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ORIGINAL RESEARCH ARTICLE



# Palynological analysis of Brazilian red propolis samples

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## Summary

Seven samples of Brazilian red propolis were analyzed using palynological methods. The samples originated from the northeastern coastal region of Brazil; three from Alagoas, three from Bahia and one from the Paraíba state. Glandular trichomes were absent in the red propolis, as were sand, earth, quartz and starch grains that normally occur in geopropolis. The presence of pollen grains of *Schinus* ("aroeira") was considered characteristic in the red propolis sediments. The quantity of pollen grains of *Protium* (Burseraceae), another possible resinous plant taxon, was very small when compared with that of *Schinus*. This tree also occurs beyond the intertidal mangrove vegetation. The three characteristic pollen types of mangrove vegetation, *Rhizophora*, *Laguncularia* and *Avicennia*, were recognized only in one sample from Bahia. Pollen grains of *Dalbergia ecastophyllum*, another red-resinous plant species occurring in the coastal areas of Alagoas, were not detected in the sediment of the propolis samples. *Cocos* pollen grains were frequent. The palynological analysis was therefore confirmed by the multivariate analysis. The red propolis may be related mainly to resin of *Schinus* in the studied samples.

## Análisis palinológico de muestras de propóleo rojo brasileño

Se han analizado siete muestras de propóleo rojo brasileño usando metodología palinológica. Las muestras son originarias de la región costera nororiental, tres de las cuales son de Alagoas, tres de Bahía y una del estado de Paraíba. Los tricomas glandulares estaban ausentes en el propóleo rojo, así como la arena, la tierra, el cuarzo y los granos rígidos que aparecen normalmente en el geopropóleo. La presencia de granos de polen de *Schinus* ("aroeira") ha sido considerada característica de los sedimentos del propóleo rojo. La cantidad de granos de *Protium* (Burseraceae), otro taxón de planta posiblemente resinosa, era muy pequeña en comparación con la de *Schinus*. Este árbol también ocurre más allá de la vegetación intermedia del mangle. Los tres tipos de polen característicos de la vegetación del mangle, *Rhizophora*, *Laguncularia* y *Avicennia*, fueron reconocidos solamente en una muestra de Bahía. Granos de polen de *Dalbergia ecastophyllum*, otra especie de planta roja-resinosa que ocurría en las áreas costeras de Alagoas, no fueron detectados en el sedimento de las muestras de propóleo. Granos de polen de *Cocos* son frecuentes. El análisis palinológico ha sido confirmado por el análisis multivariate. El propóleo rojo puede estar principalmente relacionado con la resina de *Schinus* en las muestras estudiadas.

**Key words:** pollen analysis, red propolis, cluster analysis, environment, Brazil

## Introduction

In tropical and subtropical countries, bees such as honey bees (*Apis*) and stingless bees (Meliponinae) prepare a glue which has various uses within the bee hive, and is made mainly of wax and plant

exudates or resin. It is named propolis when produced by honey bees and geopropolis when produced by stingless bees. For many years, these products have been used by native peoples in their daily life. They have been found as gifts, like hard balls, in native tombs, and have been used to seal holes and to join stone and wood pieces

together (Barth *et al.*, 2008). Propolis has acquired a high economic value today as a component in pharmacological products against inflammations and infections (Ghisalberti, 1979; Peña, 2008) in humans. It is also used in cosmetics such as hand and face creams (Marcucci and Bankova, 1999).

Black, brownish and green propolis samples from Brazil were formerly analysed using specific palynological methods (Barth, 1998; Barth *et al.*, 1999; Barth and Luz, 2003; Barth, 2004, 2006; Bastos, 2001) in order to characterize the regional vegetation from which the propolis was obtained. Chemical analysis of propolis revealed a high content of phenolic compounds (Ghisalberti, 1979; Tomás-Barberán *et al.*, 1993), and analysis of geopropolis was carried out by Bankova *et al.* (1998).

Red coloured propolis is produced in coastal and mangrove areas in the Brazilian Northeast, but has never previously been subject to palynological analysis. Chemical analysis of red propolis samples (Silva *et al.*, 2007) revealed a high content of flavonoids (Daugusch *et al.*, 2006a) adding to a disputed commercial interest. The resin origin of the red propolis produced in the northeastern states of Brazil is not at present precisely known. A first confirmation was obtained by chemical analysis of the red exudates from *Dalbergia ecastophyllum* ("rabo de bugiu") and the red propolis obtained in the same area (Daugusch *et al.*, 2006a, 2006b). In addition, several plant species produce red exudates such as *Anacardium occidentale* ("cajuero"), *Clusia* sp. (Kaminski and Absy, 2006), *Protium* sp. ("almécega, breu vermelho"), *Schinus terebinthifolius* ("aroeira vermelha") (Sawaya *et al.*, 2006), *Tapirira* sp. ("pau pombo, cupiúba") and *Vismia* ("lacre") (Absy and Kerr, 1977), but no more red propolis related data are available at the moment. Pollen analysis may be useful to characterize the composition of the local and regional vegetation, and to investigate red resin producing plant species.

## Material and methods

### Description of the samples

Seven red propolis samples were analysed. Three originated from the state of Alagoas, three from state of Bahia and one from state of Paraíba: Sample Alagoas-1: from Ilha do Porto, next to Maceió - A dark-reddish coloured glued sample obtained in 2005; Sample Alagoas-2: from a mangrove area in Marechal Deodoro - A dark-reddish sample obtained in 2005; Sample Alagoas-3: from Praia do Francês, Marechal Deodoro - A red coloured sample obtained in 2006; Sample Bahia-1: from a mangrove area in Mucuri - A red-brownish coloured sample obtained in 2004; Sample Bahia-2: from Prado - A red coloured sample obtained in 2008; Sample Bahia-3: from Camamú - A red coloured sample obtained in 2005; Sample Paraíba: from a mangrove (*Rhizophora mangle*) area in João Pessoa, in which a cashnut (*Anacardium occidentale*) orchard was found - A dark-reddish sample obtained in 2005.

### Methodology

The red propolis samples were processed using the standard methodology for propolis (Barth, 1998; Barth *et al.*, 1999), comprising extraction with ethanol followed by sediment treatment with KOH and an acetolysis mixture. Microscope slides were prepared before and after acetolysis using glycerin jelly. All samples were observed under traditional light microscopy. More than 400 pollen grains of each sample were counted (Table 1). Interpretation of the data utilised all pollen grains of plant taxa presenting 3% or more of the total count.

The multivariate analysis was performed through Principal Component Analysis (PCA) in order to verify the pollen occurrence in the samples. The matrix comprised the absolute values of all taxa found in each sample. The absolute numerical variables were transformed into natural logarithms [ $\log(x+1)$ ] using the FITOPAC program (Shepherd, 1996) and thereafter the ordination was done through a covariance matrix using PC-ORD 4.0 (Mc-Cune and Mefford, 1999).

## Results

The propolis sediments commonly presented plant tissue and debris (Fig. 2p), cell fragments and few woody pieces. Small rounded dark-brown-reddish fragments persisted after the chemical treatment, attributed to resinous cell contents. Few tector trichomes and dispersed raffides, little wax, few hyphae and dark fungal spores were recognized. A fine granulous colourless matrix occurred. 72 pollen types were recognized including family, genus and species taxa (Table 1).

### Sample Alagoas-1

Twenty five pollen types were identified. The *Mimosa scabrella* pollen type (66.5%) was dominant. In addition there were: *Borreria* (6.5%) (Fig. 2a,b,d,e,f); *Mimosa caesalpiniaefolia* (5.5%); *Cocos* (5.1%) (Fig. 2a,b,d,e); *Myrcia* (4.6%) and *Schinus* (3.2%) (Fig. 2b). Unidentified pollen types comprised 1.8%.

### Sample Alagoas-2

Twenty pollen types were identified. The most frequent pollen type was *Cocos* (35.7%), followed by *Borreria* (15.8%), *Anacardium* (8.5%) (Fig. 2n,o), *Mimosa scabrella* (4.8%), Solanaceae (4.0%), *Symphonia globulifera* (3.2%) (Fig. 2g,h) and *Tapirira* (3.1%) (Fig. 2i-l). Unidentified pollen types comprised 4.1%.

### Sample Alagoas-3

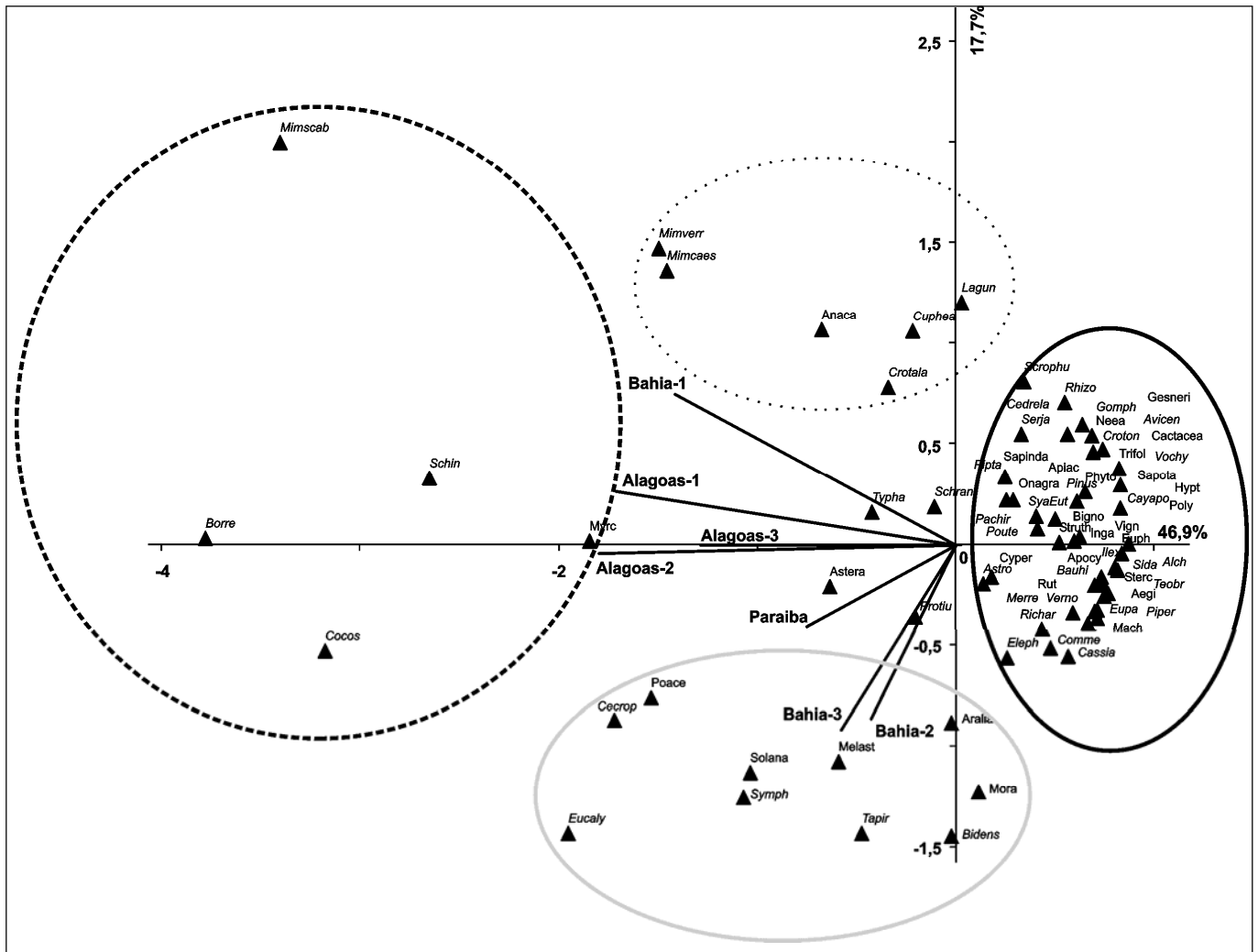
Twenty five pollen types were identified. There was no dominant pollen type. The most frequent pollen type was *Mimosa scabrella* (30.0%), followed by *Mimosa caesalpiniaefolia* (11.6%), *Borreria*

**Table 1.** Counted and identified pollen types in seven red propolis samples from North-eastern Brazil.  $\Sigma$  = sum of several pollen types.

Pollen types	Samples						
	Alagoas-1	Alagoas-2	Alagoas-3	Bahia-1	Bahia-2	Bahia-3	Paraíba
Amaranthaceae: <i>Gomphrena</i>				4			
Anacardiaceae: <i>Anacardium</i>		50		80			
<i>Schinus</i>	34	9	55	96	12	3	25
<i>Tapirira</i>	1	18			26	57	
Apocynaceae					4		1
Aquifoliaceae: <i>Ilex</i>						3	
Araliaceae			10		6	21	
Arecaceae: <i>Cocos</i>	55	210		191	4	267	54
<i>Astrocaryum</i>			25		8		
<i>Syagrus/Euterpe</i>			30				
Asteraceae ( $\Sigma$ )		4	15	2			60
<i>Bidens</i>					4	30	36
<i>Elephantopus</i>			5		10	3	
<i>Eupatorium</i>						12	
<i>Vernonia</i>						9	
Bignoniaceae				3	8		
Bombacaceae: <i>Pachira aquatica</i>	7					3	
Burseraceae: <i>Protium</i>	2	4	5		4	3	
Cactaceae				2			
Caesalpiniaceae: <i>Bauhinia</i>					2		3
<i>Cassia</i>					2	6	1
Cecropiaceae: <i>Cecropia</i>	3	11	55	6	12	111	
Clusiaceae: <i>Symphonia globulifera</i>		19	20		6	3	68
Combretaceae: <i>Laguncularia</i>	2			36			
<i>Combretum</i> /Melastomataceae	1	6	5		24	4	6
Commelinaceae: <i>Commelina</i>					4		16
Convolvulaceae: <i>Merremia</i>						2	6
Cucurbitaceae: <i>Cayaponia</i>		1					
Cyperaceae			20		4		
Euphorbiaceae: <i>Alchornea</i>						3	
<i>Croton</i>	1			1			
<i>Euphorbia</i>					2		
Fabaceae: <i>Crotalaria</i>	1	7		22			2
<i>Machaerium</i>					20		
<i>Trifolium</i>				1			
<i>Vigna</i>					2		
Gesneriaceae				3			
Lamiaceae: <i>Hyptis</i>					2		
Loranthaceae: <i>Struthanthus</i>			5		2		
Lythraceae: <i>Cuphea</i>	9			25	2		
Malvaceae: <i>Sida</i>					2		2
Meliaceae: <i>Cedrela</i>	3			1			

Table 1. (Continued).

Pollen types	Samples						
	Alagoas-1	Alagoas-2	Alagoas-3	Bahia-1	Bahia-2	Bahia-3	Paraíba
Mimosaceae: <i>Inga</i>	2				4		
<i>Mimosa caesalpiniaefolia</i>	59	1	115	16			
<i>Mimosa scabrella</i>	710	28	295	420	4		
<i>Mimosa verrucosa</i>	17	4	20	93			1
<i>Piptadenia</i>			35				
<i>Schrankia</i>	2			8	6		7
Moraceae	1				6	129	
Myrtaceae: <i>Eucalyptus</i>	3	16	60	2	310	3	33
<i>Myrcia</i>	49	11	15	11	8	21	
Nyctaginaceae: <i>Neea</i>				2			
Onagraceae	2					3	
Pinaceae: <i>Pinus</i>			5				
Piperaceae						9	
Phytolacaceae: <i>Phytolacca</i>				2	2		
Poaceae	5	5	25	4	6	21	13
Polygonaceae: <i>Polygonum</i>					2		
Rhizophoraceae: <i>Rhizophora</i>				7			
Rubiaceae: <i>Borreria</i> ( $\Sigma$ )	70	93	100	195	2	36	98
<i>Richardsonia</i>							67
Rutaceae					5		
Sapindaceae:				11	2	2	
<i>Serjania</i>			5	3			
Sapotaceae:	1						
<i>Pouteria</i>		16					
Scrophulariaceae	3			5			
Solanaceae		24	35		16	3	8
Sterculiaceae					14	1	
Typhaceae: <i>Typha</i>			25	6	2		7
Umbeliferae (Apiaceae)			5				
Verbenaceae: <i>Avicennia</i>				3			
<i>Aegiphila</i>					2	3	
Vochysiaceae				1			
Unidentified	19	24	5	15	6	18	4
<b>Total of pollen grains counted</b>	<b>1068</b>	<b>588</b>	<b>995</b>	<b>1284</b>	<b>560</b>	<b>789</b>	<b>492</b>
Pollen types identified	27	21	26	34	30	28	20
Exclusive pollen types	5	2	4	12	5	5	5



**Fig. 1.** Analysis of the main components (PCA) of the red propolis samples, using the absolute value variables per sample: Alagoas-1; Alagoas-2; Alagoas-3; Bahia-1; Bahia-2; Bahia-3; Paraíba.

(10.0%), *Eucalyptus* (6.0%) (Fig. 2m), *Schinus* (5.5%), *Cecropia* (5.5%), *Piptadenia* (3.5%), Solanaceae (3.5%) and *Syagrus* (3.0%). Unidentified pollen types comprised 0.5%.

**Sample Bahia-1**

Thirty one pollen types were identified. There was no dominant pollen type. The most frequent pollen type was *Mimosa scabrella* (32.7%), followed by *Borreria* (15.2%), *Cocos* (14.9%), *Schinus* (7.5%), *Mimosa verrucosa* (7.2%) and Anacardiaceae (6.2 %). Unidentified pollen types comprised 1.2%.

**Sample Bahia-2**

Forty one pollen types were identified. The *Eucalyptus* pollen type (55.3%) was dominant. Also identified were *Tapirira* (4.6%), *Combretum* / Melastomataceae (4.3%) and *Machaerium* (3.6%). Unidentified pollen types comprised 1.1%.

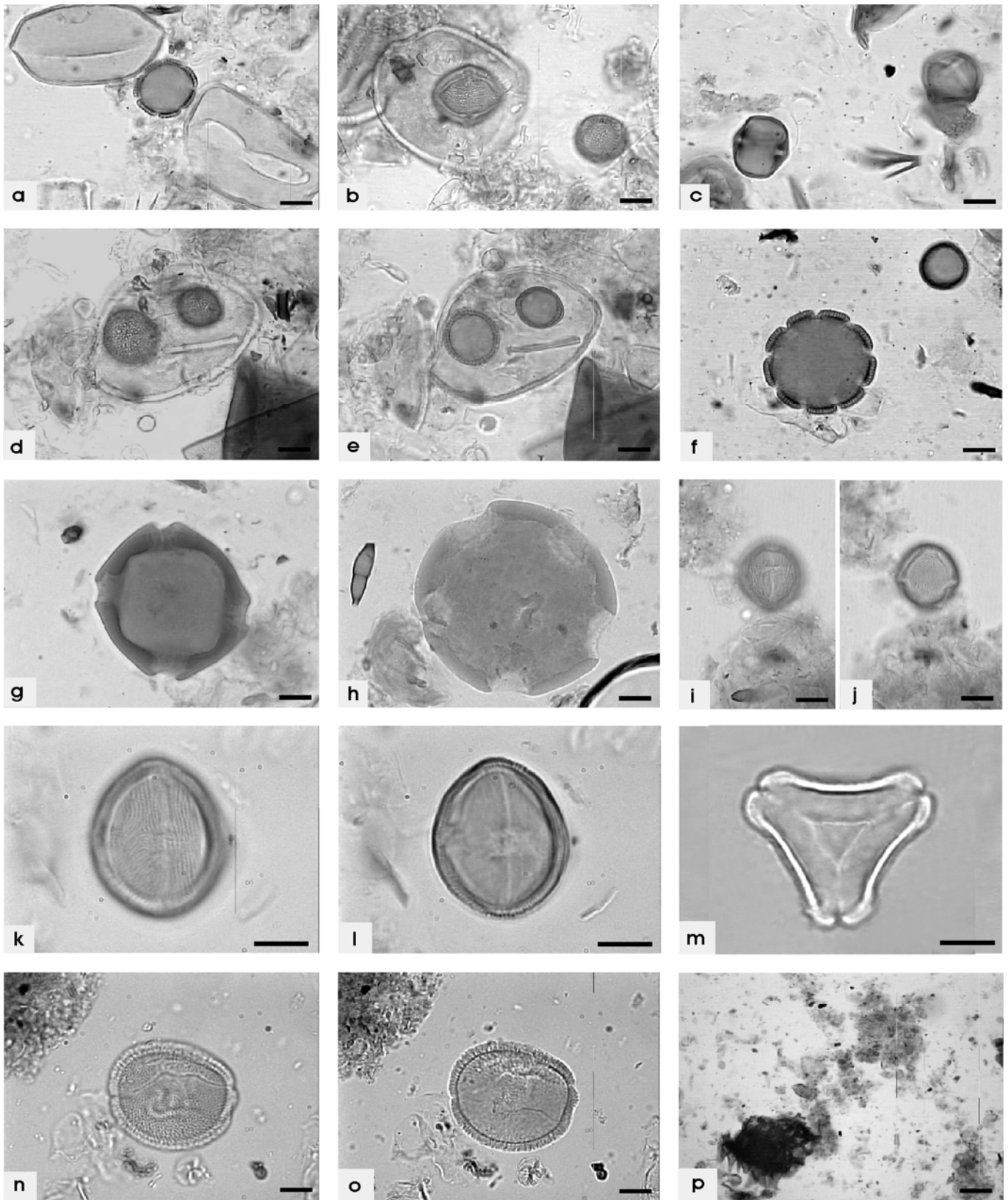
**Sample Bahia-3**

Twenty eight pollen types were identified. There was no dominant pollen type. The most frequent pollen type was *Cocos* (33.8%), followed by Moraceae (16.3%), *Cecropia* (14.0%), *Tapirira* (7.2%), *Borreria* (4.6%) and *Bidens* (3.8% ). Unidentified pollen types comprised 2.3%.

**Sample Paraíba**

Twenty one pollen types were identified. There was no dominant pollen type. The following pollen types were identified: *Borreria* (19.9%); *Symphonia globulifera* (13.8 %); *Richardsonia* (13.6%); Asteraceae (12.2%); *Cocos* (11.0%), *Bidens* (7.3%); *Eucalyptus* (6.7%); *Schinus* (5.1%) and *Commelina* (3.3%). Unidentified pollen types comprised 0.8%.

The variability between the samples of red propolis comprised 64.6% on the two first axes in the Principal Component Analysis (PCA) (Fig. 1). The axis 1 alone comprised 46.9%. Considering the



**Fig. 2.** Most frequent pollen types observed in red propolis samples from Brazilian northeast coastal areas: a. two pollen grains of *Cocos* and one of *Borreria densiflora*; b. a pollen grain of *Schinus* laying inside a *Cocos* pollen grain and *Borreria verticillata*; c. *Protium* on the left side and Anacardiaceae; d. *Cocos*, *Borreria densiflora* and *B. verticillata*, surfaces; e. *Cocos*, *Borreria densiflora* and *B. verticillata*, optical section; f. *Borreria latifolia*; g. *Symphonia globulifera*, 4-porate pollen grain; h. *Symphonia globulifera*, 5-porate pollen grain; i. *Tapirira*, equatorial view, aperture, low magnification; j. *Tapirira*, equatorial view, optical section, low magnification; k. *Tapirira*: equatorial view, ornamentation, high magnification; l. *Tapirira*, equatorial view, optical section, high magnification; m. *Eucalyptus*, polar view; n. *Anacardium occidentale*, ornamentation; o. *Anacardium occidentale*, optical section; p. Plant debris. Bar = 10  $\mu\text{m}$  (original magnifications: 400 x and 1000 x).

correlation among the pollen types, the red propolis samples showed great similarity with respect to the occurrence of the *Borreria*, *Cocos* and *Schinus* that were the main characteristic components.

## Discussion

The red propolis sediments frequently contained pollen grains of *Mimosa* (pollen types of *M. scabrella*, *M. verrucosa* and *M. caesalpiniaefolia*), *Arecaceae* (*Cocos nucifera* and others), *Cecropia* (*Cecropiaceae*), three pollen types of *Borreria* (*B. densiflora*, *B. latifolia* and *B. verticillata*, *Rubiaceae*), *Symphonia globulifera* (*Clusiaceae*), *Myrcia* (*Myrtaceae*), *Solanaceae*, *Tapirira* and *Schinus terebinthifolius* (*Anacardiaceae*).

The presence of pollen grains of *Schinus* ("aroeira") was considered a characteristic in the red propolis sediments. In two samples only (Bahia-2 and Bahia-3) its proportion was below 3%. This plant occurs commonly in the "restinga" vegetation beyond the mangroves, and may be utilised for resin supply by the bees (Sawaya *et al.*, 2006). *Cocos* pollen grain frequency remained below this limit in one sample only (Bahia-2). In all sample localities, *Cocos nucifera* was growing along the coastal areas, and its pollen may easily be identified by the presence of long raffides inside the sediments of pollen loads, honey and propolis.

Apart from sample Bahia-2, pollen grains of *Borreria* indicated areas of field vegetation. Taxa of *Mimosa*, *Pachira aquatica* (*Bombacaceae*), *Symphonia globulifera* (*Clusiaceae*) and *Cedrela* (*Meliaceae*) are representative of marsh areas, besides a great number of ruderal (field) plants, comprising several species of *Asteraceae*, *Cuphea* (*Lythraceae*), *Chenopodiaceae*, *Poaceae*, *Borreria* and *Richardsonia* (*Rubiaceae*). This assembly of taxa indicate an open-land vegetation with trees, and of hydrophytic preference.

The quantity of pollen grains of *Protium* (*Burseraceae*) (Fig. 2c), another possible resinous plant taxon, was very small when compared to that of *Schinus*. This tree occurs also beyond the intertidal mangrove vegetation. The three characteristic pollen types of mangrove vegetation, *Rhizophora*, *Laguncularia* and *Avicennia*, were recognized in one sample only (Bahia-1). Although thoroughly examined, pollen grains of *Dalbergia ecastophyllum*, another red-resinous plant species occurring in the state of Alagoas (Daugusch *et al.*, 2006b), were not detected in the sediments of the investigated red propolis samples.

The pollen types assembled inside the dashed circle using the multivariate analysis (Fig. 1: left side of axis 1) were commonly detected in all propolis sediment samples, mainly *Schinus*. Those inside the grey circle (Fig. 1: below axis 1) occurred mainly in samples Bahia-2, Bahia-3 and Paraíba, and those inside the dotted circle occurred in samples Bahia-1 and Alagoa-1, including *Laguncularia* (*Lagun*). The majority of pollen types located inside the black ellipse (Fig. 1: right side of axis 1) were of low frequency. Samples Alagoas-

1, Alagoas-2 and Alagoas-3 showed a strong similarity, as well as samples Bahia-2 and Bahia-3. The sample Paraíba was located between the samples of Alagoas and Bahia presenting common pollen types of these two states. The palynological analysis was therefore confirmed by the multivariate analysis.

Glandular trichomes were absent in the red propolis samples, but not in sediments of dark, brownish or green propolis samples (Barth, 1998; Bastos, 2001). On the other hand, when compared with sediments of geopropolis from native stingless bees (Barth and Luz, 2003), no sand, earth, quartz and starch grains could be detected. The red-brownish colour of the propolis samples may be related to resin ducts of *Schinus* formerly opened by Meliponinae, and thus introducing plant tissue fragments into the sediment.

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