The seminiferous tubules are lined by the germinative epithelium which comprises spermatogenic line cells. Cells at different phases of division can be observed (Figure 1B). Spermatogonia are found at the base of the seminiferous epithelium and are characterized as round cells with round nuclei and loose chromatin. They undergo mitosis and yield primary spermatocytes, which show dark cytoplasm with evident nucleolus and loose chromatin (Figure 1B). Spermatids are predominant in the seminiferous epithelium and found in groups close to the lumen of the seminiferous tubule. Young spermatids are cylindrical or filamentous, and their nuclei range from round to oval, with more condensed nuclei and pale cytoplasm. In the lumen, basophilic spermatozoa are found (Figure 1B).

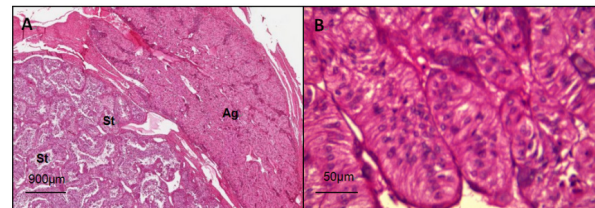
Sertoli cells are also identified in the testes. They show large pleomorphic and pale nuclei with one or two prominent nucleoli (Figure 1B). Figure 1C shows some cells in the interstitial compartment, most likely Leydig cells. These cells are polyhedral with acidophilic cytoplasm and few vacuoles, round nucleus with one or two more prominent nucleoli and loose chromatin. A particularity finding in these animals is groups of clonic spermatocytes (Figure 1C) formed by primary spermatocytes in the basal compartment of the seminiferous tubules. Secondary spermatocytes are also found at the base of the germinative epithelium. However, they are difficult to observe as they undergo a quick second meiotic division. Some spermatogonia may also be found in this location.

The ductus efferent and epididymis were not easily definable on gross dissection but extend from the outer lateral margin of the testis. The epididymis (Figure 1A, D) is composed by a thin convoluted duct, lined with tall cuboidal epithelium with stereocilia and portions of pseudostratified cells. In the lumen of the duct, slightly basophilic spermatozoa are found. The epithelium is surrounded by a layer of circularly oriented smooth muscle fibres.

A structure with glandular characteristics is attached to the dorsomedial portion of the testes, surrounded by a large amount of connective tissue (Figure 2 A, B). This gland probably represents the adrenal gland.

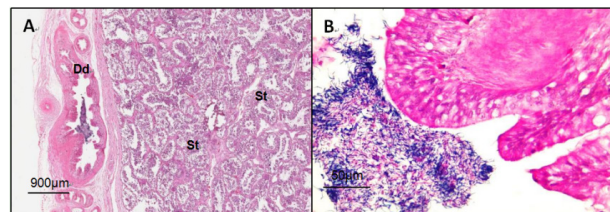


**Figure 1.** Photomicrograph of the testis and epididymis of *Paleosuchus palpebrosus*. (A) Testis and epididymis; (B, C) Seminiferous tubule; (D) Epididymis. Seminiferous tubule (St); Epididymis (Ep); Tunica albuginea (Ta); Spermatogenic cells (Sc); Sertoli cell (St), Clonic spermatocytes (Cs), Leydig cells (Lc). Amplification of 10 X (Figures A, C) and 40 X (figures B, D). Hematoxylin/eosin colouration. Photos by: Adrielly Lopes.



**Figure 2.** Photomicrograph of the adrenal gland of *Paleosuchus palpebrosus*. Seminiferous tubule (St); Adrenal gland (Ag). Amplification of 10 X (Figure A) and 40 X (figure B). Hematoxylin/eosin colouration. Photos by: Adrielly Lopes.

The epididymis continues in the deferent duct (Figure 3 A, B). It is characterized by cylindrical pseudostratified epithelium with stereocilia and is surrounded by smooth muscle fibres. In the lumen, basophilic spermatozoa are found.



**Figure 3.** Photomicrograph of the duct deferens of *Paleosuchus palpebrosus*. Seminiferous tubule (St); Deferent duct (Dd). Amplification of 10 X (figure A) and 40 X (figure B). Hematoxylin/eosin colouration. Photos by: Adrielly Lopes.

### 4. Discussion

The shape of the testes with a tunica albuginea well

vascularised is similar the other alligators, like *Crocodylus porosus* [14]. The capsule of dense fibromuscular tissue surrounding the testis, epididymis and the adrenal gland of *Paleosuchus palpebrosus*, and forms trabeculae in the interstitial compartment, similar to the description by Cabrera [7].

In this study, we have verified the same observation of Gribbins [4,9] who described seminiferous tubules with germinal cells at different phases of division, primary spermatocytes which show dark cytoplasm with evident nucleolus and slightly basophilic spermatozoa in the lumen. In the base of the seminiferous tubules, groups of clonic spermatocytes were observed (Figure B), similar to the structures found in *C. crocodilus* by Cabrera [7], who also characterized secondary spermatocytes as cells with slightly acidophilic cytoplasm and round nuclei. The Leydig cells (Figure C) present in the interstitial compartment, as described by Cabrera [7] in *C. crocodilus*, are similar to those of reptiles, birds and mammals.

Similar histological characteristics of the epididymis (Figure A, D) were reported in other alligators [7,14-17]. Similarly to *Paleosuchus palpebrosus*, in *Crocodylus porosus* the epithelium is formed by non-ciliated and ciliated cells structured into a simple columnar lining of the ductus efferent through to the high pseudostratified columnar epithelium of the epididymis and ductus deferens [5]. Other reptiles, such as sea snakes, also show the same epididymal lining as crocodilians [18]. A similar structure covering the epididymis was found by Guerrero and Saccucci [8,19], which divided this organ in a cranial, middle and caudal portion. In soft-shelled turtle *Pelodiscus sinensis*, the epididymis of the have three distinct regions, cranial, middle and caudal were identified in the based on anatomical characteristics, with epithelium consists of five different cell types: principal, narrow, apical, clear and basal cells [10].

The glandular structure in the dorsomedial portion of the testis that was characterized as an adrenal gland in this study, was similar to adrenal gland identified in other reptiles [7,11,18]. In *C. crocodilus*, similar to that verified in this research, the adrenal gland is closely related to the gonads, with a capsule of fibrous connective tissue and consists of pale cells, corresponding to cortical cells and basophilic cells corresponding to medullary cells [20]. The adrenal gland is formed by two portions: a steroidogenic tissue and a chromaffin tissue, and its distribution in crocodilians is abundantly intermingled, and the organ does not present a clear cortical and medullary division [11,20].

The deferent duct of *Paleosuchus palpebrosus* presented similar characteristics to the descriptions of other caimans [7,8,19], who showed that the deferent duct was covered by the testicular capsule but separated from the

testis by dense connective tissue and its lumen was lined by a cylindric or columnar pseudostratified epithelium, surrounded by a layer of smooth muscle fibres arranged in a circular direction and an outer layer composed of dense and irregular connective tissue.

## 5. Conclusions

The structure of the reproductive tract of *Paleosuchus palpebrosus* is histologically similar to that of other caiman species. A peculiarity of the germinal epithelium is groups of clonic spermatocytes in the basal compartment of the seminiferous tubule. The epididymis is very small compared to the size of the testis and the adrenal gland is found in the dorsomedial portion of the testis.

## Author Contributions

- Teresinha Inês de Assumpção: Conceptualization, Methodology, Investigation, Resources, Data Curation, Writing, Supervision, Project administration.
- Adrielly Julien Silva Lopes: Methodology, Investigation, Data Curation
- Lilja Fromme: Conceptualization, Methodology, Investigation, Data Curation, Writing
- André Luís Quagliatto Santos: Methodology, Investigation, Resources, Writing

## Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## ARTICLE

# The Growth Parameters and Mortalities of Five Species of *Synodontis* in the Lower River Benue at Makurdi

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## ABSTRACT

The growth parameters and mortalities of five species of *Synodontis* in the lower river Benue at Makurdi, Benue State were studied from January, 2016 to December, 2018. The asymptotic length ( $L_{\infty}$ ) calculated for the five species ranged from 18.80cm in *S.clarias* females to 37.04cm in *S.membranaceus* females. The  $t_0$  values were all negative in the combined sexes of *S.clarias*, *S.omias*, *S.gambiensis* and *S.membranaceus*. In both combined sexes of *S.membranaceus* and *S.schall*, the  $t_0$  values were positive. The growth rate (K) was low in *S.clarias* and *S.omias* (0.301 - 0.497, 0.171 - 0.310) respectively and higher in *S.membranaceus* (0.310 - 0.640), *S.schall* females (0.430 - 0.580); *S.schall* males (0.573), *S.gambiensis* (0.500 - 0.571). Growth performance index ( $\phi'$ ) was 2.212 in *S.gambiensis* and 2.946 in *S.schall* combined. Natural Mortality (M) ranged from 0.5422 in *S.omias* females to 1.3340 in *S.membranaceus* males. Fishing Mortality (F) was 0.8214 in *S.omias* combined and 3.0934 in *S.membranaceus* females. Total mortality (Z) ranged from 1.52 in *S.omias* combined to 4.078 in *S.membranaceus* combined. Mean Exploitation (E) ratios was 0.61 in *S.clarias*, 0.64 in *S.omias*, 0.53 in *S.gambiensis*, 0.70 in *S.membranaceus*, and 0.66 in *S.schall*. The rate at which these species survived in the River was low (from 0.147, in *S.omias* combined, to 1.482 in *S.membranaceus* combined).

## 1. Introduction

The growth of a fish like any other organism results from its food consumption and assimilation<sup>[1]</sup>. The growth of fishes can be affected by many factors which include food availability (amount and quality), population density, food consumption, genetic composition, photoperiods, and length of growing periods. Temperature affects the rate of metabolism of fishes as they are ectothermic. Changes in temperature from season to season affect the growth rate<sup>[2,1]</sup>. Other factors include salinity, amount of dissolved oxygen, the physiological condition of the fish, parasitism,

competition, predation and presence of adequate anions and cations in the water body<sup>[3-5]</sup>.

Age determination and growth are important aspects of fisheries management<sup>[6,7]</sup>. The study of factors influencing fish growth which include maturation, migration, food and feeding habits, spawning frequency, stock responses to changes in habitats are essential to the fishery biologist<sup>[8]</sup>.

Fish growth can be used to describe the status of a given fish population as well as for predicting the potential yield of the fishery. It also facilitates the assessment of production, stock size and recruitment to adult stock<sup>[9]</sup>. According to<sup>[10]</sup>, fish growth can be used to measure

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characteristics of fish stocks, species and is fundamental to the understanding of the life histories of demographics, ecosystem dynamics and sustainability of fisheries. The Walford plot analysis for fish is used to determine the Von Bertalanffy growth parameters for different sexes of fishery stock in a given body of water. However, <sup>[10]</sup> observed that information on growth parameters of many tropical fishes were either scarce, few or no records were available at all in Fishbase. There are no published works on the determination of true asymptotic length ( $L_{\infty}$ ) of *Synodontis* species in this River. This research therefore, focus on the determination of the true asymptotic length ( $L_{\infty}$ ) and length-at-age ( $L_t$ ) of five *Synodontis* species in River Benue at Makurdi.

## 2. Materials and Methods

### 2.1 The Study Area

The study area was the Lower Benue River at Makurdi. The Lower Benue River is part of the Benue River contained within Benue state of Nigeria to the confluence at Lokoja <sup>[11]</sup>.

Benue state is located in the middle belt area of Nigeria. It is located between Latitudes 6° 25'N and 8° 8'N and Longitudes 7° 47'E and 10°E. (<http://www.absoluteastronomy.com>).

River Benue is an inland water body which originates in the Adamawa Plateau of Northern Cameroun as the Benoue and flows to the west across east-central Nigeria as the Benue. It extends from the Adamawa mountains of Cameroun some 500 km beyond the Nigerian territory where it flows west through the town of Garoua and Lago Reservoir into Nigeria south of the Mandara mountains, and through Jimeta, Ibi and Makurdi before it joins with River Niger at Lokoja, about 1400 km long <sup>[11]</sup>.

Makurdi town, the capital of Benue state and the headquarters of Makurdi Local Government Area are situated on both banks of this River within the flood plain. The town is between Latitudes 7° 38'N and 7° 50'N and Longitudes 8° 24'E and 8° 38'E. Its mean elevation is 92 meters above sea level <sup>[11]</sup>.

The flood plain stretches for as much as 187 km with a width ranging between 3-10 km <sup>[12]</sup>. The flood plain is flooded mainly along the south bank. The large area of flood plain is flooded every year in the rainy season when it rises extending to long distances along the shores <sup>[12]</sup>. The flooded plain forms breeding grounds for fish species.

At bankfull, the flooded plain can cover 129,000 ha, but when flooded, the area can rise to 310,000 ha. <sup>[11]</sup> observed that the highest water levels of the River were in August-September and the lowest were in March and April.

### 2.2 Length at Age

The length at age of the five species of *Synodontis* was calculated using the <sup>[13]</sup> growth model of in the LSA/Fi-SAT computer programmes using the formula below:

$$L(t) = L_{\infty}(1 - e^{-k(t-t_0)})$$

Where  $L(t)$  = length at age  $t$

$L_{\infty}$  = the asymptotic or maximum attainable length, assuming fish growth is indefinite.

$k$  = the rate at which the asymptotic length is approached.

$t_0$  = the time in the growth history of fish at which the fish would be zero sized

$e$  = exponential and

$t$  = age in years.

Growth curves were fitted to the Von-Bertalanffy model and tested with Walford plots to obtained  $L_{\infty}$  trials <sup>[7]</sup>.

The gradient  $\text{Log}_e L_{\infty} + Kt_0$ , is the intercept, then  $t_0 = \frac{Y - \text{intercept} - \text{Log}_e L_{\infty}}{K}$ .

$K$  is the gradient of the line of best fit from the trial plots of  $\text{Log}_e (L_{\infty} - L_t)$  against age.

### 2.3 Mortality

#### 2.3.1 Natural Mortality (M)

Natural mortality ( $M$ ) for the five species of *Synodontis* was estimated using empirical formula below:

$$\text{Log}_{10} M = -0.0066 - 0.279 \text{Log}_{10} L_{\infty} + 0.6543 \text{Log} K + 0.463 \text{Log}_{10} T \quad [17]$$

Where,

$L_{\infty}$  = the asymptotic length of the fish species in cm

$K$  = the Von Bertalanffy parameters,

$T$  = the mean environmental temperature which was 27.6 in this study.

#### 2.3.2 Total Mortality (Z)

Total Mortality ( $Z$ ) was calculated on FiSAT II from the lengths of fish samples using the formula below:

$$Z = K [L_{\infty} - L_{\text{mean}}] / [L_{\text{mean}} - L']$$

where

$Z$  = Beverton and Holt function,  $K$  = curvature parameter of the VBGF,  $L_{\infty}$  = asymptotic length of fish,  $L_{\text{mean}}$  = mean length of fish samples and  $L'$  = cut off or lower limit of the smallest length class.

#### 2.3.3 Growth Performance Index

The growth performance index  $\Phi'$  was calculated using the formula below:

$$\Phi' = \text{Log} K + 2 \text{Log} L_{\infty} \quad [14]$$

Where,  $K$  and  $L_{\infty}$  are parameters of VBGF.

### 3. Results

The growth parameters and mortalities of the five species of *Synodontis* as obtained from the Walford and Ricker plots and estimations were shown in Table 1.

The growth rates (K) of the five species was generally low in *S.clarias* and *S.omias* in male and female sexes. Growth rate of 0.640 was the highest in *S.membranaceus* males, followed by 0.580 in *S.schall* females, then 0.573 in *S.schall* males and 0.571 in *S.gambiensis* males.

The growth performance index ( $\phi$ ) of all the species ranged between 2.212 in *S.gambiensis* females to 2.946 in *S. schall* males and females. In *S. clarias*, the growth performance index ( $\phi'$ ) in length was 2.453, 2.321 and 2.693 in females and males sexes respectively. In *S.omias*, the values were 2.440 and 2.321 in the females and males sexes respectively. *S.gambiensis* had the growth performance index of 2.212 and 2.374 for females and males respectively. The growth performance index was 2.562 in *S.membranaceus* females and 2.689 for the males. The growth performance of 2.756 was observed in *S.schall* females and 2.64 for the males. The growth performance was highest in *S.schall* followed by *S.membranaceus* and lowest in *S.gambiensis*.

The asymptotic lengths ( $L_{\infty}$ ) for the five species ranged between 18.80cm in *S.clarias* females to 37.04cm in *S.membranaceus* females. In *S.clarias*, the  $L_{\infty}$  ranged between 18.80cm -28.50cm. *S.omias* had the range of 28.0cm - 28.50cm, *S.gambiensis* 23.0cm - 23.70cm, *S.membranaceus* 28.05cm-37.04cm and *S.schall* 28.05cm - 30.05cm (Table 1).

The hypothetical age at which length is zero ( $t_0$ ) was negative in *S.clarias*, *S.omias*, *S.gambiensis* and *S.membranaceus* and positive in both sexes of *S.membranaceus* and *S.schall*.

The natural mortality (M) was 0.5422 in *S.omias* females and 1.3340 in *S. membranaceus* males. Natural mortalities were higher in *S.schall* and *S.gambiensis* than all the other species (Table 1).

The total mortality (Z) of the five species ranged between 1.5120 in *S.omias* combined to 4.078 in *S.membranaceus* females. The total mortality of the five species was generally high (Table 1).

The fishing mortality (F) for the five species ranged between 0.8214 in *S.omias* combined to 3.0934 in *S.membranaceus* females. The Fishing mortality for the species was higher than natural mortality (Table 1).

**Table 1.** Growth Parameters and Mortalities of the Five Species of *Synodontis* in the Lower River Benue.

Species	Sex	Growth Parameters						Mortality Coefficients				
		$L_{\infty}$ (cm)	K (1/yr)	$t_0$ (cm)	$L_t - L_{t_0}$ (cm)	Back-calculated	$\phi$	S	M (1/yr)	F (1/yr)	Z (1/yr)	E(F/Z)
<i>S.clarias</i>	♀	18.80	0.385	-0.723	7.85	7.46	2.453	1.232	0.7403	1.1427	1.883	0.76
	♂	28.50	0.301	-0.440	5.50	8.55	2.331	0.961	0.8242	1.2238	2.048	0.60
	Comb.	23.00	0.497	-0.090	6.55	8.18	2.693	0.659	1.2036	1.0844	2.288	0.47
<i>S.omias</i>	♀	28.05	0.171	-0.706	4.43	9.16	2.440	0.287	0.5422	1.6518	2.194	0.75
	♂	28.00	0.310	-0.419	8.50	8.66	2.321	0.375	0.8188	1.3882	2.207	0.63
	Comb.	28.50	0.240	-0.542	10.90	9.82	2.649	0.147	0.6906	0.8214	1.512	0.54
<i>S.gambiensis</i>	♀	23.50	0.500	-0.714	9.84	6.38	2.212	0.389	1.1774	1.0096	2.187	0.46
	♂	23.70	0.571	-0.464	9.84	6.43	2.374	0.279	1.3070	2.1540	3.461	0.62
	Comb.	23.00	0.523	-0.641	9.53	8.61	2.445	0.556	1.2444	1.3376	2.582	0.52
<i>S.membranaceus</i>	♀	37.04	0.320	0.344	8.90	9.86	2.562	0.945	0.9846	3.0934	4.078	0.77
	♂	28.25	0.640	0.890	12.65	9.53	2.689	1.466	1.3340	1.9850	3.319	0.60
	Comb.	35.10	0.310	-0.032	6.70	8.46	2.869	1.482	0.8312	2.2108	3.042	0.73
<i>S.schall</i>	♀	30.05	0.580	0.830	9.10	7.78	2.756	0.431	1.2351	2.2029	3.438	0.64
	♂	30.00	0.570	0.332	9.50	7.55	2.645	0.327	1.2267	2.3553	3.582	0.66
	Comb.	28.50	0.430	0.000	7.70	7.71	2.946	0.614	1.0453	2.3597	3.405	0.69

$L_{\infty}$  = Asymptotic Length, K = Growth curvature,  $t_0$  = length at time 0,  $L_t - L_{t_0}$  = Change in length,  $\phi$  = Growth Performance Index, S=Survival Rate, M = Natural Mortality, F = Fishing Mortality, Z = Total Mortality, E= Exploitation Ratio

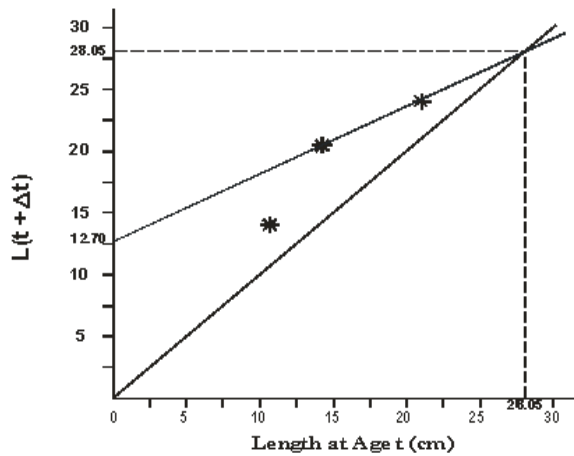


Figure 1a. WALFORD PLOT FOR DETERMINATION OF  $L_{\infty}$  FOR *S.membranaceus* (MALES)

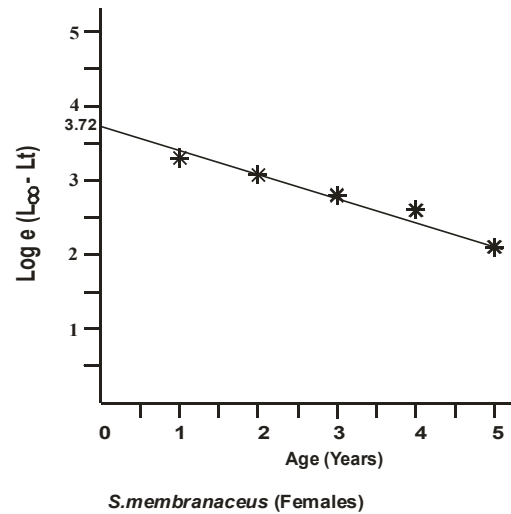


Figure 2b. RICKER PLOT FOR DETERMINATION OF TRUE  $L_{\infty}$

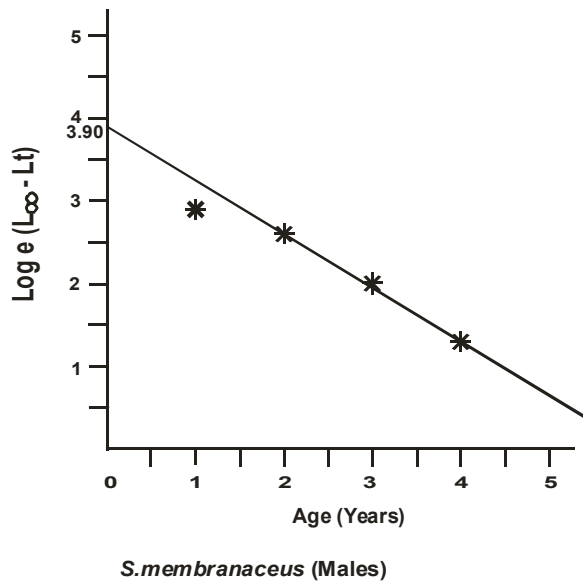


Figure 1b. RICKER PLOT FOR DETERMINATION OF TRUE  $L_{\infty}$

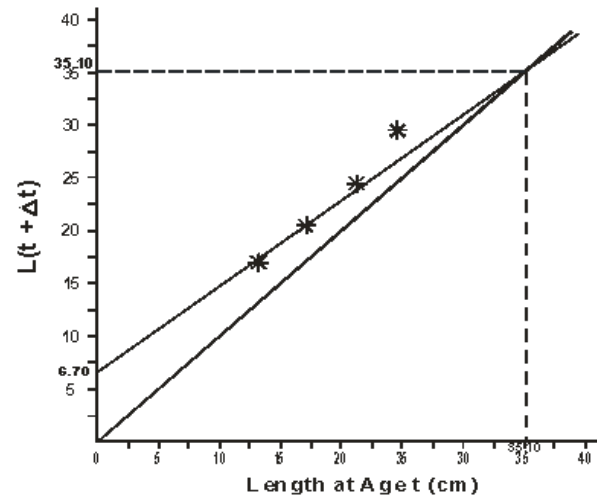


Figure 3a. WALFORD PLOT FOR DETERMINATION OF  $L_{\infty}$  FOR *S.membranaceus* COMBINED

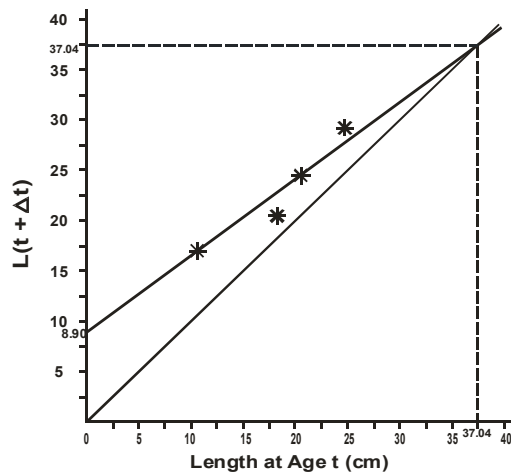


Figure 2a. WALFORD PLOT FOR DETERMINATION OF  $L_{\infty}$  *S.membranaceus* (FEMALES)

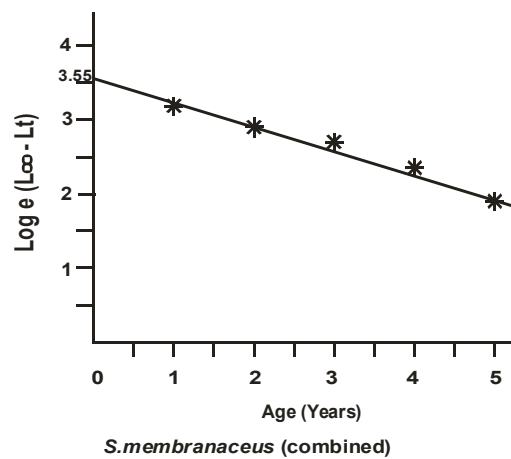


Figure 3b. RICKER PLOT FOR DETERMINATION OF TRUE  $L_{\infty}$



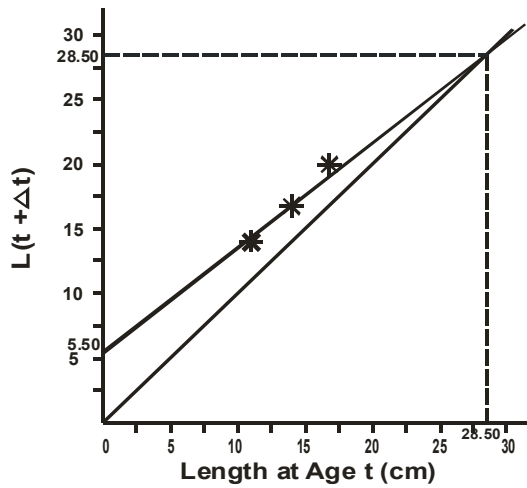


Figure 4a. WALFORD PLOT FOR DETERMINATION OF  $L_{\infty}$  FOR *S. clarias* (MALES)

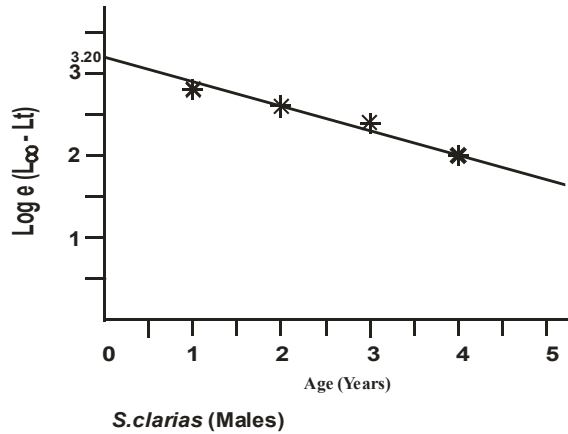


Figure 4b. RICKER PLOT FOR DETERMINATION OF TRUE  $L_{\infty}$

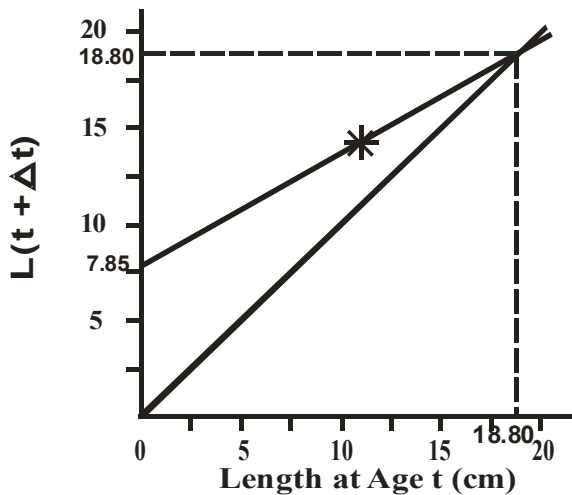


Figure 5a. WALFORD PLOT FOR DETERMINATION OF  $L_{\infty}$  FOR *S. clarias* (FEMALES)



Figure 5b. RICKER PLOT FOR DETERMINATION OF TRUE  $L_{\infty}$

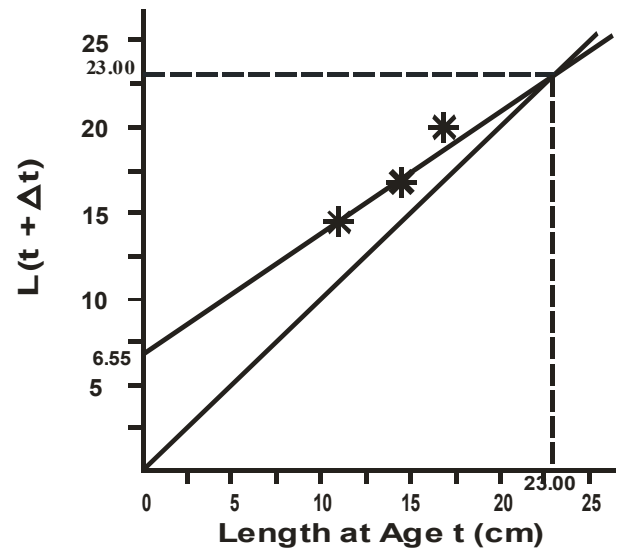


Figure 6a. WALFORD PLOT FOR DETERMINATION OF  $L_{\infty}$  FOR *S. clarias* combined

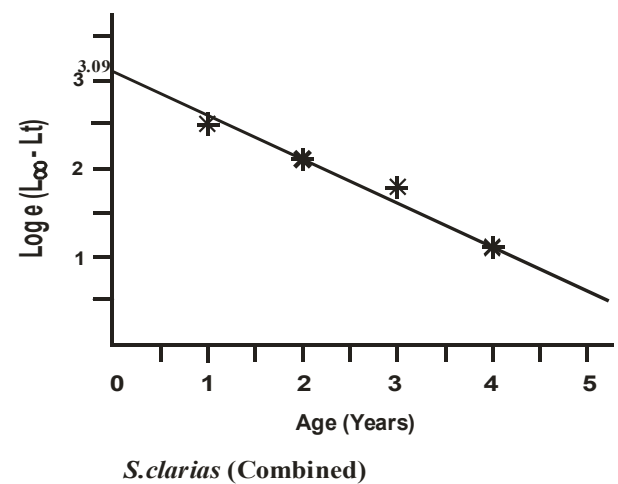


Figure 6b. RICKER PLOT FOR DETERMINATION OF TRUE  $L_{\infty}$

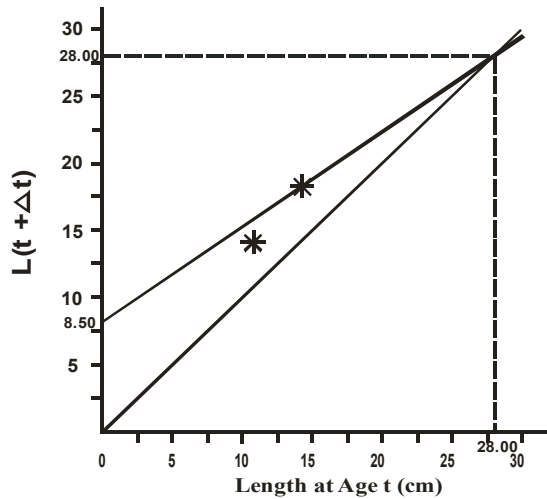


Figure 7a. WALFORD PLOT DETERMINATION OF  $L_{\infty}$  FOR *S.omias* (MALES)

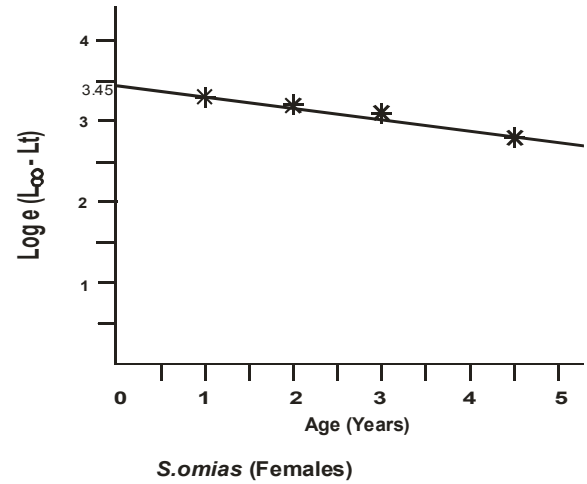


Figure 8b. RICKER PLOT FOR DETERMINATION OF TRUE  $L_{\infty}$

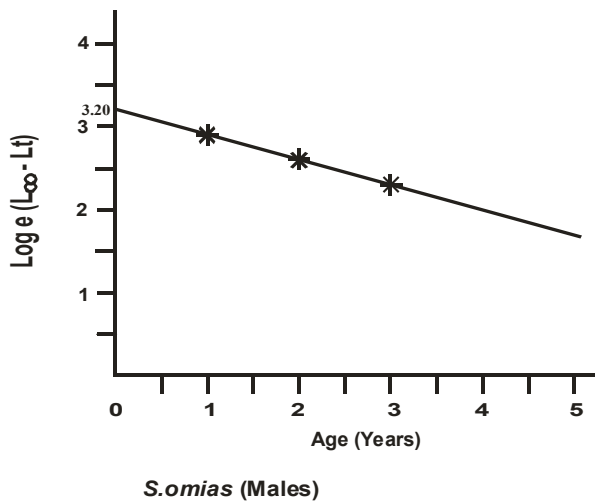


Figure 7b. RICKER PLOT FOR DETERMINATION OF TRUE  $L_{\infty}$

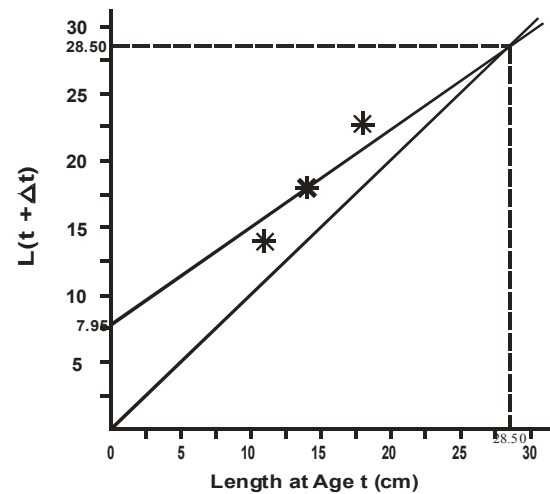


Figure 9a. WALFORD PLOT FOR DETERMINATION OF  $L_{\infty}$  FOR *S.omias* COMBINED

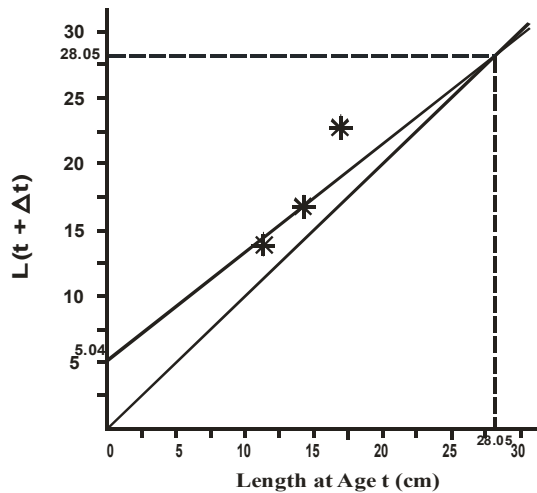


Figure 8a. WALFORD PLOT FOR DETERMINATION OF  $L_{\infty}$  FOR *S.omias* (FEMALES)

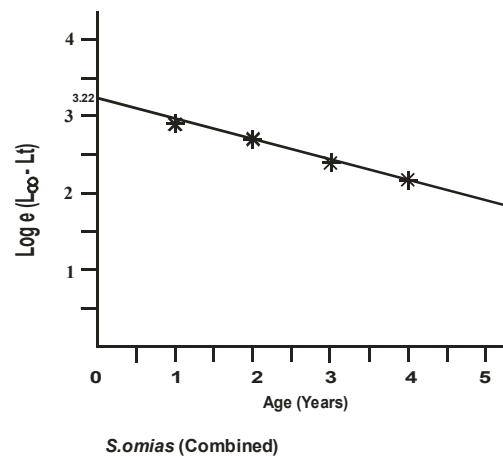
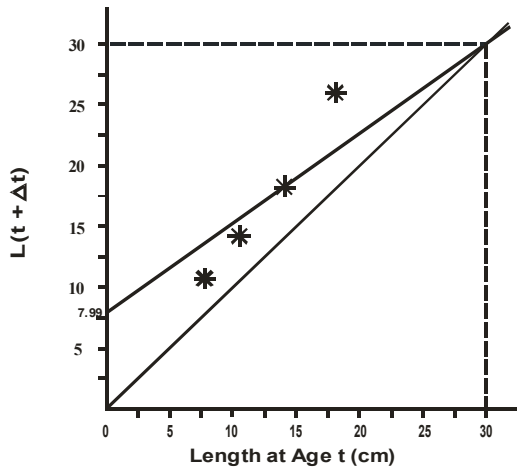
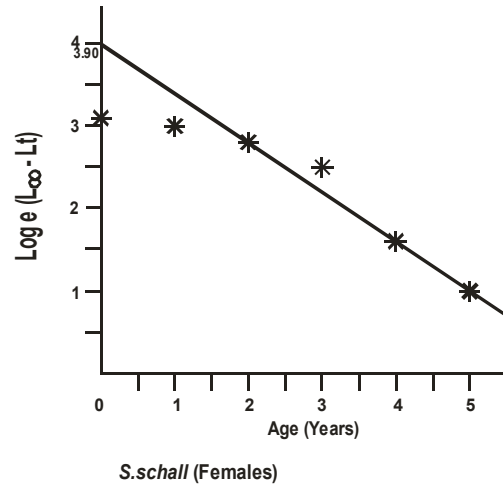


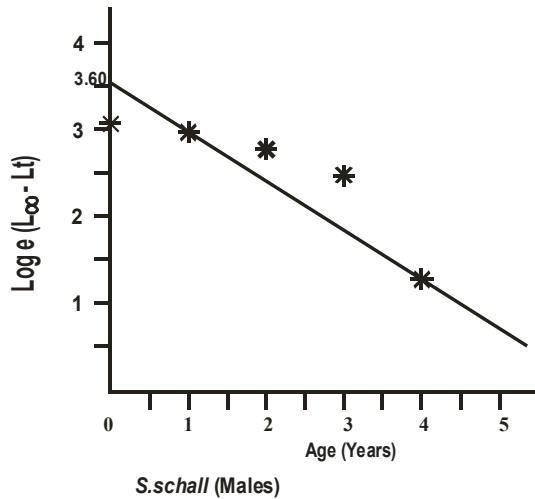
Figure 9b. RICKER PLOT FOR DETERMINATION OF TRUE  $L_{\infty}$



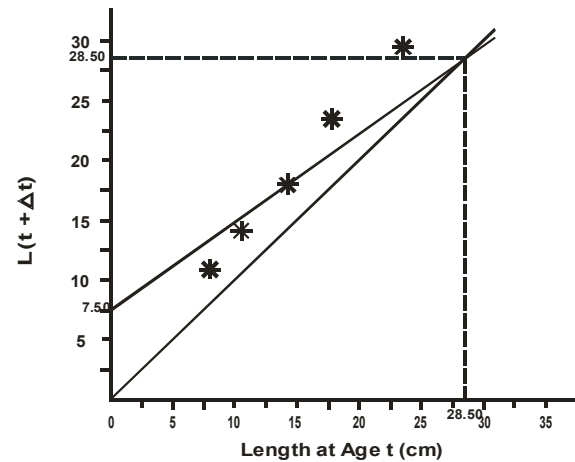
**Figure 10a:** WALFORD PLOT FOR DETERMINATION OF  $L_{\infty}$  FOR *S.schall* (MALES)



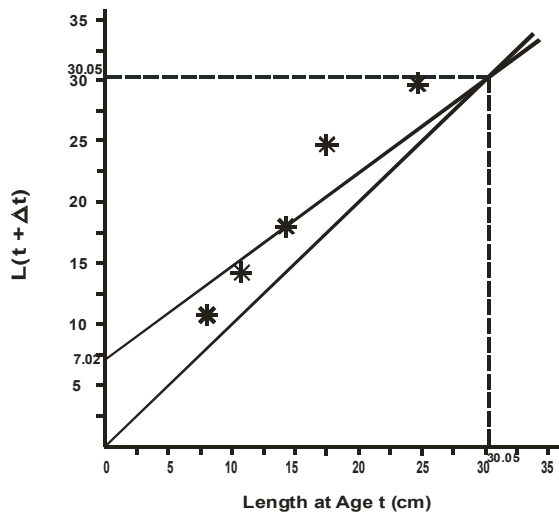
**Figure 11b:** RICKER PLOT FOR DETERMINATION OF TRUE  $L_{\infty}$



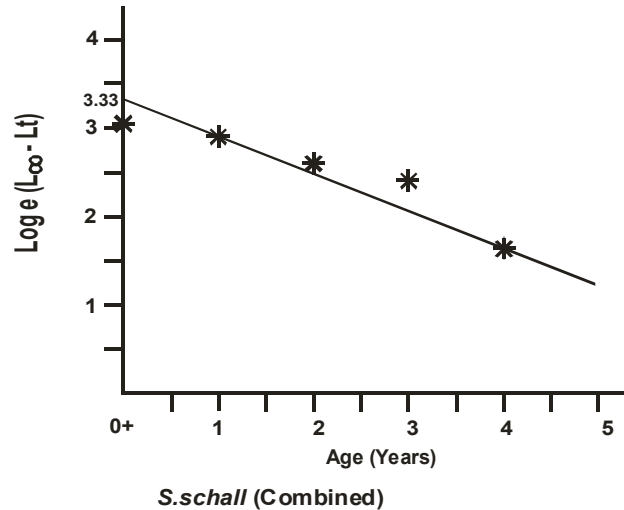
**Figure 10b:** RICKER PLOT FOR DETERMINATION OF TRUE  $L_{\infty}$



**Figure 12a:** WALFORD PLOT FOR DETERMINATION OF  $L_{\infty}$  FOR *S.schall* COMBINED



**Figure 11a:** WALFORD PLOT FOR DETERMINATION OF  $L_{\infty}$  FOR *S.schall* (FEMALES)



**Figure 12b:** RICKER PLOT FOR DETERMINATION OF TRUE  $L_{\infty}$

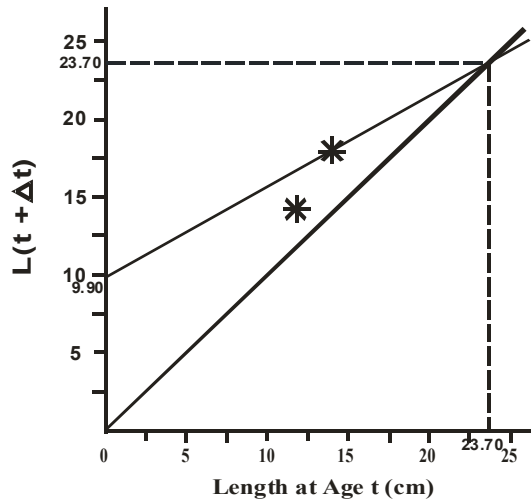


Figure 13a. WALFORD PLOT FOR DETERMINATION OF  $L_{\infty}$  FOR *S.gambiensis* (MALES)

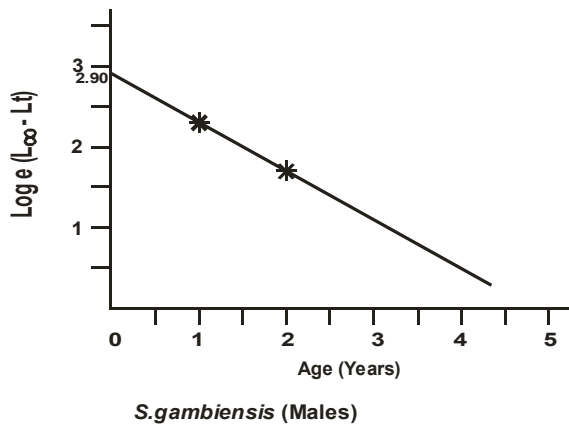


Figure 13b. RICKER PLOT FOR DETERMINATION OF TRUE  $L_{\infty}$

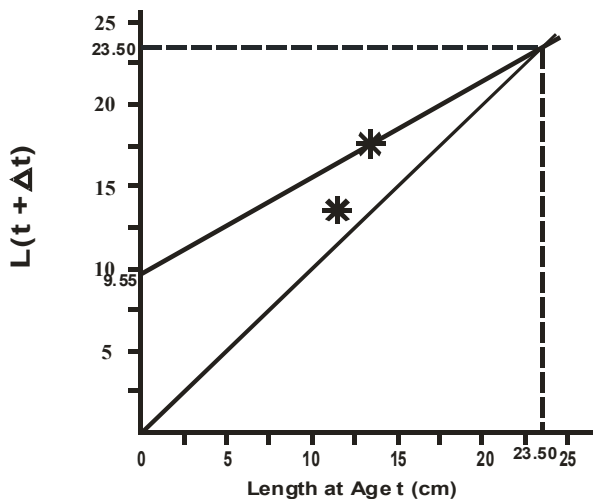


Figure 14a. WALFORD PLOT FOR DETERMINATION OF  $L_{\infty}$  FOR *S.gambiensis* (FEMALES)

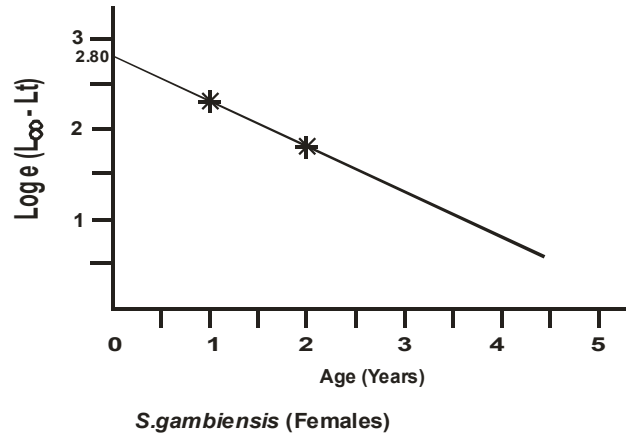


Figure 14b. RICKER PLOT FOR DETERMINATION OF TRUE  $L_{\infty}$

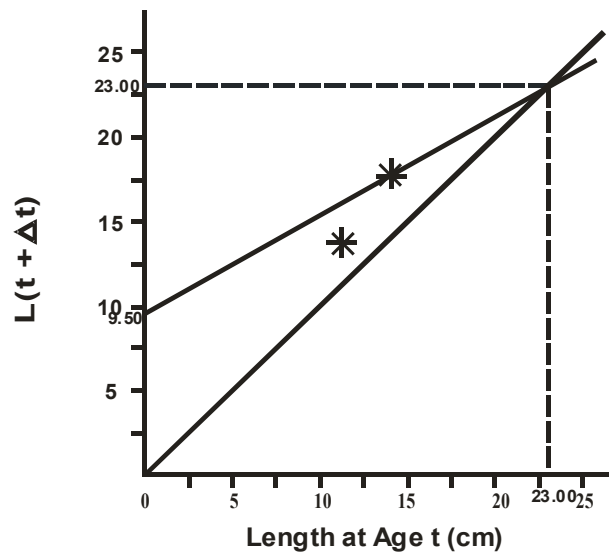


Figure 15a. WALFORD PLOT FOR DETERMINATION OF  $L_{\infty}$  FOR *S.gambiensis* COMBINED

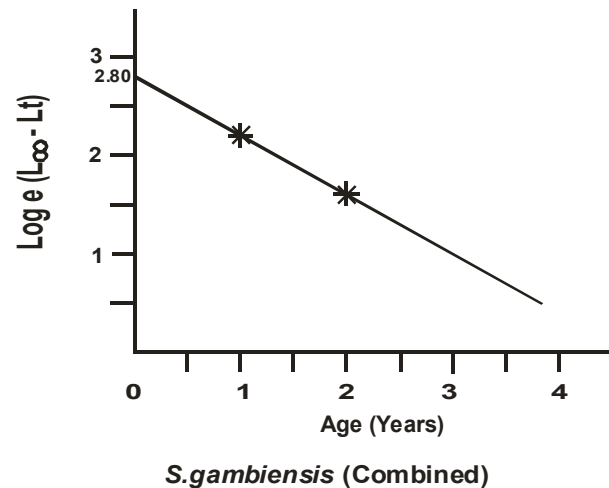


Figure 15b. RICKER PLOT FOR DETERMINATION OF TRUE  $L_{\infty}$



#### 4. Discussion

The asymptotic lengths ( $L_{\infty}$ ) calculated for the five species ranged from 18.80 cm in *S.clarias* females to 37.04 cm in *S.membranaceus* females. The highest  $L_{\infty}$  of 28.50 cm was observed in *S.clarias* males, while the maximum length of fish of this species was 30.0cm also a male. In *S.omias* the highest  $L_{\infty}$  of 28.50 cm was observed in the combined, the maximum length of 22.8 cm was found in female. The highest  $L_{\infty}$  of *S.gambiensis* was 23.70 cm in the males and the maximum length of 18.1cm was still in male. The  $L_{\infty}$  of *S.membranaceus* was 37.04 cm in the females where as the maximum length was 28.8 cm in female. The highest  $L_{\infty}$  of female *S.schall* was 30.05 cm with the maximum length of 30.40 cm. The  $L_{\infty}$  in *S.omias*, *S.gambiensis* and *S. membranaceus* were higher than the maximum length of other species of Synodontis under this research. This means that *S.omias*, *S.gambiensis* and *S. membranaceus* had a tendency to live long and grow bigger if the physico-chemical parameters of River Benue are favourable.

The results of this study were different from <sup>[15]</sup> which computed the  $L_{\infty}$ , back calculated, and integrated methods in *S.schall* as 50.4 cm, 49.5 cm and 50.0 cm respectively. Again, the results of this study is different from <sup>[16]</sup> who observed the  $L_{\infty}$  of 62.74cm, 64.24cm and 63.45 cm for males, females and combined sexes of *S.schall* respectively in Egypt at Gizza. Further, this study is similar to <sup>[17]</sup> who reported the  $L_{\infty}$  of *S.schall* to be 38.7 cm, *S.clarias* 35.56 cm and *S.membranaceus* 43.8 cm from the Lower Nun River, Niger Delta.

According to <sup>[18]</sup>, growth of fishes differed from species to species and from stock to stock even within the same species as a result of different environmental conditions. <sup>[19]</sup> posited that the maximum size attained in fishes was generally location specific. A study conducted by <sup>[20]</sup> revealed that the differences in the maximum size of *Chrysichthys nigrodigitatus* in the Lower Nun River was due to high fishing pressure, environmental pollution and degradation. <sup>[10]</sup> observed that the variations in the growth parameters of some of the species sampled in the central Amazon appeared to accord with the high degree of environmental variations in the ecosystem. The smaller sizes obtained in this river could also be attributed to fishing pressure and environmental pollution and degradation.

In this study, *S.clarias*, *S.omias*, *S.gambiensis* and *S.membranaceus* had negative hypothetical age at which length was zero ( $t_0$ ) and positive in *S.membranaceus* male and female and *S.schall* male and female. The results of this study was different from the work of <sup>[21]</sup> who observed a negative  $t_0$  of -1.543 in *O.niloticus* from a tropical shal-

low Lake in Mexico. Similarly the result of this study was different from <sup>[22]</sup> who obtained a negative  $t_0$  of -3.93 for *Pellnula leonensis*. The results of this study was similar to the work of <sup>[23]</sup> who reported positive  $t_0$  values of 0.12 for *O.niloticus* males, 0.66 for females; 0.30 for *S.galilalaeus* males, 0.86 for females and 0.50 for *B.bayad* males, 0.85 for females.

The growth rates (K) of the five species was generally low in *S.clarias* and *S.omias* in male and female sexes. The results of this study showed that the rate at which the males and females of *S.schall* approach  $L_{\infty}$  is similar. Whereas in *S.membranaceus*, *S.gambiensis* and *S.clarias*, the males had a faster growth rate than the females. The findings of this study was similar with that of <sup>[21]</sup> and <sup>[24]</sup>.

Growth performance indices of the studied species were very good (Table 1). The results of this work on Growth performance index ( $\phi'$ ) was in conformity with <sup>[9]</sup> who reported the length performance index ( $\phi'$ ) of 2.63 in *Hemisyndontis membranaceus*. More so, the results was similar to <sup>[24]</sup> who observed the length performance indices of 2.71 in *S.schall*, 2.63 in *S.membranaceus*, and 3.23 in *S.clarias*. Further, the result was similar to <sup>[19]</sup> who reported the growth performance indices in length of *S.schall* as 2.689, 2.692 and 2.709 in the males, females and combined sexes respectively. In addition, this research was similar to <sup>[28]</sup> who observed the performance index of 2.62 and 2.51 in *Schilbe mystus* in Asejire and Oyan Lakes respectively. <sup>[30]</sup> stated that  $L_{\infty}$ , K and  $t_0$  were highly related or correlated as K depended on the values of  $L_{\infty}$  and  $t_0$ . With all else held constant, K would be larger for smaller values of  $L_{\infty}$ .

The results of this study showed high mortality for all the species. Their Natural mortality (M) ranged from 0.5422 in *S.omias* females to 1.3240 in *S.membranaceus* males. Fishing mortality (F) ranged from 0.8214 in *S.omias* combined to 3.094 in *S.membranaceus* females. Total mortality (Z) of the studied fish was low. The results of this study were similar to that of <sup>[20]</sup> who observed the total mortality (Z) for *S.schall* and *S.clarias* as 2.1, 1.5 in *S.membranaceus*. In addition, this study was similar to <sup>[25]</sup> who observed the Z of 2.54, M of 0.88 and F of 1.66 in *Clarias gariepinus* and <sup>[23]</sup> who obtained low values of mortalities in *S.schall*. This study is different from <sup>[28]</sup> who obtained the total mortality (Z) of 0.61-1.25 for some fish species in River Katsin-Ala. Again, this work is different from <sup>[28]</sup> who reported Z, M and F as 4.03, 2.77 and 1.26 respectively in *Pellonula leonensis*. Fish mortality in this study could be attributed to factors such as fish age, fish predation, environmental stress, parasites, diseases and fishing activities by man <sup>[22,30,9]</sup>.

## 5. Conclusions

The studied species had potentials to grow more if the existing conditions in the river were favourable. However, among the studied species the growth of *S.gambiensis* was very poor while that of *S.schall* and *S.membranaceus* was good.

The values of the asymptotic lengths obtained in this study from the Walford plots were appropriate for these species in the River Benue.

In the species studied, the hypothetical age at which length is zero ( $t_0$ ) were all negative in *S.clarias*, *S.omias*, *S.gambiensis* and *S.membranaceus* combined and positive in *S.membranaceus* male and female and *S.schall* male, female and combined.

## Recommendation

Based on the findings of this research which showed that growth performance of *S.gambiensis*, *S.clarias* and *S.omias* in the river was very poor, I recommend that a minimum mesh size of 7.5cm should be enforced to allow these species to reproduce at least once before they are captured.

## Acknowledgments

We are most grateful to the laboratory technicians, Mr. Waya, J. I.; Mrs. Shiriki. D.; Mrs. Tyona E.; Mr. Adanu, P. and all the other Laboratory Assistants for their support during the practical work. We are also grateful to Mr. Atooyough and Mr. Richard Bul who used to drive us to the market landing sites to purchase the fishes and go for water sampling. We appreciate you and may God bless you all.

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ARTICLE

## Habitat Suitability Index Modelling for Bluebull (*Boselaphus tragocamelus*) in Pench Tiger Reserve, M.P. India

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ABSTRACT

The habitats for the wild animals are shrinking due to the clearance of forests for agriculture and industrialization. The idea of wildlife conservation begins with the identification of their acceptable habitat. Since this crucial information helps in the development and maintenance of the protected areas. The requirement of habitat varies with different landscapes. The bluebull (*Boselaphus tragocamelus*) is Asia's largest antelope, widespread throughout the northern Indian subcontinent. Peter Simon Pallasin (1766) described it as the only member of the genus *Boselaphus*. The Wildlife (Protection) Act of 1972 lists it as a Schedule III animal, while the IUCN lists it as Least Concern (LC). Our goal was to design a habitat appropriateness model for blue bull so that it could reduce the conflict with farming community due to crop damage. Model will be develop using RS & GIS technique to protect the species inside the Pench Tiger Reserve (77° 55' W to 79° 35' E and 21° 08' S to 22° 00' N) the central highlands of India. The satellite data from LANDSAT-8 of 4th April 2015, Path- 144, Row- 45, with a ground resolution of 30 meters, were collected from the USGS website. This satellite image was then transferred in image format to ERDAS IMAGINE 2013 for further analysis. The data from satellites were gathered and analysed. The purpose of the field survey was to gather information about the presence of various ungulates. A ground truthing exercise was also carried out. For data processing and GIS analysis, ERDAS IMAGINE 13 and Arc GIS 10 were used. Analytical Hierarchy Process (AHP) was used Factors were identified who were influencing the spatial distribution of the species for conservation planning. The linear additive model was used for HSI. The results show that 242 km<sup>2</sup> (29.48 percent) of Pench Tiger Reserve forest was recognized to be highly suitable for bluebull, while 196 km<sup>2</sup> (23.87 percent) was moderately suitable, 231 km<sup>2</sup> (28.14 percent) was suitable, 109 km<sup>2</sup> (13.28 percent) was least suitable, and about 43 km<sup>2</sup> (5.249 percent) of PTR was completely avoided by bluebull.

### 1. Introduction

The rate of deforestation has increased the change in forest cover and density to fulfill the demand of human needs, particularly agriculture expansion has shrunk the habitat for the wild species. Some habitat is protected in

the protected areas, and it is a need to understand the habitat requirement of the wild species. For the conservation and management of many species, it is essential to understand the relationship between their geographical distribution and their habitats<sup>[1]</sup>.

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The bluebull (*Boselaphus tragocamelus*) is Asia's largest antelope, distributed across the northern Indian subcontinent. Peter Simon Pallasin (1766) described it as the only member of the genus *Boselaphus*. According to the Wildlife (Protection) Act of 1972, the Bluebull is a Schedule - III animal and is classed as Least Concern (LC) by the IUCN. Conflicts with local farming communities over bluebull crop damage have generated conservation challenges, and we aimed to establish a habitat appropriateness model for bluebull to safeguard within protected areas.

Wildlife management encompasses both the conservation of wildlife species and the management of an entire ecosystem<sup>[2]</sup>. Remote Sensing (RS) and Geographic Information Systems (GIS), are powerful tools of geospatial technology that can be used to evaluate natural resources. It is possible with remote sensing data to monitor, regular, real-time evaluation and administration of large areas<sup>[5]</sup>. For a range of species, the US Fish and Wildlife Service has developed habitat suitability index (HSI) models, which are crucial for wildlife and habitat management<sup>[3,4]</sup>. The outcomes of such models are relatively simple and easy to understand, and they can be used to evaluate environmental impacts or prioritized conservation initiatives in a fast and cost-effective manner<sup>[5,6]</sup>.

We can evaluate the qualities of the habitat using this technique, which evaluates detailed ecological information about the species<sup>[4,7]</sup>. As discussed in the beginning section, numerous studies have been conducted to assess the habitat of diverse species in various parts of the world, as well as the Indian subcontinent<sup>[5,8-11]</sup>.

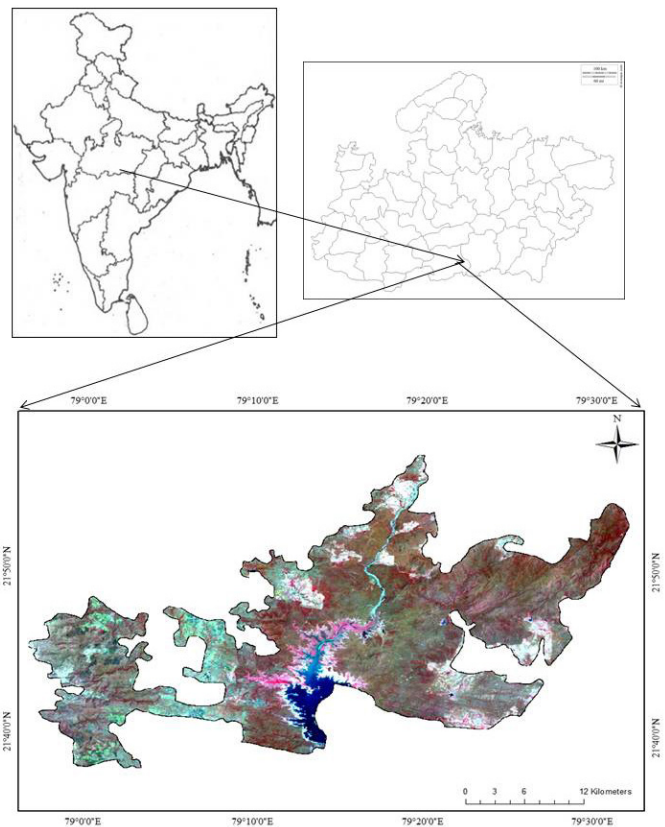
The Pench Tiger Reserve (PTR) in Central India represents tropical dry deciduous and tropical moist deciduous ecosystems. The quantitative and qualitative structure of vegetation types in PTR (2012) was initially described by Sankar et al. (2001)<sup>[12]</sup>, Areendran (2007)<sup>[13]</sup>, and Basu (2007)<sup>[14]</sup>. The impact of changes in the landscape on large mammal habitats in Pench Tiger Reserve in Madhya Pradesh, India was examined by Basu (2012)<sup>[14]</sup>. In his study, he evaluated the different types of vegetation of the PTR and generated the habitat suitability model for all the large mammals including spotted deer (*Axis axis*), Sambar (*Rusa unicolor*), wild boar (*Sus scrofa*), and carnivores like tiger (*Panthera pardus*), and dhole (*Cuon alpinus*).

Geospatial technology was applied in the study to anticipate the appropriate habitats for bluebull in the Pench tiger reserve due to its scale and utility.

## Study area

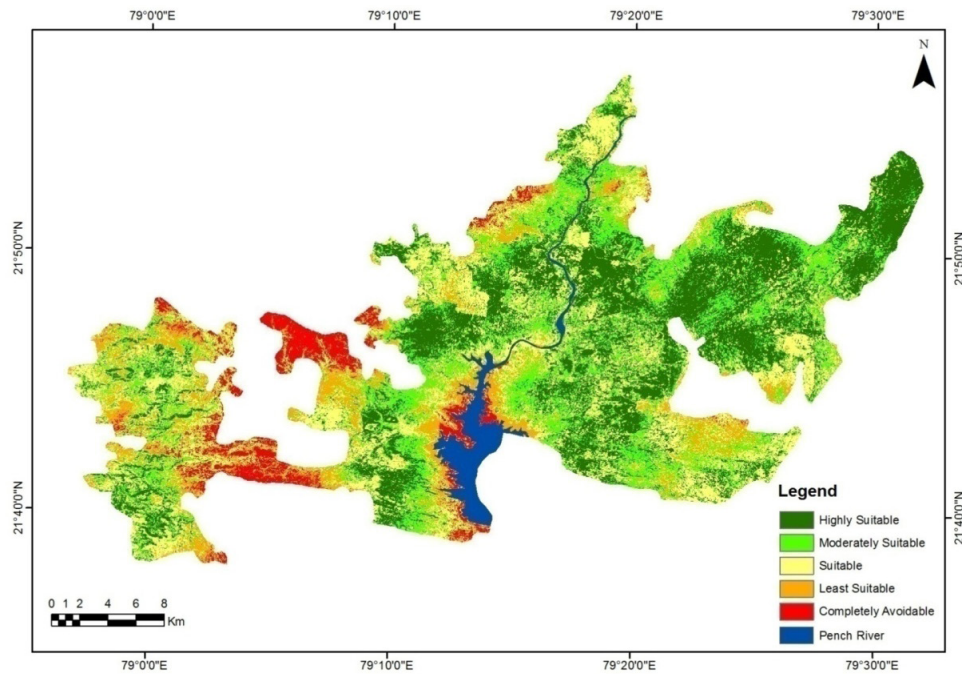
In the Satpura-Maikal ranges of Central India, the Pench Tiger Reserve in Madhya Pradesh is one of the

most prominent conservation areas. The Sanctuary was established in 1977, covering around 449.392 square kilometers. In 1983, an area of 292.857 sq km was declared as Pench National Park, while the remaining 118.473 sq km was recognized as Pench Sanctuary. In 1992, India's government designated 757.85 square kilometers, encompassing the National Park and Sanctuary, as the country's 19th Tiger Reserve (77° 55' W to 79° 35' E and 21° 08' S to 22° 00' N) (Figure 1).



**Figure 1.** Map of the Pench Tiger Reserve.

The extreme weather conditions prevail in the sanctuary. Temperature varies from 0°C in winters to 45°C in summer. The average annual rainfall is 1400 mm and seasons are categorized as summer, monsoon, post-monsoon, and winter. The PTR is a dry deciduous woodland, dominated by *Tectona grandis*, which includes *Tectona grandis*, *Anogeissus latifolia*, *Boswellia serrata*, *Sterculia urens*, and *Gardenia latifolia* with good predator and prey populations. The major carnivore species of the Reserve are leopard (*Panthera pardus*), Tiger (*Panthera tigris*), dhole (*Cuon alpinus*), small Indian civet (*Viverricula indica*), jungle cat (*Felis chaus*), striped hyena (*Hyaena hyaena*), sloth bear (*Melursus ursinus*), common palm civet (*Paradoxurus hermaphroditus*) and golden jackal (*Canis aureus*). Among herbivores, apart from sambar, the major species are chital (*Axis axis*) gaur (*Bos frontalis*),



**Figure 2.** Habitat suitability map for Bluebull

bluebull (*Boselaphus tragocamelus*), chowsingha (*Tetraceros quadricornis*), barking deer (*Muntiacus muntjac*), and sambar (*Rusa unicolor*).

## 2. Materials

### Satellite Data

It was obtained from the Landsat-8 OLI Data of 4<sup>th</sup> April 2015 and Digital Elevation Model ASTER DEM.

### Ancillary Data

Topographic maps and Digital boundary of the Pench Tiger Reserve obtained from the Forest Department.

### Software's

ArcGIS 10; ERDAS IMAGINE 2013.

## 3. Methodology

The aim of the study was to evaluate the appropriateness of the habitat suitability index for Bluebull in the Pench Tiger Reserve. For this, satellite data were analyzed. A field survey was carried out to collect evidence of the occurrence of various ungulates. Ground Truthing exercise was also carried out. The post-field effort included the establishment of a database and the modeling of the habitat appropriateness index. In the present study, ERDAS IMAGINE 13 and ArcGIS 10 software were deployed for processing data and GIS analysis.

## Factors influencing the habitat suitability

For establishing an effective conservation strategy and habitat suitability evaluating methods it is essential to identify factors determining species distribution<sup>[15]</sup>. Therefore, in the present study, parameters related to the distribution of ungulates from the previously published literature, statistical data from the field surveys, and suggestions from the conservation experts were determined and used in developing a model. These variables were operational, representative, and indicative for the analysis. Along with this these variables can provide the large amount of information needed for the assessment. These included slope, vegetation types, distance from water resource, and distance from the road were developed using topographic sheet based on some prior habitat evaluation analyses<sup>[16,17]</sup>. These variables were selected since they effectively represent the main features of appropriate habitats for ungulate species and can be used as variables in a habitat suitability model. After analyzing remote sensing imagery in the GIS domain, the aforesaid parameters were discovered<sup>[11]</sup>.

## Data collection and data processing

The satellite data from LANDSAT-8 of 4<sup>th</sup> April 2015, Path- 144, Row- 45, with a ground resolution of 30 meters, were collected from the USGS website. This satellite image was then transferred in image format to ERDAS IMAGINE 2013 for further analysis. An area of interest

(AOI) was identified using satellite data, and an FCC was created. ASTER (Advanced spaceborne thermal emission and reflection) data were downloaded from the USGS website, and a digital elevation model (DEM) was developed in the domain of ERDAS using this data. A slope layer was created using a DEM.

The topographic maps of the study area (55 O/2, 55 O/5, 55 O/6, 55 O/9, 55 K /13, and 55 K /14) were collected and collated from the Survey of India (SOI), Jabalpur, while the forest border map was obtained from the Pench Tiger Reserve, Seoni, Madhya Pradesh. These topographic maps were scanned and exported to ERDAS IMAGINE and were geo-referenced with a one-third pixel root mean square error and re-sampled using the nearest neighborhood methodology. For further analysis, the data was re-projected into the UTM-WGS 84 (Universal Transverse Mercator geodetic system-84 projection).

### Field Survey

From 2013 to 2015, a field study was conducted throughout the year (except during the monsoon). The Pench Tiger Reserve is comprising of major three ranges namely Kurai, Karmajhiri, and Gumtara. Pench Mowgli Sanctuary is formed by the Kurai range, whereas Pench National Park is formed by the Karmajhiri and Gumtara ranges. Because it was difficult to cover all three ranges, therefore, the Karmajhiri range was selected as an intensive study region for data gathering. And for the long-term study distinct habitats within the Karmajhiri range were chosen. In the selected habitats, 15 line transects were laid down with 10 circular plots at every 200m distance on each transect. Thus, in total 150 sampling plots were determined and species occurrence data were collected from these plots in different seasons for 2 years from 2013 to 2015 along with the GPS coordinates. Other data such as vegetation cover (tree cover, shrub cover), distance from the nearest human settlement, distance from waterhole were also collected during field surveys. Slopes and road distances data were obtained using remote sensing as they could be more accurately calculated and analyzed in a GIS framework <sup>[11]</sup>.

### Post-field analysis *Land use/Landcover*

The LULC map of the study area was generated using unsupervised classification via geo-coded FCC of LANDSAT 8 of April 4, 2015. For differentiating spectral reflectance of various objects, the ISODATA algorithm has been used. The spectral signatures of the spectral classes present in the image were identified using multi-spectral imaging. Land use/cover types were classified using un-

supervised clustering. Maximum probability classification was used since it has been shown to be particularly effective for land use/cover mapping. The accuracy assessment was followed by the unsupervised classification. The most crucial part of assessing the accuracy of maps is to analyse their reliability. The accuracy assessment approach was utilized in this study, and the software generated random points. Cohen's Kappa Statistics were used to assess accuracy <sup>[18]</sup>.

The accuracy of the map was tested by 100 randomly picked points. These places' land cover information was compared to classified maps. Cohen's Kappa Statistics (Khat coefficient) were used to assess accuracy <sup>[18]</sup>.

### Database preparation for Habitat Suitability Analysis

In the research region, vector layers of roads and water bodies were generated, as well as a distance map. LULC maps were created through unsupervised classification and categorized them into twelve classes. Slope and elevation maps were generated using 30 m resolution ASTER DEM data. The ecological importance of the habitats for the studied species were evaluated using these different layers. In order to determine the appropriateness of habitats for different ungulates in the PTR, a linear additive approach was used after the classification of all layers. An Analytical Hierarchy Process (AHP) devised by SAATY (1980, 1991) <sup>[19,20]</sup> was implemented to assess the weight allocated to distinct base layers.

AHP is an advanced approach in providing a methodology and reducing uncertainty in the evaluation of environment and regional sustainable management processes <sup>[20]</sup>. In this method, a numerical value is designated to each factor to determine its relative importance <sup>[21]</sup>. Each factor is then, evaluated against the other through assigning a relative dominating value between 1 and 9 to the intersecting cell in the construction of a pair-wise comparison matrix (Table 2). With the help of the relative scale measure mentioned in, a pair-wise comparison matrix was created for ungulates.

In the AHP process, the eigenvector plays a crucial role in the calculation. Each element of the eigenvector denotes the relative importance of the corresponding factor <sup>[21,22]</sup> i.e., When one factor is favoured over another, the eigenvector component of the favoured factor is larger. The eigenvalue and subsequent eigenvector are calculated using the sum/product method. The final weights calculated by AHP are utilised to create the HSI model.

In order to analyse the rationality of AHP, it is necessary to determine the degree of consistency that has been applied in producing the judgments. The consistency ra-



tio (CR) is an AHP consistency metric that evaluates the probability that matrix judgments were produced randomly<sup>[22,23]</sup>.

$$CI/RI = CR,$$

Where RI is an average consistency index based on the order of the matrix given by Saaty (1977), and Consistency Index (CI) is defined as:

$$CI = (\lambda_{\max} - n) / (n - 1)$$

Major Eigen value of the matrix is  $\lambda_{\max}$ , and the order of the matrix is n.

In the present study, parameters included forest types, distance from the road, distance from water, and elevation were investigated. Expert assessments were used to compare and weigh these factors. Table 1 shows the starting scales for each variable. For calculating the final weight MS Excel was used to create a pair-wise comparison matrix (Table 3). Then, in the GIS domain, the final weight [Consistency Index (CI)] calculated for each variable is employed with the HSI equation.

**Table 1.** Rank allocated to different layers

S.No.	Layers	Assigned Rank
1	Land use/Land cover	1
2	Distance from water body	2
3	Slope map	3
4	Distance from road	4

**Table 2.** Pair-wise comparison scale for Analytical Hierarchy Process preferences (Saaty 1980)

Weightage	Preferences
9	Extremely Preferred
8	Very Strongly to Extremely Preferred
7	Very Strongly preferred
6	Strongly to Very Strongly
5	Strongly Preferred
4	Moderately to Strongly Preferred
3	Moderately Preferred
2	Equally to Moderately preferred
1	Equally Preferred

**Table 3.** Synthesized Matrix of different layers for Bluebull

Class Name		1	2	3	4	Consistency Index (CI)
Vegetation	1	0.66	0.87	0.44	0.36	2.33
Distance from Water Bodies	2	0.07	0.10	0.44	0.36	0.97
Slope	3	0.13	0.02	0.09	0.21	0.45
Distance from Road	4	0.13	0.02	0.03	0.07	0.25

Habitat Suitability Index was calculated by multiplying the total of habitat suitability variables with AHP-determined weights<sup>[24,25]</sup>.

$$HSI = \sum_{i=1}^n W_i$$

Where,

$W_i$  = weight of the factor and

$I$  = I rating factor.

The final weights of the above-mentioned variables were assessed from the aforesaid analysis, and the HSI for various ungulates was calculated as follows:

$$HSI \text{ for Bluebull} = (2.33 \times LULCI) + (0.97 \times DFWI) + (-0.45 \times SI) + (0.25 \times DFRI)$$

Where,

HSI = Habitat Suitability Index,

LULCI= Land use/Land cover Index,

DFWI= Distance from water-body Index;

SI= Slope Index;

DFRI= Distance from road Index

## 4. Results and Discussion

Habitat Suitability Index for blue bull in Pench Tiger Reserve was generated using the linear additive model. On the basis of assigned suitability weightage, calculated output layers were reclassified. The reclassified raster layer displays areas based on their suitability categories for blue bull, as well as the area of each category, as indicated in Table 4.

**Table 4.** Area wise Habitat Suitability status for Bluebull in PTR

S. No.	Category	Area (Km <sup>2</sup> )	Percentage (% Area)
1	Highly Suitable	242	29.48
2	Moderately Suitable	196	23.87
3	Suitable	231	28.14
4	Least Suitable	109	13.28
5	Completely Avoidable	43	5.24
Total Area in Km <sup>2</sup>		821	100

## Habitat Suitability Index

The Habitat Suitability Index for blue bull in Pench Tiger Reserve was created using a linear additive model. Calculated output layers were classified depending on the suitability weightage assigned. The reclassified raster layer displays areas according to their appropriateness categories for blue bull, as well as the area of each category, as given in Table 4.

According to the HSI model developed for bluebull, 81.49 percent of PTR's forest area is appropriate for the species. It has been observed that a large portion of the



tiger reserve's northeastern region offers very appropriate habitats for the bluebull. In most cases, highly appropriate habitats are surrounded by either a moderately suitable or a suitable environment, creating a buffer zone. PTR's north-eastern part was also connected to other PAs, which may explain why its supports are suitable for bluebull. There are three major and a variety of small portions of appropriate land on the western side of the river. The species, however, avoid or avoids the areas near the reservoir. The HSI map shows that the species avoids the north-central area of the tiger reserve entirely. This could be due to the presence of human habitations and activities. The far western sector of the region, which is connected by a corridor, is significantly less appropriate than the eastern portion of PTR because to its uneven shape and proximity to human settlement and agricultural land. When we look at the narrow corridor, we can observe that bluebull totally avoiding it, which is most likely owing to manmade activity. In the Pench Tiger Reserve, however, the model indicates a clear HSI for bluebull. PTR is one among Madhya Pradesh's best-managed protected areas, with little disturbance. To develop a suitable habitat for the bluebull and other allied species, the less appropriate environment in the north-central area of the tiger reserve must be explored and strengthened.

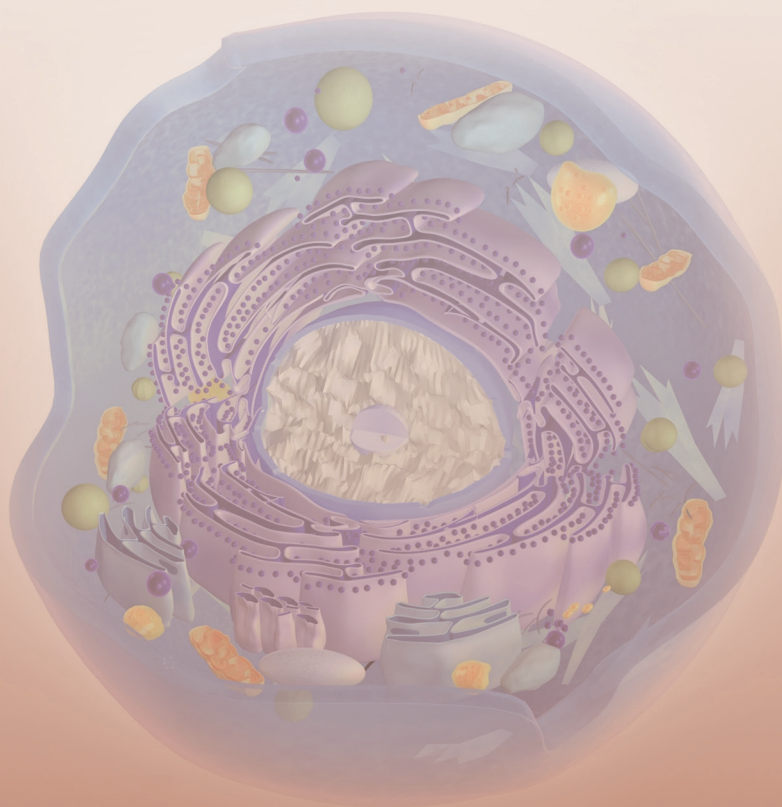
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