Understanding dietary differences in Indian dugongs through opportunistic gut sampling of stranded individuals

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We analysed gut samples of stranded dugongs from Tamil Nadu and Gujarat, India, to understand their dietary preferences. We quantified seagrass fragments from the gut as leaf, stem and rhizome, and identified leaf fragments up to genera level by their morphological features and epidermal cell characteristics using an inverted microscope. The overall abundance of aboveground fragments (leaf, stem) was higher in all samples, which may suggest the dugongs use a cropping mechanism to forage. The ingested seagrass generic diversity was higher in Tamil Nadu (n = 5) dugong individuals than those in Gujarat (n = 2). A total of five genera were recorded from all samples, viz. *Halophila* spp., *Halodule* spp., *Cymodocea* spp., *Enhalus* sp. and *Syringodium* spp. In Tamil Nadu, *Cymodocea* spp. (46.24%) was the most dominant, followed by *Halophila* spp. (26.49%), *Syringodium* spp. (14.83%) and *Halodule* spp. (12.16%), with a low occurrence of *Enhalus* spp. (0.19%). In Gujarat, *Halodule* spp. (61.48%) was the most dominant, followed by *Halophila* spp. (30.20%). The recorded plastic and wood fragments suggest fine spatial scale threat mapping in dugong habitats.

Keywords: Dugongs, foraging pattern, megaherbivore, necropsy, seagrass.

DUGONGS (*Dugong dugon*, Müller, 1776; order: Sirenia) are globally threatened marine mammals that primarily forage on seagrass[1,2]. Despite their vast global distribution range from the East African coast to Australia (Indo-Pacific Ocean region), their population is declining due to various human-mediated drivers[2]. These drivers include mortalities from incidental capture in fishing nets, boat strikes, hunting for meat and seagrass habitat loss due to increased sedimentation and pollution[3], which have led to local extinction of the species[4–6].

For species monitoring and conservation, it is crucial to understand dugong distribution and the factors influencing the same. The availability of seagrass is one of the limiting factors for dugong distribution. Thus, understanding dugong foraging patterns is crucial for mapping their critical habitats. Dugongs use two major feeding techniques, viz. cropping and excavation of seagrass, depending on species morphology and substratum[7–9]. So far, dugong foraging preferences are known through direct observations of feeding or by analysis of stomach contents[10–13].

Indian dugong populations are at risk due to various threats, with an estimated population of fewer than 300 individuals left in the wild[14,15]. Recent studies across isolated pockets of their distribution along the Indian coastline (Gulf of Kutch in Gujarat, Gulf of Mannar and Palk Bay in Tamil Nadu, and Andaman and Nicobar Islands) have helped generate crucial ecological data on their distribution, habitats, genetic diversity, connectivity and threats[6,15–17]. Limited studies exist on dugong feeding biology from India[13,18], given the difficulty of observing them in the wild. Thus, stranded dugongs provide a critical opportunity to understand their dietary composition through gut sampling. In this study, we utilize the gut contents collected from stranded dugongs to understand the differences in their foraging pattern within the study sites. This study helps fill the research gap on dugong feeding behaviour from the Indian

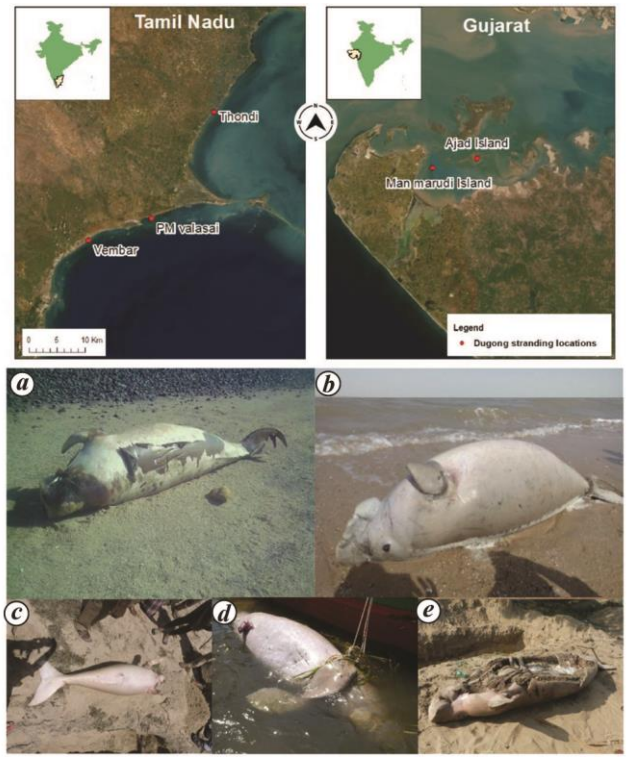

Figure 1. (Top) Location of stranded dugongs in the Gulf of Mannar and Palk Bay, Tamil Nadu and Gulf of Kutch, Gujarat, India. (Bottom) ***a***, Adult female dugong found in Ajad Island, Gulf of Kutch on 4 February 2018. ***b***, Adult male dugong found in Man Marudi Island, Gulf of Kutch on 20 May 2018. ***c***, Juvenile male dugong found in P.M. Valsai, Palk Bay on 16 June 2018. ***d***, Adult male found in Thondi, Gulf of Mannar on 20 June 2018. ***e***, Adult individual found in Vembar, Palk Bay on 7 December 2018.

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Table 1. Details on location, carcass condition, cause of mortality of dead stranded dugong from the coast of Tamil Nadu and Gujarat

Dugong individual	Date	Location	Sex	Age class	Carcass condition	Cause of mortality	Informant group	Body length (m)
Gujarat coast (N = 2)								
Dugong 1	04–02–2018	Ajad Island	Female	Adult	Decomposed	Net entanglement	Fisherman	2.6
Dugong 2	20–05–2018	Man Marudi Island	Male	Adult	Fresh	Net entanglement	Fisherman	2.4
Tamil Nadu coast (N = 3)								
Dugong 3	16–06–2018	P.M. Valsai	Male	Juvenile	Fresh	Boat strike	Fisherman	1.5
Dugong 4	20–06–2018	Thondi	Male	Adult	Fresh	Poached	Forest Department and Marine Police	2.4
Dugong 5	07–12–2018	Vembar	Not identified	Adult	De-composed	Not known	Fisherman	2.9

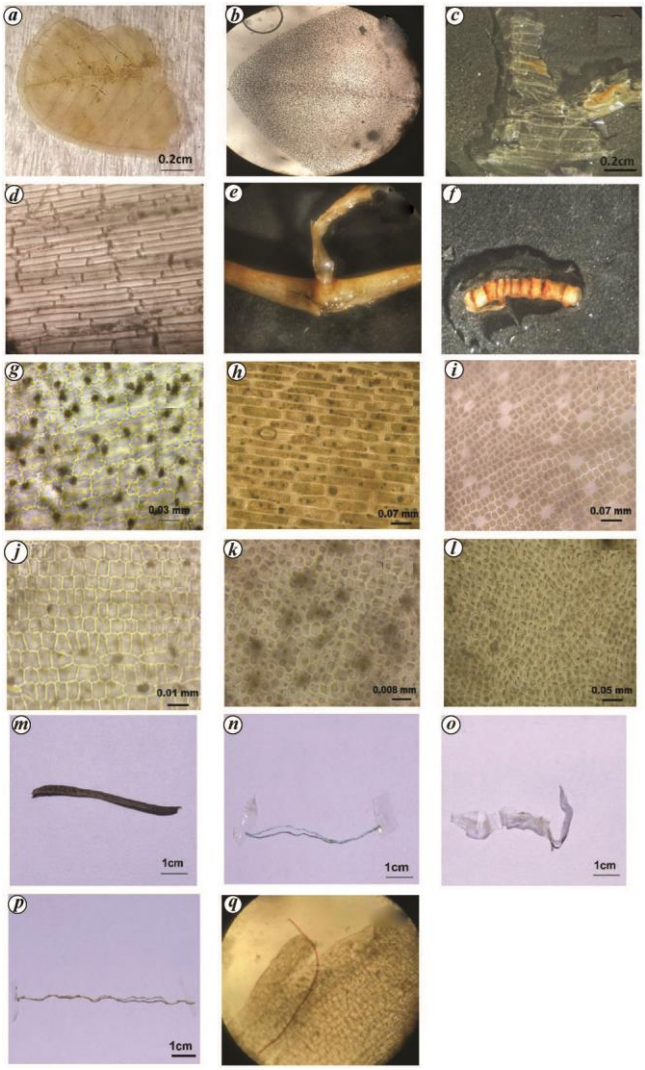

Figure 2. Fragments and epidermal cell structures of seagrass and non-biological materials from dugong gut samples. ***a***, ***b***, Leaf fragment of *Halophila* spp. with venation under a stereo-microscope and compound microscope (4×). ***c***, Fibroid structure of vertical stem under stereo-microscope. ***d***, Epidermal cell structure of stem under compound microscope (20×). ***e***, Rhizome fragment. ***f***, Presence of leaf scars in rhizome fragment under stereo-microscope. ***g***, Epidermal cell structure of *Halophila* spp. (40×). ***h–l***, Epidermal cell structure: ***h***, *Halodule* spp. (40×); ***i***, *Cymodocea* spp. (40×); ***j***, *Enhalus* spp. (40×); ***k***, *Syringodium* spp. (40×) and ***l***, Algal fragment (40×). ***m***, Wooden fragment (4 cm). ***n***, Fishing net filament (4 cm). ***o***, Polythene fragment (4 cm). ***p***, Fishing net fragment (9 cm). ***q***, Red-coloured microfilament (20×).

waters and presents the gut content analysis report to supplement existing knowledge on dugong feeding biology on a regional scale.

In 2018, we sampled gut contents of stranded dead dugongs from the coasts of Tamil Nadu (n = 3 individuals) and Gujarat (n = 2 individuals) (Figure 1). Strandings were informed by the local dugong volunteer network involving fishermen and personnel from the Coastal Security Police and State Forest Department. Details of dead animals, stranding locations, cause of mortality and condition of the carcass are given in Table 1. Gut samples were collected, secured in ethanol in airtight containers and preserved at –20°C until further processing. Dugong carcasses were sexed, measured and necropsies were conducted based on carcass conditions using standard protocols (Table 1).

To estimate the proportion of seagrass consumed by dugongs from the gut samples, we categorized seagrass fragments as leaf, stem and rhizome based on their morphological features (Figure 2)[19]. We used the point-intercept method to calculate the abundance of leaf, rhizome and stem fragments[20]. The samples were further divided into ten subsamples of 1 g each and later homogeneously spread on a Petri plate of size 100 × 15 mm. The Petri plate was divided into 24 quadrates of 1 cm^2 each and gut material was observed under a stereo-microscope (10× magnification).

We identified seagrass leaf fragments based on apex structure, visible venation, and stem fragments from their fibroid structure. Rhizomes were identified by their hard structure with nodes and the presence of leaf scars[19].

Further, to find the preferred seagrass by dugongs, we identified seagrass fragments only up to the genus level. Species identification of different seagrass species belonging to the same genera was not possible due to similarity in their epidermal cell shape and size[19,21,22]. Only leaf fragments were considered for genera-level identification, as stem and rhizome fragments are conserved in appearance through most genera[21]. Validation of genus was done referring to prepared reference slides of seagrass species collected from Tamil Nadu, Gujarat, and Andaman and Nicobar Islands; the present distribution range of dugongs in Indian waters. The leaf and epidermal cell features were studied using a stereo-microscope (10×) and inverted microscope at various magnifications (4×, 10×, 20× and 40×) (Supplementary Figure 1).

Table 2. Details of the percentage contribution of fragments of seagrass present in gut samples of dead, stranded dugong from the coast of Tamil Nadu and Gujarat, India

Dugong individual	Gut content weight (g)	Percentage of fragments of seagrass in/g sample (mean ± SD)			Percentage of above ground seagrass fragments (leaf and stem) (%)	Percentage of below ground seagrass fragments (rhizome) (%)	Percentage of fragments (in parenthesis) of seagrass genera in/g of samples (mean ± SD)					Algal fragments
		Leaf	Stem	Rhizome			*Halophila* spp.	*Halodule* spp.	*Cymodocea* spp.	*Syringodium* spp.	*Enhalus* spp.	
Dugong 1	64.77	12.13 ± 8.1 (43.29%)	7.05 ± 3.4 (25.22%)	8.80 ± 3.80 (31.48%)	68.51	31.48	23.8 ± 14.1 (79.20%)	3.55 ± 4.4 (11.81%)	0	0	0	2.70 ± 5.2 (8.98%)
Dugong 2	159.03	14.2 ± 9.15 (40.86%)	9.30 ± 6.0 (26.76%)	11.25 ± 6.1 (32.37%)	67.62	32.37	4.2 ± 4.8 (6.28%)	53.6 ± 35.5 (85.36%)	0	0	0	5.00 ± 5.1 (7.95%)
N = 2 (Gujarat coast)	–	13.15 ± 8.6 (52.44)	8.17 ± 5.8 (22.08%)	10.02 ± 5.2 (25.47%)	74.52	25.47	14 ± 14.3 (30.20%)	25.8 ± 35.6 (61.48%)	0	0	0	3.85 ± 5.2 (8.30%)
Dugong 3	76.832	7.45 ± 7.43 (41.45%)	4.60 ± 4.6 (25.59%)	5.92 ± 3.77 (32.94%)	67.04	32.94	8.82 ± 9.2 (44.08%)	5.72 ± 6.8 (28.59%)	1.02 ± 7.6 (5.10%)	4.35 ± 6.2 (21.74%)	0.1 ± 0.3 (0.50)	0
Dugong 4	25.94	16.55 ± 1.0 (55.29%)	8.05 ± 6.5 (26.89%)	5.30 ± 2.55 (17.80%)	82.19	17.80	16.45 ± 6.8 (49.76%)	1.86 ± 1.5 (5.63%)	2.50 ± 1.6 (7.56%)	12.1 ± 8.5 (36.45%)	0.1 ± 0.3 (0.30)	0.1 ± 0.3 (0.30%)
Dugong 5	30.73	21.65 ± 7.3 (60.81%)	5.25 ± 4.4 (14.74%)	8.70 ± 2.61 (24.43%)	75.55	24.43	5.50 ± 2.96 (7.68%)	4.15 ± 2.5 (5.80%)	61.8 ± 14.1 (86.31%)	0	0	0
N = 3 (Tamil Nadu coast)	–	13.3 ± 10.0 (41.95%)	5.60 ± 5.2 (26.06%)	6.46 ± 3.46 (31.97%)	68.01	31.97	9.50 ± 4.9 (26.49%)	4.36 ± 5.3 (12.16%)	16.6 ± 27.3 (46.24%)	5.32 ± 7.4 (14.83%)	0.07 ± 0.3 (0.19)	0.025 ± 0.2 (0.069%)

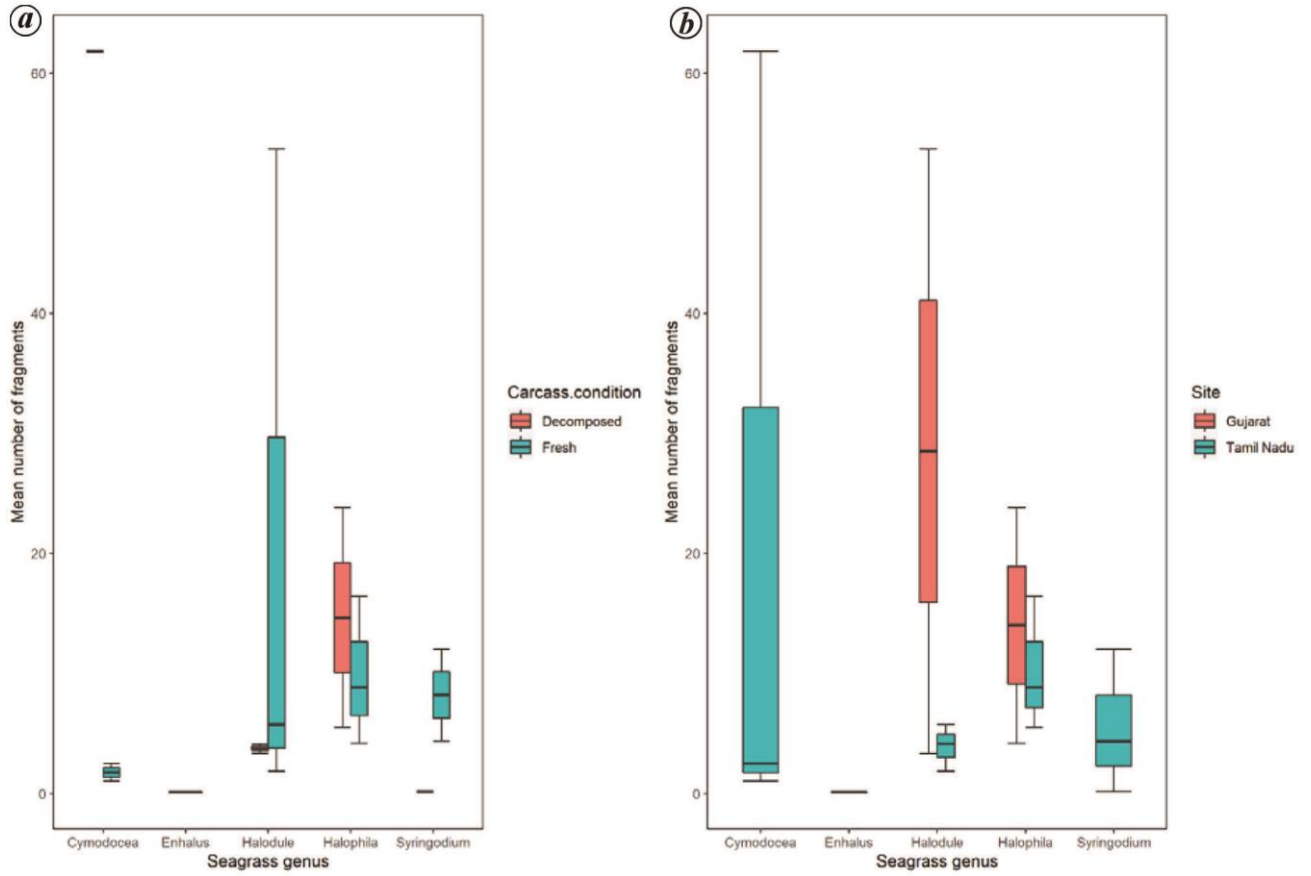

Figure 3. Mean number of seagrass fragments obtained from the gut of stranded dugongs sorted according to (***a***) carcass condition and (***b***) sampling site.

Epidermal cell characteristics and tannin cell arrangements were used to identify macerated seagrass to the genus level, using the quadrat method[21], where one-quarter of the petri plate (25% of grids) was chosen randomly to count the leaf fragments. Gross morphological features (venation, size, apex structure and shape of leaves) were considered for intact leaves[19]. Fragments were identified at different magnifications (4×, 10× and 40×) using seagrass identification keys[21,23–25].

Since the gut material consisted of seagrass and algae, to independently quantify both, we identified algal fragments (Figure 1) based on their cellular structure (40×) using the peer-reviewed published literature[21].

Mann–Whitney *U* test was performed for *Halophila* spp. and *Halodule* spp. to check the differences in their occurrence between sites, i.e. Tamil Nadu and Gujarat, as well as between fresh and decomposed carcass. This test was possible only for these two genera, as they commonly occurred in all the gut samples.

A total of five genera were recorded from the gut samples across sites, viz. *Halophila* spp., *Halodule* spp., *Cymodocea* spp., *Enhalus* sp. and *Syringodium* spp. The overall proportion of seagrass leaf fragments (>40%) was higher in all the samples analysed. The percentage of above-ground fragments (leaf, stem) was higher than the below-ground fragments (rhizome) (Table 2). Of the two genera recorded from the Gujarat samples, viz. *Halophila* spp. and *Halodule* spp. (Figure 2), the latter was more abundant (61.48%) than the former (30.20%), along with some algal fragments (8.30%) (Table 2). Five seagrass genera, namely *Halophila* spp., *Halodule* spp., *Cymodocea* spp., *Enhalus* spp. and *Syringodium* spp. and algal fragments were recorded from Tamil Nadu dugong individuals (Figure 2). Overall, leaf fragments of *Cymodocea* spp. (46.24%) were dominant in the samples, followed by *Halophila* spp. (26.49%), *Syringodium* spp. (14.83%) and *Halodule* spp. (12.16%). Low occurrence of *Enhalus* spp. (0.19%) and algal fragments (0.069%) were found in the samples (Table 2).

We found a difference in the occurrence of *Halodule* spp. between Tamil Nadu and Gujarat samples ($U = 987.5$, $P < 0.001$), but not for *Halophila* spp. ($U = 1411$, $P > 0.05$; Figure 3). The occurrence of *Halophila* spp. differed according to carcass condition ($U = 1214$, $P < 0.05$), but not for *Halodule* spp. ($U = 1840$, $p = >0.05$) (Figure 3).

In addition to seagrass, plastic and wooden fragments were found in the gut content of two individuals (one each from Gujarat and Tamil Nadu) (Figure 2). Two fishing net filaments (~9.4 and ~4 cm in length), one polythene fragment

(~4 cm length) and one wooden fragment (~4 cm in length) were obtained from the Tamil Nadu individual, while a plastic microfilament was retrieved from the Gujarat individual (Figure 2).

Globally, studies on dugong gut content have highlighted selective consumption of seagrass species like *Halophila ovalis*, *Halodule uninervis*[1,26–28], *Enhalus* sp.[29], *Thalassia hemprichii*, *Syringodium isoetifolium*[11] and *Cymodocea serrulata*[11,28]. Percentage contribution of above- and below-ground plant material was proportionate in the gut samples of Gujarat individuals (Table 2), which possibly suggests dugongs excavating the whole seagrass plant. Our findings suggest that dugongs might exhibit a cropping mechanism over an excavation in Tamil Nadu (percentage of above-ground seagrass fragments more than that of below-ground seagrass fragments) (Table 2), which needs further validation with more ecological observations and a larger sample size. A limited percentage of algal material (3.58) could be due to incidental ingestion while feeding on seagrass. In the present study, more generic diversity of seagrass in the gut content of individuals from Tamil Nadu ($n = 5$) than in Gujarat ($n = 2$) could be attributed to the high regional generic diversity of seagrasses in Tamil Nadu than in Gujarat[30].

The dominance of *Halophila* spp. in dugong gut samples from Ajad Island, Gujarat, is in line with field observations of dominant *H. ovalis* and *H. beccarii* meadows in the region[31]. *Halodule* spp. was found to be dominant in the samples of Man Marudi Island (close to Beyt-Dwarka), Gujarat. Thus, considering the low population size of dugongs in the Gulf of Kutch[14], locating *Halodule* spp. meadows would help in identifying critical foraging grounds in the area.

Stranded dugongs in Tamil Nadu showed a differential rate of digestion from fresh carcasses in comparison to the highly decomposed state (Tables 1 and 2). Fresh carcasses were recorded with a higher mean occurrence of *Halophila* spp., while decomposed carcasses were found with *Cymodocea* spp. in dominance. This could be attributed to the carcass condition (fresh/decomposed), as Thayer *et al.*[32] and Preen[33] have reported dugongs showing species-specific differential digestion rates in their digestive tracts. Further, we speculate that differential digestion is affected by the carcass condition and decomposition rate of individual species. Additionally, the role of plant morphology and composition is crucial in affecting the time required for digestion and in turn occurrence in the gut[1]. More fibrous species like *Cymodocea* spp. take a longer time for decomposition and digestion than smaller leaved, less fibroid plants like *Halophila* spp.[9]. Thus, differences in *Halophila* spp. fragments in fresh and decomposed carcasses can be attributed to their availability and differential digestion rate[34].

This study is a first report on the dietary preferences (seagrass) of dugongs from Indian waters which provides key baseline information from two important dugong distribution ranges in the Indian sub-continent. The occurrence of plastic, fishing net fragments and wood debris reveals a potential risk to dugongs in their foraging grounds. Thus, we recommend enhanced monitoring of seagrass habitats and fine spatial-scale threat mapping in the entire dugong distribution range in India.

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A new species of Indian kino tree from the Early Eocene forests of northwestern India

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Two impressed leaflet remains described here as a new species *Pterocarpus emarginaticus* Patel, Rana and Khan sp. nov., showing close resemblance with the extant leaflets of *Pterocarpus marsupium* Roxb. (Fabaceae), commonly known as the Indian kino tree, have been recorded from the Early Cenozoic sedimentary sequences of the Gurha opencast lignite mine (Early Eocene, Palana Formation), Rajasthan, northwestern India. The diagnostic macromorphological characteristics of the fossil leaflets are elliptical to obovate shape, microphyll size, acute base, characteristic emarginate apex, pulvinate petiolule, entire margin, *brochidodromous* secondary veins, presence of thin intersecondary veins and reticulate tertiary veins. This is reliable fossil evidence of leaflets similar to modern *P. marsupium* from India and abroad. The occurrence of this species and the earlier reported angiosperm, including Fabaceae taxa from the same formation, suggest the existence of a tropical, warm and humid climate during deposition.

Keywords: Fossil leaflets, opencast mine, *Pterocarpus emarginaticus*, *Pterocarpus marsupium*, sedimentary sequences.

PTEROCARPUS Jacq. is a pantropical tree belonging to the family Fabaceae, subfamily Papilionoideae and tribe Dalbergieae[1,2]. The genus is subdivided into two groups based on

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