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Paleobiological Significance of Trichome Inclusions in Baltic Amber from Goa, India

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Abstract

Baltic amber (succinate) is a fossilized resin belonging to the Eocene period (44-49 million years old) derived from the Araucariaceae and Leguminosae families of trees containing up to 8% of succinic acid and compounds such as terpenoids and phenolic derivatives. Amber is primarily yellow in colour, having different shades ranging from light yellow to dark yellow to brown to black. This is the first report on the trichome bioinclusions from imported Baltic amber in Goa. Samples of imported Baltic amber were validated and studied for the presence of bioinclusions. Trichomes, also known as "stellate hairs," are present in the vast majority of angiosperms and are considered for some time to be of importance in comparative systematic studies. Trichomes have an important role as a defensive structure for plants. The trichomes are common in Baltic amber and have been considered as a characteristic of this type of amber. Higher flow of tourists from Russia to Goa by chartered flights has introduced many traded commodities like semi-precious stones and Baltic amber. Since amber of this quality was not easily available in India, the Baltic amber traded in Goa was utilized for scientific studies focused on the identification of biological inclusions after sorting and classifying the purchased samples from authentic traders. This paper reports preliminary analysis of fossilized trichome elements in locally purchased samples of Baltic amber imported from Russia. Specimens were subjected to stereomicroscopic and phase contrast microscopic analysis, and interesting inclusions entombed in amber were studied. Two types of trichomes were found to be present in the Baltic amber stellate hair type and glandular types. The stellate hair type of trichomes was minute and radially branched, ranging from 12 to 17 radii. Glandular trichomes were erect, with a unicellular glandular head and possessed a stalk which was both unicellular and multicellular.

Keywords: Baltic amber, Fossils, Trichome, Bioinclusions, Goa, India

Introduction

Amber (Succinate=$C_6H_6O_4$) is the fossilized resin produced from the trunks and the roots of certain trees, mainly belonging to the family Pinaceae. The plant that secreted Baltic amber has already been identified as *Pinites succinifer*. Amber is a complex mixture of diverse compounds, such as terpenoids and phenolic derivatives (Poinar, 1992) is found distributed globally in Russia, France, Germany, Lebanon, Spain, Dominican Republic, Austria, USA, Myanmar, Japan, and Mexico (Schmidt *et al*., 2014). The age of all amber specimens has been determined to be minimum 4 to maximum 300 million years old. Amber primarily yellow in colour displays shades ranging from light yellow to dark yellow to brown to black (Aranda *et al*., 2007). Nathanael Sendel (1686 - 1757) is a pioneer in research on plants, microbes, and animals embedded in Baltic amber. Plants secrete resin when they suffer injury, and the biota, such as microbes, plant parts and animals get trapped inside this resin. Over the years, the process of fossilization occurs, and resin is converted into amber, and the bioinclusions remain inside the amber (Pontin and Celi, 2000). Organisms that were trapped in resin and are preserved in amber are called bioinclusions, and some of these are especially informative about various taphonomic processes, paleoenvironmental conditions, and important paleobiological aspects (Speranza *et al*., 2015). Amber has been used since prehistory in the manufacture of jewellery and ornaments. The scent of amber and amber perfumery is well known. Amber oil has been used for medicinal purposes (Poinar, 1992). Differences between "true/ natural/ biogenic" and false/ synthetic/ artificial amber can be identified by some classical tests, for example, true amber produces sweet, pine smell when burnt and does not dissolve in acetone (Pionar, 1992). True amber floats in salt water, and this is called as saltwater test (Raducanu, 2006). Amber burns with a black smoke like incense and does not melt and this forms the basis of the validation of amber by the burning test

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(Raducanu, 2006). Other tests of amber analysis include the fluorescent test, refractive index test, IR spectroscopy, and polarized light test (Raducanu, 2006). Various scientists (Stubblefield *et al*., 1988; Pionar and Celi, 1992; Pontin *et al.*, 2000; Smejkal *et al.*, 2011; Hartl *et al.*, 2015) have reported the presence of palaeomicroflora in the amber. Particularly interesting among these findings are the sooty mould morphospecies described by Schmidt *et al.* (2014). In addition, glandular trichomes of *Cannabis sativa* have been reported (Punja *et al*., 2023). Flowers of Apocynaceae have been documented in amber from the early Eocene of India (Singh *et al.*, 2020). There are reports on the palynological study of the subsurface sediments in Borehole AK-10 of the Wardha Valley coalfield, Maharashtra, detailing the sequence. Palynofloral evidence from Subsurface Sediments of the Bhadrawati Area, Wardha Valley Coalfield, Central India, is also discussed. The palynoassemblage recovered showed a predominance of radial monosaccates, along with some algal elements (Sarate and Murthy, 2017; Murthy *et al*., 2019). Some Cyclostome (Bryozoa) are found in the Maastrichtian Kallankurichchi Formation, Ariyalur Group, South India (Sonar *et al.*, 2025). Quamar and Bera (2016) have reported modern pollen assemblages in tropical deciduous forests of Chhattisgarh, central India, and this study helped in the interpretation of palaeoclimate in these areas. The main objective of this study was to study validated Baltic amber samples imported in Goa as standard reference material and specific studies for the detection of trichome bioinclusions in Baltic amber samples

Fig.1. a. Areas of Baltic amber deposition and Baltic Sea (Weitschat and Wichard, 2002); **b.** Baltic amber samples imported from Russia and marketed in local markets; **c.** Baltic amber collection

Materials and Methods

Most of the Baltic amber from Russia is dated to the Eocene period (44-49 million years old). Areas of Baltic amber deposition and the Baltic Sea are shown in figure 1. The Baltic amber samples used for this study were officially imported from Russia in Goa for sale to tourists. Higher flow of tourists (32392 in 2006 to 162746 in 2013 and 104890 in 2014-2015) from Russia and Ukraine to Goa by chartered flights has also introduced many traded commodities like semi-precious stones and Baltic amber. Since amber of this quality was not easily available in India, the Baltic amber traded in Goa was utilized for scientific studies. Identification of specimens with bioinclusions was carried out; further confirmation and validation were done by FTIR spectroscopy. Baltic amber samples obtained were studied using a stereomicroscope (Olympus SZ51) and tested and confirmed by standard salt water and acetone tests (Poinar, 1992). The specimens were randomly mounted on the slide under a stereomicroscope and were visualized under a phase contrast microscope (Olympus BX41TF) equipped with a photomicrographic attachment and studied for the presence or absence of bioinclusions. The specimens with bioinclusions were further studied for the presence of bioinclusions using standard keys (Poinar, 2022), and those showing the presence of different forms were photographed. Specimens exhibiting trichome inclusions were labeled as *GU-Myco/2020/01–15* and deposited in the culture collection of the Mycological laboratory, Botany program, School of Biological Sciences and Biotechnology, Goa University. The image color summarizer was used to produce descriptive color statistics for an image. This app reports the average, median, minimum, and maximum of each component of RGB, HSV, LCH, and Lab (CIELAB, L*a*b*). Average hues are calculated using the mean of circular quantities.

Results and Discussion

The size of the Baltic amber specimens ranged from 1-2 cms (Fig. 2 a-d). This is the first study of imported Baltic amber in India and claimed to be the first modest study to report the detection of trichomes bioinclusion. Earlier studies were on palynoflora from amber in Gujarat, India (Singh *et al*., 2013). Baltic amber specimens were microscopically ranged from 1-2 cms. Out of all the processed fragments or microamber 70% showed presence of unidentified microbial inclusions, whereas the other 20% showed unidentified plant and animal inclusions, and 10% were void with no inclusions.

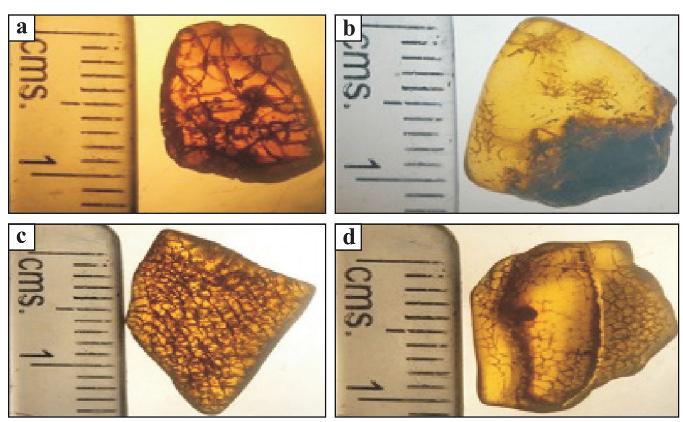

Fig.2. a-d: Typical Baltic amber specimens scrutinized for bioinclusions

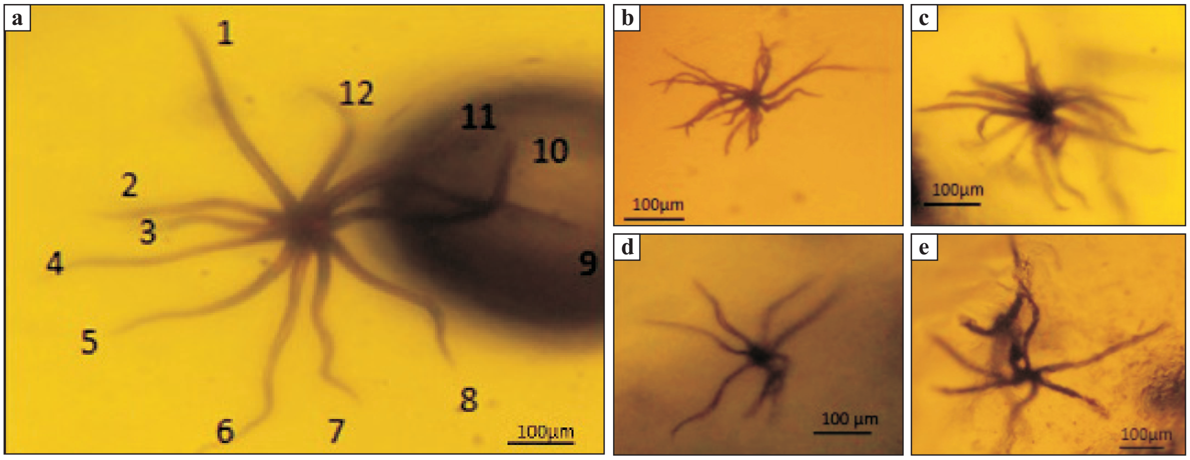

Fig.3. a-e: a-Outline of Baltic trichome with indication of the number of radii. b, c- trichomes with more than 10 radii, d-e: Trichomes with less than 10 radii radially branched.

Similar observations were noticed by Mukherjee *et al.*, (2005) in amber from the lower Eocene in Gujarat. Two types of trichomes were found to be present embedded inside Baltic amber, the stellate radially branched hair and glandular types with a stalk and a glandular head. Figure 3a gives the outline of the Baltic trichome with indication of the number of radii. The stellate radially branched hair type of trichomes was minute and had radii ranging between 7 to 17 (Fig.3b-e). Glandular trichomes were erect, with a unicellular glandular head and possessed a stalk which was unicellular and multicellular, as shown in figures 4a-b. Glandular trichomes are made up of three differentiated parts, which include the base, stalk, and head or gland. The base connects the stalk to the surrounding epidermal cells, the stalk supports the head, and the head secretes the specialized metabolites. All three parts can be clearly seen. Advanced color analysis helped to reveal that the trichome has 8 color groups and 31 colors (Table 1). The length of the radii also differs from trichome to trichome. The length of each radius varies from 2 to 5 µm (Table 2). This study marks a significant contribution to the understanding of trichome bioinclusions within Baltic amber imported into India. The findings offer valuable insights into the preservation and paleobiological significance of plant structures such as stellate and glandular trichomes, which have not been previously studied in this context in India. The detection of both stellate and glandular trichomes provides critical evidence for reconstructing the ancient ecosystems and plant distribution patterns that contributed to the formation of Baltic amber. Stellate trichomes, with their characteristic radial branching, suggest their role in defensive mechanisms, while glandular trichomes highlight potential metabolic pathways preserved within the amber. These

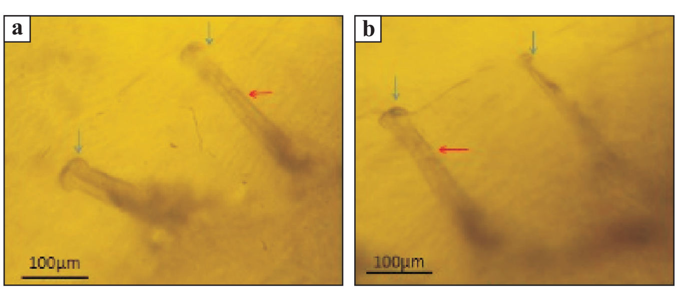

Fig.4. a-b. Glandular trichomes: a, b- unicellular glandular head and multicellular stalk

Table 1: Color analysis of the trichome

Color Name	Color Group	Red	Green	Blue
Snow	Red	254	248	252
Snow	Red	254	248	252
Baby Pink	Red	231	213	198
Golden Brown	Orange And Brown	132	90	16
Golden Brown	Orange And Brown	132	90	16
Chocolate	Orange And Brown	118	74	3
Sepia	Orange And Brown	118	74	9
Brown	Orange And Brown	132	89	11
Seashell	Orange And Brown	254	248	242
Desert	Orange And Brown	176	154	108
Desert Sand	Orange And Brown	223	197	167
Copper	Orange And Brown	172	130	46
Chamoisee	Orange And Brown	166	135	84
Champagne	Orange And Brown	245	235	210
Khaki	Orange And Brown	203	185	139
Raw Umber	Orange And Brown	131	95	42
Wheat	Orange And Brown	238	219	187
Burlywood	Orange And Brown	214	193	155
Dark Brown	Orange And Brown	119	87	36
Sand Dune	Orange-Yellow	158	115	37
Sand Dune	Orange-Yellow	158	115	37
Dark Tan	Orange-Yellow	166	135	73
Pearl	Orange-Yellow	245	235	220
Sand	Orange-Yellow	182	165	123
Dark Goldenrod	Orange-Yellow	178	126	24
Cornsilk	Orange-Yellow	253	241	225
White	White	251	255	255
White	White	251	255	255
Ghost White	Blue	245	244	248
Ghost White	Blue	245	244	248
Auburn	Red-Orange	91	55	5
Auburn	Red-Orange	91	55	5
Bole	Red-Orange	125	91	37
Cinereous	Red-Orange	165	144	107
Beige	Yellow	245	235	229
Beige	Yellow	245	235	229
Ivory	Yellow	253	250	241
Pastel Gray	Yellow	229	215	197
Brass	Yellow	177	144	74
Dark Khaki	Yellow	200	170	106
Lavender Blush	Magenta-Pink	251	246	245
Lavender Blush	Magenta-Pink	251	246	245
Alice Blue	Cyan-Blue	237	247	247
Alice Blue	Cyan-Blue	237	247	247
Ash Grey	Green-Cyan	198	189	171
Ash Grey	Green-Cyan	198	189	171

Table 2: Length of each radii

Number of radii	Length of radii in μm				
	T1 (Trichome with 12 radii)	T2 (Trichome with 16 radii)	T3 (Trichome with 13 radii)	T4 (Trichome with 8 radii)	T5 (Trichome with 7 radii)
1	3	5	3	3.5	4.5
2	2.5	4	3	3	4.5
3	2	2	3	3	3
4	3	2	3	3.5	3
5	3	2	3.5	3.5	3
6	4	2	3	3	4
7	3.5	2	3	3	4.5
8	3.5	2.5	2.5	3	
9	4	2.5	2.5		
10	4	3	3		
11	3	3	3		
12	2	3.5	3		
13		3	3		
14		3			
15		3			
16		3			

findings align with earlier studies that have identified trichomes in amber samples from other geographical regions. However, this study provides a unique regional perspective, contributing to the broader understanding of bioinclusions in amber. Future studies could focus on expanding the database of bioinclusions by employing advanced imaging technologies, such as confocal laser scanning microscopy and spectroscopic techniques, to gain deeper insights into the morphological and chemical characteristics of these inclusions. Comparative analyses of trichomes across different amber specimens worldwide could further enhance our understanding of ancient plant evolution and their ecological roles. Earlier studies were carried out on mosses belonging to the group of Lejeuneaceae and Pleurocarpous (Heinrichs *et al*., 2016). More work has been carried out on the zoological aspects such as on the insects (Rust *et al.*, 2010). Dabolkar and Kamat (2016, 2017) reported the presence of fossilized microbial structures in both Baltic amber and micro-amber fragments isolated from tropical beach sands in Goa, India. Using a novel sieving-microscopy technique, Amber fragments as small as 70-150 μm that contained morphologically identifiable fungal and actinobacterial inclusions were recovered. These observations suggest that tropical coastal sediments may preserve an overlooked record of ancient microbial diversity. Baltic amber specimens were microscopically ranged from 1-2 cms. Out of all the processed fragments or microamber 70% showed the presence of unidentified microbial inclusions, whereas the other 20% showed unidentified plant and animal inclusions, and 10% were void with no inclusions. Similar observations were noticed in amber from the lower Eocene in Gujarat (Alimohammadian *et al.,* 2005). Two types of trichomes were found to be present embedded inside Baltic amber the stellate hair and glandular types. The stellate hair type of trichomes was minute and radially branched, ranging between 7 to 17 radii. Stellate trichomes are made up of a central cell that can be thick, elongated, forming porrect rays, followed by a stalk made up of three to six cells, playing the role of supporting and lastly is the radiating cells that radiate out from the central cell. Stellate trichomes are star-shaped and can be found on various parts of plants, such as leaves, stems, and capsules. Although some of the trichomes studied had fewer than ten radii, the form of these trichomes is similar to that found in the Euphorbiacean angiosperm Croton (Webster *et al.*, 1996). Glandular trichomes were erect, with a unicellular glandular head and possessed a stalk that was both unicellular and multicellular. Similar observations were made on Mexican amber by using confocal microscopy, where the hair trichomes from oak trees, which led to the formation of Baltic amber millions of years ago (Clark and Daly, 2010). The trichomes studied were stellate form with seventeen radii.

Conclusions

This preliminary study of trichome bioinclusions in Baltic amber samples marketed in Goa demonstrates the potential of using such amber for paleobotanical research in India. The detection of both stellate and glandular trichomes suggests significant paleoecological and paleobotanical implications, paving the way for further research on plant-insect interactions, ancient climates, and resin-producing flora. The findings underscore the importance of developing standardized methodologies for analyzing amber bioinclusions. Moreover, this study highlights the value of interdisciplinary approaches combining microscopy, spectroscopy, and chemical analysis to enhance the resolution of paleobiological investigations.

It is necessary to develop a comprehensive database of bioinclusions from Baltic amber marketed in India and to utilize advanced imaging and analytical tools such as confocal laser scanning microscopy, FTIR and Raman spectroscopy to identify and classify inclusions with greater precision, and also to explore the paleobiogeography of resin-producing plants to understand their distribution and ecological significance during the Eocene.

Its important to foster collaborations with international amber research laboratories to share methodologies and findings, and investigate the possibility of detecting genetic material or biochemical residues in fossilized inclusions for insights into ancient plant biochemistry and genetics and conduct comparative studies with other amber types from different regions to identify unique and shared features of inclusions, and encourage citizen science initiatives to document and collect amber samples for scientific analysis and recognize that the fossilized organic forms entombed in Baltic samples have significant potential for further systematic collection and microscopic examination, aiding in reconstructing ancient environments and in analyzing distribution patterns of plants, animals and microorganisms. The detection of fossilized organic form has shown potential for further comprehensive collection and microscopic examination of such entombed Baltic samples. Such studies could help in reconstructing ancient environments and in analyzing the distribution patterns of plants, animals and microorganisms.

Authors' Contributions

SD: Data Curation, Writing- Original Draft Preparation, Visualization, Investigation, Writing- Reviewing, Editing, Software. **NMK:** Conceptualization, Supervision**,** Editing.

Conflict of Interests

There is no conflict of interest

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