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ENAMEL HYPOPLASIA AS A STRESS SIGNAL: UNVEILING PALEOENVIRONMENTAL STRESS EPISODES IN THE SIWALIK'S SUIDS OF PAKISTAN FROM THE MIDDLE MIOCENE TO EARLY PLEISTOCENE

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ABSTRACT

Numerous paleontologists have used research on tooth enamel hypoplasia as a stress signal when assessing paleoenvironments. The goal of the current study was to identify and evaluate stress periods that occurred in the middle Miocene to early Pleistocene eras of the Siwaliks of Pakistan by analyzing enamel hypoplasia in eight extinct suids species: *Listriodon pentapotamiae, Tetraconodon minor, Sivachoerus prior, Hippohyus lydekkeri, Hippohyus sivalensis, Hippopotamodon sivalense, Tetraconodon magnus, Propotamochoerus hysudricus.* All suid species from all time intervals between 14.2 and 0.6 million years old (Myo) were analyzed, with the exception of those from the 11.2-9.0 Myo time frame (late Miocene), when no suids were found to show enamel hypoplasia. Enamel hypoplasia was the most common in suids from the middle Miocene Siwalik (63%), followed by those from the early Pliocene to the Pleistocene (43%), the late Miocene to the Pliocene (25%), and the late Miocene to the early Pleistocene (33%). The percentage of incidence of E.H with respect to each species was 62.50% in *Listriodon pentapotamiae,* 50% in *Sivachoerus prior* and *Hippohyus sivalensis,* 33.33% in *Hippohyus lydekkeri* and *Propotamochoerus hysudricus* and 0% in *Tetraconodon minor* and *Tetraconodon magnus*. The prevalence of enamel hypoplasia during the Neogene and Quaternary in the Siwalik region provides evidence for the occurrence of stress episodes, while the proportion of hypoplastic enamel illustrates the relative intensity of these stresses. These stressful events brought on by a lack of food or nutrition happened because of the historical periods' drastic shifts in weather, vegetation, ecology, and wildlife. These stress periods, which occurred from the early Miocene to the Pleistocene, may have spurred the evolution and speciation of the Siwalik fauna, notably the mammals.

Key Words: Enamel hypoplasia, Stress signals, Paleoenvironment, Siwaliks, Suids

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INTRODUCTION

The study of paleoenvironmental conditions and their impact on ancient populations has been a subject of paramount importance in paleontological and anthropological research (Ogilvie *et al*., 1989). Enamel hypoplasia, a structural anomaly in dental enamel, has emerged as a reliable indicator of stress events experienced by individuals during their developmental years (Goodman *et al*., 1990). This is particularly relevant when examining suid populations from the Siwalik Hills region of Pakistan, an area renowned for its rich fossil record spanning the Middle Miocene to Early Pleistocene epochs. The Siwalik suids, comprising an assemblage of extinct pig-like mammals, offer a unique opportunity to unravel the intricate interactions between paleoenvironmental stressors and the adaptive responses of these taxa (Khan *et al*., 2019).

Enamel hypoplasia has been well-documented in various species and contexts, serving as an invaluable tool for discerning episodes of physiological stress in the lives of ancient organisms (Guatelli-Steinberg *et al*., 2012; Guatelli-Steinberg *et al*., 2004). Such stressors encompass a spectrum of environmental factors, including nutritional deficiencies, disease outbreaks, climatic fluctuations, and ecosystem perturbations (Goodman *et al*., 1980). In this study, we focus on the Siwalik suids as bioindicators to investigate the presence and implications of enamel hypoplasia within their dental records.

The Siwalik Hills, stretching along the northern edge of the Indian subcontinent, offer an exceptional archive of terrestrial paleoenvironments and evolutionary history (Badgley, 1986). These hills have yielded a treasure trove of suid fossils representing diverse species, making them a promising avenue for paleostress analysis. Previous studies have demonstrated the utility of dental enamel hypoplasia in unraveling the impact of ecological and environmental stressors on various taxa (Khan, 2017). However, the application of this method to

Siwalik suids remains relatively unexplored, leaving significant gaps in our understanding of the stress episodes these animals endured.

This research aims to bridge these gaps by systematically examining enamel hypoplasia in Siwalik suids across different temporal intervals, ranging from the Middle Miocene to the Early Pleistocene. By integrating detailed dental analyses with established paleoenvironmental proxies, we seek to decode the nature and intensity of stressors that may have influenced the lives of these suids. Furthermore, this investigation holds the potential to contribute to broader discussions regarding the adaptability of suid populations in the face of changing paleoenvironments.

Studies on enamel defects can offer a special viewpoint on the environmental stressors prevalent during vanished animal's formative years. Since enamel is the body's hardest tissue and enamel hypoplasia (E.H) marks are unaffected by fossilization, they can serve as a very valuable stress indicator for an extinct animal's life history (Goodman, 1980).

A form of enamel defect known as E.H is characterized by enamel thinning (Goodman and Rose, 1990). Two stages can be distinguished in the development of enamel: the secretary stage and the maturation stage. During the secretary stage, enamel begins at the top of the dental crown and gradually moves to the sides. During the maturation stage, mineralization of the tooth occurs (Fig. 1). Secretary life is where you will find E.H.

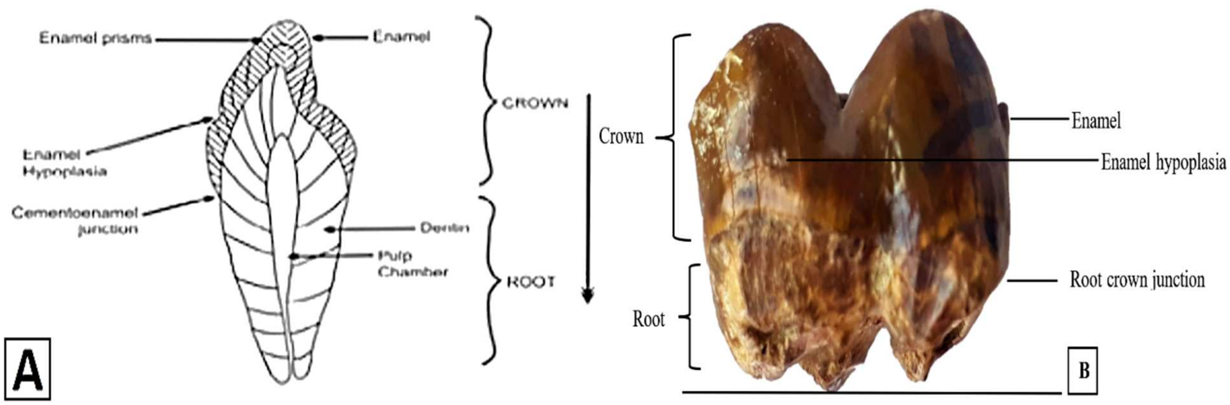


Figure 1: (A) A theoretical enamel growth diagram, (B) In an investigated model of suids, the enamel creation process is displayed. The direction of arrow indicates the path in which enamel forms.

There are three distinct forms of enamel hypoplasia (E.H) recognized by Mohamed *et al*., (2010): enamel hypoplasia due to an area of lacking enamel, E.H due to a single pit, and E.H due to many pits. The enamel-deficient variety has an advantage over the other two in that it may be researched at a macro level. The time of life of an organism during a certain stress episode in its ecosystem can be determined by analyzing the position of the region of lost enamel on dental crowns in respect to the tooth-crown junction (Suckling, 1989). Areas with missing enamel can be classified as either linear (L.E.H) or semi-circular (S.E.H). Skinner and Goodman (1992) describe L.E.H as one or more horizontal furrows on the surface of a tooth, while S.E.H is a semicircular depression on a tooth that runs horizontally. E.H occurs when the ameloblasts are physically harmed during tooth development. This issue is commonly linked to systemic and general stress (Franz-Odendaal *et al*., 2004). Hunger and malnutrition are major contributors to these systemic stresses. Stress of a physiological or environmental type is linked to nutritional deficiency.

Many researchers have examined E.H in extinct and living species of ungulates (Franz-Odendaal, 2004; Niven *et al*., 2004; Roohi *et al*., 2015) to learn more about the ecological circumstances these species confronted during their formative years. The objective of this research was to investigate the presence of ecological stresses affecting extinct suids in the Siwaliks of Pakistan, as well as to assess the varying degrees of severity of these pressures across different Siwalik formations. There had been no previous investigations of E.H on the suids in Siwalik hence this investigation was the first attempt to assess E.H in suids native to the Siwalik Potwar Plateau.

In this paper, we present the results of our comprehensive study on enamel hypoplasia as a stress signal in Siwalik suids, shedding light on the palaeoecological challenges they encountered and their potential implications for evolutionary trajectories. By combining paleontological, palaeoecological, and dental analyses, we aim to unravel the intricate relationship between paleoenvironmental stress and the recorded responses within the dental enamel of these enigmatic creatures.

This E.H study includes the genera *Listriodon, Tetracondon, Sivachoerus, Propotamochoerus, Hippopotamodon,* and *Hippohyus* from the order Artiodactyla and the family suidae to predict climatic changes and environmental disturbances in the Neogene and Quaternary Siwaliks.

MATERIALS AND METHODS

The investigated material collected from the early Miocene to Pleistocene sedimentary layers of Siwalik belong to the eight species of suids. *Listriodon pentapotamiae* belongs to genus *Listrodon*, *Tetraconodon magnus* and *Tetraconodon minor* is species of genus *Tetraconodon, Sivachoerus prior* belongs to genus *Sivachoerus, Propotamochoerus hysudricus* belongs to genus *Propotamochoerus, Hippopotamodon sivalense* belongs to genus *Hippopotamodon*, *Hippohyus sivalensis* and *Hippohyus lydekkeri* are species of genus *Hippohyus*. Table 1 gives a detailed description of the investigated material as well as the findings regarding the presence of E.H in these fossils. The well-preserved teeth were examined and damaged or highly worn were excluded. These samples were analyzed in the Palaeontology and Ecology Lab (Institute of Zoology, University of the Punjab, Lahore Pakistan).

Table 1: The Siwalik suids were studied from the early Miocene to the early Pleistocene.

Chronological range of studied species	Studied Species	Catalogue Number	Type of Dentition	Presence/Absence of E.H
Middle Miocene	*Listriodon pentapotamiae*	PUPC12/51	M1-3	01 L.E.H
		PUPC 94/16	p3-m2	01 L.E.H
		PUPC 16/94	M2	01 L.E.H
		PUPC16/102	m2-3	01 L.E.H
		PUPC 12/26	p3	………………
		PUPC 15/354	m2	01 L.E.H
		PUPC08/86	m1-3	………………
		PUPC16/99	M2	………………
		PUPC16/96	M2	01 L.E.H
		PUPC16/97	p4	02 L.E.H
		PUPC16/101	m1-2	02 L.E.H
		PUPC85/186	m3	01 L.E.H
		PUPC85/185	p1-m2	………………
		PUPC85/190	p3-m1	………………
		PUPC16/88	i1	………………
		PUPC85/187	p2-m1	01 L.E.H
Early Late Miocene	*Tetraconodon minor*	PUPC94/11	m1	………………
Ealy Pliocene to Pleistocene	*Sivachoerus prior*	PUPC 95/8	m1	01 L.E.H
		PUPC 96/61	M2	………………
		PUPC 15/41	m3	………………
		PUPC 95/7	p3	01 L.E.H
	Hippohyus lydekkeri	PUPC 15/27	m3	………………
		PUPC15/40	P3	………………
		PUPC 15/259	m3	01 L.E.H
		PUPC 15/351	m2-3	………………
		PUPC 15/257	p4-m1	01 L.E.H
		PUPC14/144	m1	………………
	Hippohyus sivalensis	PUPC15/350	M3	01 L.E.H
		PUPC94/11	m2	………………
		PUPC15/356	M1	01 L.E.H
		PUPC97/89	M3	………………
Late Miocene to Pliocene	*Hippopotamodon sivalense*	PUPC94/3	m1	………………
		PUPC16/72	P3	01 L.E.H
		PUPC15/43	I2	………………
		PUPC16/74	p3	………………
		PUPC15/45	M3	01 L.E.H
		PUPC16/68	P3	01 L.E.H
		PUPC96/60	p3	………………
		PUPC15/346	M3	………………
		PUPC16/77	I1	………………
		PUPC95/19	p4- m3	………………

		PUPC15/352	M1	01 L.E.H
		PUPC15/347	m1-2	……………….
		PUPC16/80	I1	……………….
		PUPC96/81	p4	……………….
	Tetraconodon magnus	PUPC 98/2	m3	……………….
		PUPC 15/345	m2	……………….
Late Miocene to Early Pleistocene	*Propotamochoerus hysudricus*	PUPC99/1	m2-3	01 L.E.H
		PUPC16/75	i2	……………….
		PUPC16/187	m2	……………….

With the aid of magnifying glasses, each tooth was examined for the presence or absence of E.H. Each linear or circular depression on the tooth crown and jaw surface were noted. The dental terminology of suids teeth follows Khan *et al.* (2010). A vernier caliper was used to measure the location of E.H on the tooth crown heights from the root-crown joints. All sizes were measured in millimeters.

RESULTS

Among 63 samples, only 21 (33%) were found to have E.H. 28% of premolars, 39% of molars, and none of the incisors were found to have E.H. These fossils with E.H are attributed to species that are thought to have spread throughout a considerable region of the Siwalik Hills. In Figure 2, we see a comparison of the frequencies of E.H in suid fossils from Neogene and Quaternary deposits in the Siwalik Hills of Pakistan. Figure 3 shows that E.H was present in seven studied species. Based on the results of this investigation, single or multiple cases of L.E.H and/or S.E.H on teeth are possible (Fig. 3). The incidence (%) of E.H with respect to each suid species is given in the figure 4. Among all the species the incidence of E.H was detected in 62.50% *Listriodon pentapotamiae* species. 28.58% *Hippopotamodon sivalense* were positive for E.H teeth disease.

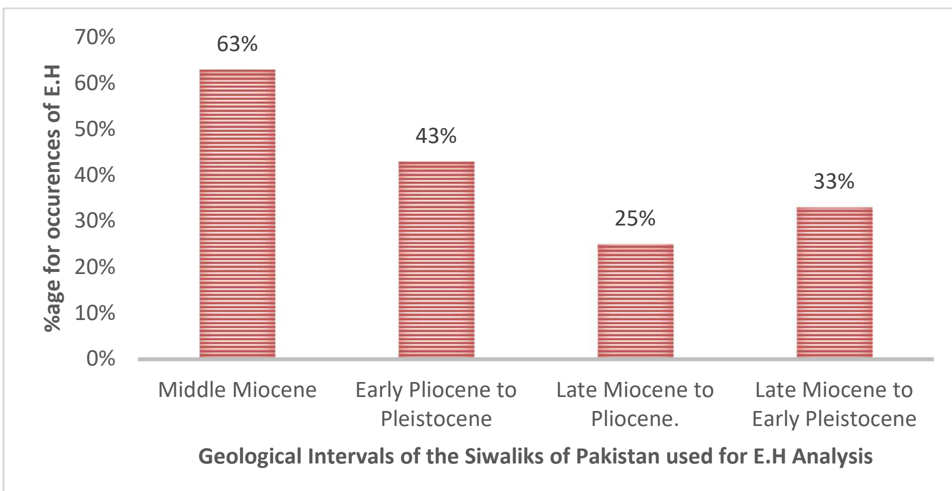
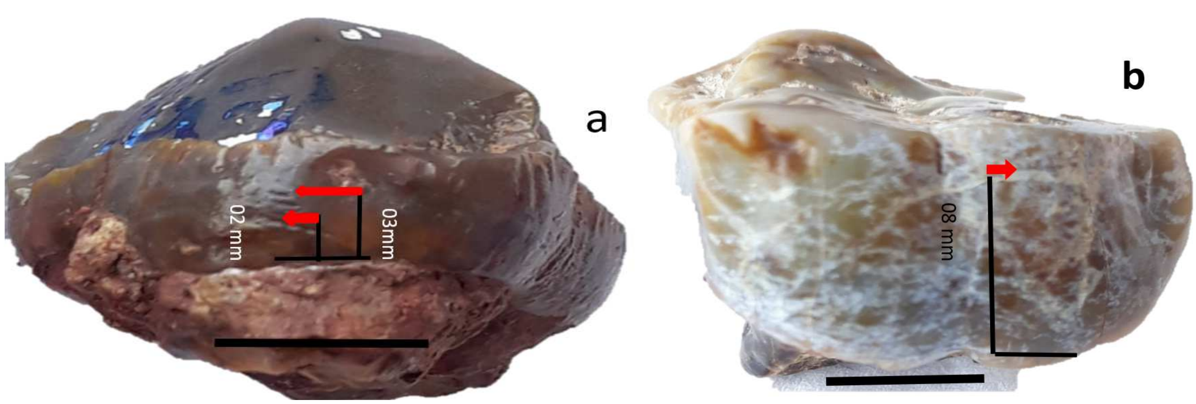


Figure 2: Incidence of enamel hypoplasia among the Siwalik suids between the middle of the Miocene and the late Miocene to the early Pleistocene.



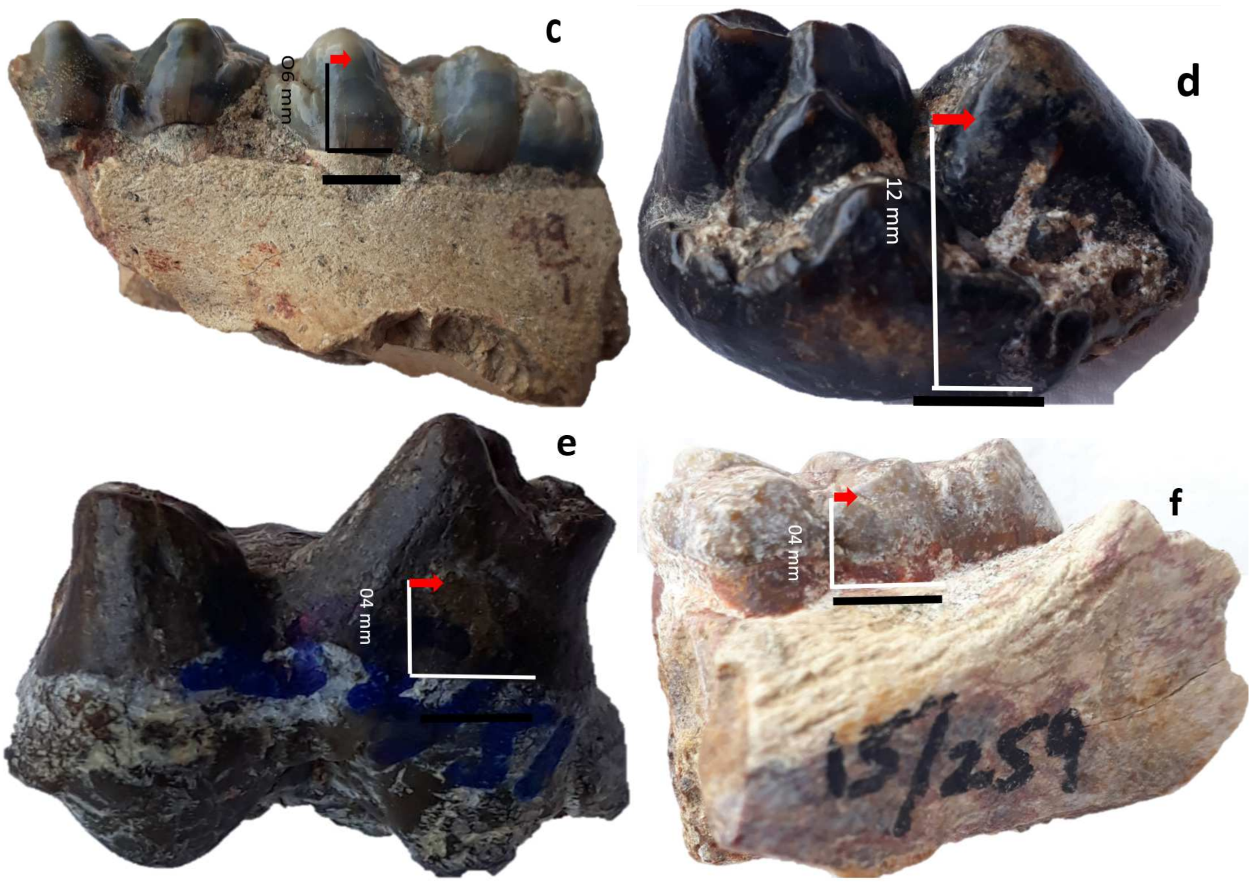


Figure 3: E.H in suids species of the Siwaliks: a, *Listriodon pentapotamiae*; b, *Sivachoerus prior*; c, *Propotamochoerus hysudricus*; d, *Hippopotamodon sivalensis*; e, *Hippohyus sivalensis*; f, *Hippohyus lydekkeri*.

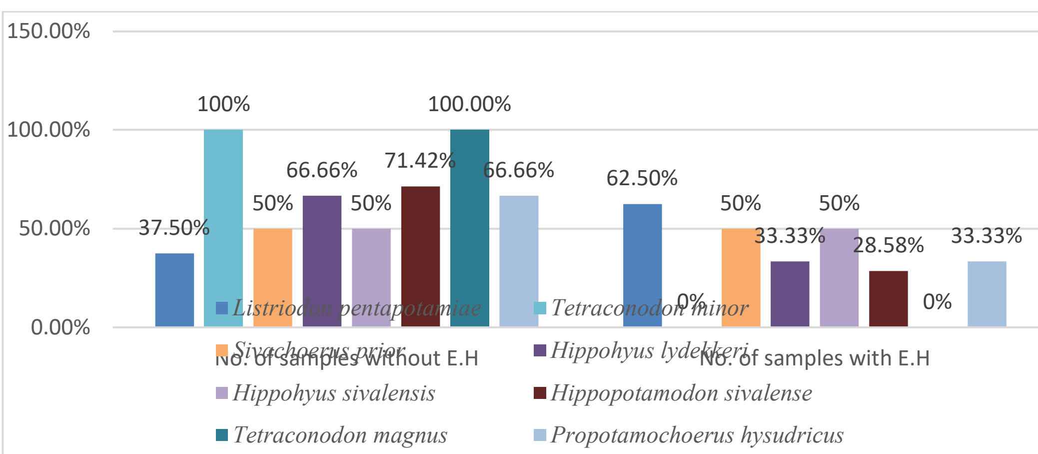


Figure 4: Percentage of incidence of E.H with respect to each suid species

DISCUSSION

If E.H was a hereditary condition in extinct suids, then the animal carrying the E.H gene should experience E.H symptoms in all of its teeth (Stewart and Poole, 1982; Franz-Odendaal *et al*., 2004). Six out of the eight jaw fragments in this investigation had E.H. No species had a jaw where every tooth was afflicted by E.H, showing that the condition was not inherited but rather a result of a nutritional or environmental stressor during the animals' growth and development. According to Mead's (1999) research of E.H in *Teleoceras* (Miocene rhinoceroses), this species had 19% E.H. A comparison of E.H in Siwalik giraffids revealed that 34% of Giraffe teeth have E.H (Ahmad *et al*., 2018). While relatively equal E.H was found in studied suid species, 33% of their teeth were affected, demonstrating that E.H is also common in suids just like to giraffid members. This finding suggests that environmental change over geological time scales in various regions has caused suids to experience comparatively more stress. There are no instances of vertical E.H among the observed E.Hs (Fig. 3). The fact that Franz-Odendaal *et al*., (2004) only noticed horizontal E.H signs in extinct suids is supported by these findings.

Data interpretation in regional context: The species from the middle Miocene is called *Listriodon pentapotamiae*. The proportional percentage of E.H in individuals of middle Miocene Siwalik species, which is 63%, indicates a middle Miocene epoch of severe strain events throughout the evolution of Siwalik mammals. The middle Miocene was a challenging time period because of jarring and unpredictable climate shifts that happened around 13 Ma. The middle Miocene species' nutritional and ecological strain was ultimately attributable to a fast change in the environment, as indicated by the larger ratio of disappearances to appearances during this time period (Barry and Flynn, 1990; Barry *et al*. 2002).

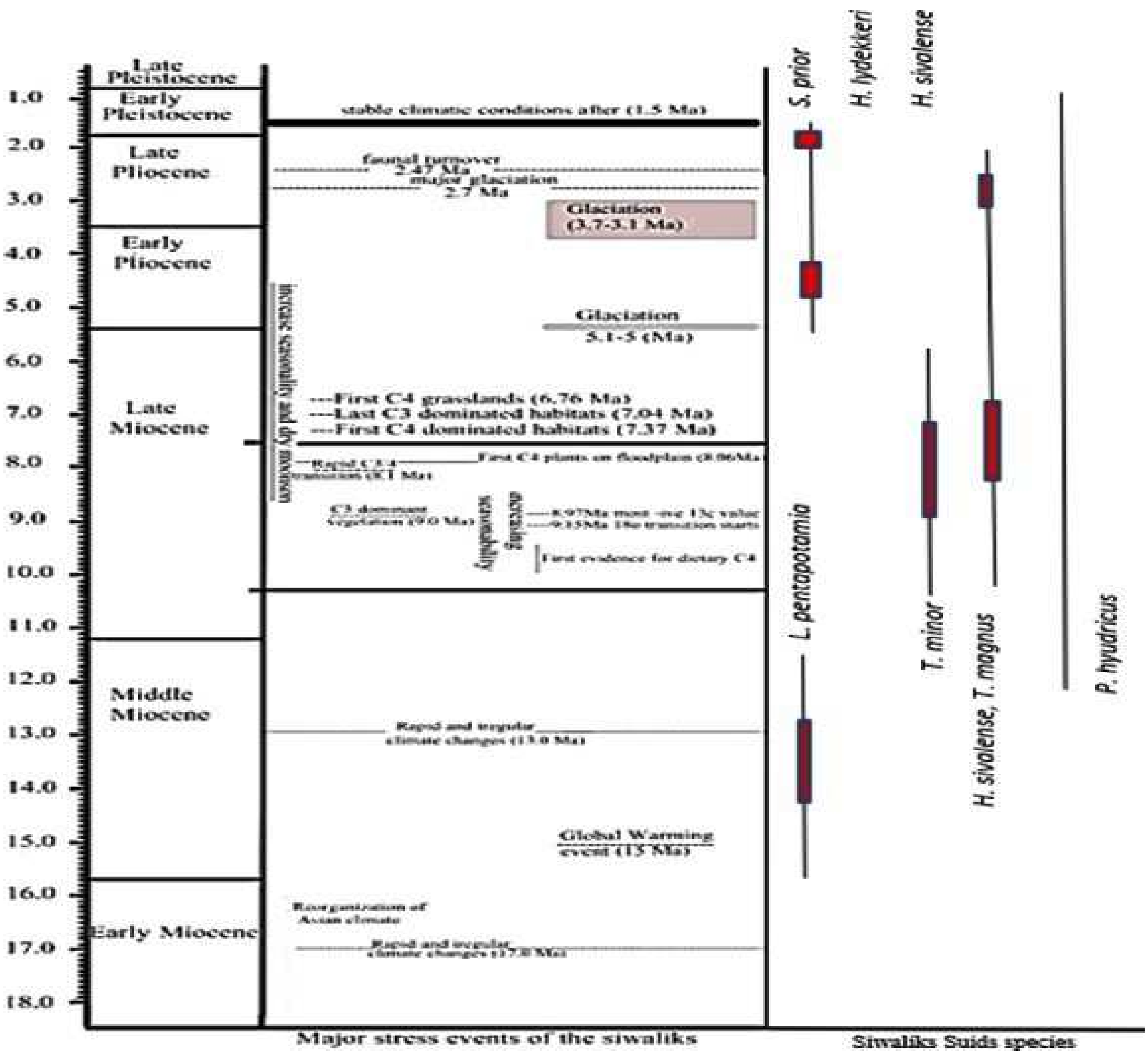


Figure 4: Colored areas in the picture represent the geological time for the presence of E.H in these species. Comparison of E.H in suids species with distinct chronological ranges in the Siwaliks of Pakistan. (Data for events and ages is from Barry and Flynn, 1990; Barry *et al*., 2002; Dennell *et al*., 2006; Lisiecki and Raymo 2007)

Fossilized *Hippopotamodon sivalensis* from the Late Miocene to Pliocene eras have an E.H of 25%, which is equivalent to the modern species. Several species experienced tooth defects as a result of various stresses during the late Miocene and early Pliocene. In the late Miocene, as a result of an increase in environmental stressors, the average lifetime of animals declined dramatically. These climatic shifts developed in large part because of a period of extreme cold that occurred after 6.5 Myo (Barry and Flynn, 1990).

In Early Pliocene to Pleistocene, investigated fossils of *Sivachoerus prior, Hippohyus lydekkeri, and Hippohyus sivalensis,* 43% of relative E.H is experienced. During the Plio-Pleistocene period, cooling tendencies and the glacial series scale increased. The uneven saw-tooth type glacial shape of those periods was one of the driving forces behind the climatic dynamics. These characteristics of the shifting climate are thought to be primarily driven by the 2.5 Myo transition (Lisiecki and Raymo 2007). According to Dennell *et al*. (2006), the Siwalik region saw shifts toward drier conditions throughout the course of 1.8, 1.7, and 1.0 Myo.

One species' (*Tetraconodon magnus*) E.H from the late Miocene period was investigated. The lack of E.H in this species sheds light on the largely stress-free environment that Suids lived in from 11.2-9.0 Myo, or the late Miocene epoch.

Similar E.H of 33% can be seen in *Propotamochoerus hysudricus* fossils from the late Miocene and early Pleistocene. In the late Miocene, the average species extent sharply decreased as environmental disturbances became more frequent. After 6.5 Myo, there was a massive cooling event that had a major impact on the evolution of these environmental changes. (Barry and Flynn, 1990).

Conclusion: Multiple extinction events have affected the Siwalik fauna during the Neogene and Quaternary epochs. There is no evidence of a mass extinction during the Miocene, thus the loss of numerous mammalian species throughout this time period is likely due to the animals' diminished compatibility with changing climatic circumstances. Extremely high E.H frequencies may have contributed to the extinction of these creatures in their native Siwalik habitat. Hypoplasia is likely related to environmental stress, which may have altered dietary habits and food availability. All of the observable signs of E.H in tooth development strongly corroborate this. The discovery of E.H in the fossilized remains of the suids species studied from the Neogene sediments of the Siwaliks provides further evidence that the extinction and migration of extinct Himalayan species were profoundly influenced by the many vegetational and climatic changes that occurred in these ecosystems over the course of Earth's history.

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