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Endophytic fungal assemblage of two halophytes from west coast mangrove habitats, India

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Twenty-five endophytic fungi comprising three ascomycetes, 20 mitosporic fungi and two sterile fungi were recovered from two halophytes (*Acanthus ilicifolius* and *Acrostichum aureum*) of a west coast mangrove habitat in India. Overall colonisation of tissue segments by endophytes ranged between 74.5 % (*Acanthus ilicifolius*) and 77.5 % (*Acrostichum aureum*). Analysis using the Jaccard's similarity coefficient revealed 16–25 % similarity in endophyte assemblage among different tissues, and 24.5 % between the two hosts. Out of four tissues screened, species richness and diversity were high in stems of *Acanthus ilicifolius* and roots of *Acrostichum aureum*. The most dominant endophyte was *Colletotrichum* sp. in prop roots of *Acanthus ilicifolius*, and Yeast sp. 1 in rhizomes of *Acrostichum aureum*. Among the dominant endophytes (colonisation frequency >5 %), *Acremonium* and Yeast sp. 1 were common to both hosts. *Acanthus ilicifolius* showed dominance of a single species, (*Colletotrichum* sp.), while in *Acrostichum aureum* multiple species dominance was seen (*Acremonium* sp., *Penicillium* sp. and Yeast sp. 1). Only one typical marine mitosporic fungus (*Cumulospora marina*) was recovered from the roots of *Acanthus ilicifolius*.

Key words: mangroves, halophytes, endophytes, fungi, India

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V mangrovech na západním pobřeží Indie bylo ze dvou druhů halofytů (*Acanthus ilicifolius* a *Acrostichum aureum*) zjištěno 25 druhů endofytických hub, z toho 3 druhy askomycetů, 20 druhů anamorfních hub a 2 druhy hub ve sterilním stavu. Celková kolonizace segmentů pletiv se pohybovala mezi 74.5 % (*Acanthus ilicifolius*) a 77.5 % (*Acrostichum aureum*). S použitím Jaccardova indexu podobnosti byla zjištěna 16–25-procentní podobnost ve složení společenstva endofytů mezi různými pletivy a 24.5-procentní podobnost mezi oběma hostiteli. Ze 4 studovaných typů pletiv byla nejvyšší diverzita endofytů ve kmenech druhu *Acanthus ilicifolius* a v kořenech *Acrostichum aureum*. Dominantním endofytem byl druh *Colletotrichum* sp. v oporných kořenech *Acanthus ilicifolius* a kvasinka sp. 1 ve rhizomech *Acrostichum aureum*. Z dalších dominantních endofytů (s frekvencí kolonizace >5 %) byly druhy *Acremonium* sp. a kvasinka sp. 1 společné pro oba hostitele. U *Acanthus ilicifolius* dominoval jeden druh (*Colletotrichum* sp.), zatímco u *Acrostichum aureum* bylo dominantních druhů více (*Acremonium* sp., *Penicillium* sp. a kvasinka sp. 1). Byla nalezena pouze jedna typicky mořská anamorfní houba, a sice *Cumulospora marina* v kořenech druhu *Acanthus ilicifolius*.

INTRODUCTION

The term endophyte refers to the fungi and bacteria, which throughout or part of their life cycle invade the tissues of living plants and cause unapparent and asymptomatic infections entirely within plant tissues, but cause no symptoms of disease (Wilson 1995). Although endophytes have drawn the attention of mycologists for about 80 years (Lewis 1924), serious efforts to study them begun only in the 1970s (Bernstein and Carroll 1977, Carroll et al. 1977, Carroll and Carroll 1978). Fungal endophytes have been isolated from a variety of plant species (Wilson and Carroll 1994), generally from the temperate parts of the world (Petrini 1986, 1991). Studies on fungal endophytes of the tropical region were initiated recently (Rodrigues and Petrini 1997). Endophytic fungi have been studied at different spatial scales: from different parts of a simple leaf to a geographic scale (Carroll 1995, Taylor et al. 1999). The endophytic mycoflora of tropical plants differs from that of temperate plants (Rodrigues and Petrini 1997, Taylor et al. 1999). For instance, higher numbers of xylariaceous fungi were found in endophyte assemblages from tropical palms when compared with temperate palms (Fröhlich 1997, Petrini et al. 1995). Such difference in endophyte assemblages has been connected to climatic factors (Fisher et al. 1995, Taylor et al. 1999). Besides understanding the ecology, distribution and diversity of fungi, endophytic fungi are the centres of attraction for recognising novel metabolites of agricultural and pharmaceutical value (Strobel et al. 1996).

Among 54 mangrove tree species and 60 mangrove associate plant species, up to 55 have been studied for saprophytic fungi (Jones and Alias 1997). Recently a series of papers has been published on the foliar endophytes of mangrove plant species of the east coast of India (Kumaresan and Suryanarayanan 2001, 2002; Suryanryanan et al. 1998; Suryanarayanan and Kumaresan 2000). Ananda and Sridhar (2002) investigated the diversity of root endophytic fungi of mangrove plant species of the west coast of India. In order to fill the gap in studies on endophytes of all mangrove plant species, the present investigation has been concentrated on the endophytic assemblage of an angiosperm mangrove associate (*Acanthus ilicifolius*) and a pteridophyte mangrove associate (*Acrostichum aureum*) established in a mangrove habitat of the west coast of India.

MATERIALS AND METHODS

Sampling

The angiosperm shrub, *Acanthus ilicifolius* L. and a pteridophyte, *Acrostichum aureum* L. grow abundantly in homogeneous stands along the mangrove vegetation in the Nethravathi River mouth located about 4 km south of Mangalore,

south-west coast of India. Ten plants (about 5 m apart) of *Acanthus ilicifolius* and *Acrostichum aureum* each growing in pure stands at low-tide levels were chosen for sampling during the summer season (April-May). In April 2001 five healthy mature leaves, five pieces (about 8–10 mm diameter and 3–5 cm length) of stem, prop roots and roots were randomly cut from each plant of *Acanthus ilicifolius* for the study. From each plant of *Acrostichum aureum* five healthy mature leaves, five pieces (about 1 cm thick and 5 cm length) of rhizome and roots were sampled in May 2001. The plant material was brought to the laboratory in sterile polyethylene bags in cold pack and processed within 4 hours after sampling.

Surface sterilisation and incubation

The plant material was rinsed gently in freshwater to remove debris. From each leaf 0.5 × 1 cm segments were prepared and the rest of the material (*Acanthus ilicifolius*: stem, prop root and root; *Acrostichum aureum*: petiole, rhizome and root) was cut into segments of 1 cm length. They were surface sterilised according to the method outlined by Taylor et al. (1999) with a slight modification. Each set of plant material was immersed in 95 % ethanol for 1 min. followed by immersion in 6 % sodium hypochlorite (BDH, UK) for 6 min. and again in 95 % ethanol for 0.5 min. Later the segments were rinsed three times in sterile distilled water before plating on 1.5 % malt extract agar (MEA) medium complemented with terramycin (250 $mg.l^{-1}$; Sigma, USA). The plates were incubated at 25 ± 1 °C for up to seven weeks. The light regime was 12 hours light alternated with 12 hours darkness. Periodically the plates were screened for fungal outgrowth from the plant tissue. Wherever growth occurred, the tips of growing mycelia were transferred to fresh antibiotic-free MEA. In most plant segments fungal growth was seen after 7–10 days of incubation.

Data analyses

The colonisation frequency (% CF), number of fungi per segment and contribution of dominant endophytes (% DE) (Kumaresan and Suryanarayanan 2001) were calculated as follows:

$$\text{Colonisation frequency (\% CF)} = \frac{\text{Number of tissue segments colonised by a fungus}}{\text{Total number of tissue segments assessed}} \times 100$$

$$\text{Number of fungi per segment} = \frac{\text{Total number of isolations of fungi}}{\text{Total number of tissue segments assessed}}$$

$$\text{Contribution of dominant endophytes (\% DE)} = \frac{\text{\% CF of the dominant endophyte}}{\text{Sum of \% CF of all endophytes}} \times 100$$

The independent samples t-test (MICROSTAT, Ecosoft Inc. 1984) was applied to determine significant differences in colonisation frequency of endophytes in different tissues of the same host and between the two host plant species. The diversity (Magurran 1988) and evenness (Pielou 1975) of endophytic fungi in each type of plant material was determined. The percent Jaccard's index of similarity (JI) was calculated for all pairs of host tissues and also for the two hosts (Kenkel and Booth 1992).

RESULTS

Out of 200 segments of *Acanthus ilicifolius*, 149 (74.5 %) segments yielded 160 isolates comprising two ascomycetes, 17 mitosporic fungi and two sterile fungi (Table 1). Differences in endophyte colonisation were seen between the four tissues tested (leaf, stem, prop root and root). The highest number of stem segments (88 vs. 62–84 %) of *Acanthus ilicifolius* was colonised by endophytic fungi. The number of endophytes (12 vs. 8–11), number of isolates (51 vs. 26–48) and mean number of endophytes per segment (1.02 vs. 0.52–0.96) were also higher in stem segments than in other tissues. Among the 200 segments of *Acrostichum aureum* analysed, 155 (77.5 %) segments yielded 158 isolates comprising two ascomycetes and 14 mitosporic fungi (Table 1). The highest number of petiole segments (88 vs. 70–78 %) was colonised by endophytic fungi. Species richness was highest in root segments (11 vs. 7–10), while the mean number of endophytes per segment was higher in rhizomes (1 vs. 0.56–0.88) than in other tissues. Twelve species belonging to ten genera of fungi were common to both hosts. The independent samples t-test for difference in two group means revealed no significant difference in total colonisation frequencies between the two hosts irrespective of tissues.

Five endophytes were dominant (CF, 5 % and more) in both plant species (Table 2). *Colletotrichum* sp. was the most dominant endophyte in *Acanthus ilicifolius* (DE, 32.5 %). It showed 64 % CF in prop roots followed by 32 % CF in stems (Table 1). Yeast sp. 1 was dominant in *Acrostichum aureum* (DE, 36.8 %) and colonisation was highest in rhizomes (38 % CF) followed by roots (32 % CF) (Table 2). Among the dominant endophytes, *Acremonium* sp. and Yeast sp. 1 were common to both hosts. Table 3 shows the species richness, diversity and evenness of endophytic fungi in the four tissues of the host plants. *Acanthus ilicifolius* showed the highest species richness (12) and diversity (0.917; 3.585) of endophytic fungi in stems followed by leaves. In *Acrostichum aureum*, roots showed highest species richness (11) and diversity (0.909; 3.459) followed by leaves. The percent Jaccard's index of similarity (JI) between the four tissue types of *Acanthus ilicifolius* revealed a maximum of 25 % similarity between prop roots and roots (Table 4). In the rest of the tissues, it ranged between 16 and 23.1 %. In *Acrostichum aureum* also the maximum similarity was 25 % between leaves and roots; petioles and

Table 1. Colonisation frequency (% CF) of fungal endophytes in different tissues of *Acanthus ilicifolius* and *Acrostichum aureum*.

Endophyte	*Acanthus ilicifolius*					*Acrostichum aureum*				
	Tissues				Total % CF	Tissues				Total % CF
	Leaf	Stem	Prop root	Root		Leaf	Petiole	Rhizome	Root	
Ascomycetes										
Ascotricha chartarum Berk.	0	0	0	0	**0**	4	0	0	0	**1**
Yeast sp. 1	0	0	4	16	**5**	4	10	38	32	**21**
Yeast sp. 2	0	2	0	4	**1.5**	0	0	0	0	**0**
Mitosporic fungi										
Acremonium sp.	4	8	6	6	**6**	6	16	12	12	**11.5**
Alternaria chlamydosporus Mouchacca	2	0	2	0	**1**	0	0	0	0	**0**
Alternaria sp.	0	0	0	0	**0**	8	0	0	2	**2.5**
Aspergillus sp. 1	2	4	0	0	**1.5**	0	0	0	0	**0**
Aspergillus sp. 2	0	0	0	2	**0.5**	12	0	10	10	**8**
Aspergillus sp. 3	6	2	0	0	**2**	0	0	0	2	**0.5**
Cladosporium sp.	18	0	0	0	**4.5**	6	4	4	2	**4**
Colletotrichum sp.	4	32	64	4	**26**	0	0	0	0	**0**
Cumulospora marina J. Schmidt	0	0	0	6	**1.5**	0	0	0	0	**0**
Cytospora sp.	0	26	0	0	**6.5**	4	0	0	0	**1**
Dicyma sp.	0	4	2	0	**1.5**	4	0	0	0	**1**
Fusarium oxysporum	0	8	2	0	**2.5**	0	6	2	2	**2.5**
Fusarium sp.	0	2	14	2	**4.5**	0	2	0	2	**1**
Nigrospora oryzae (Berk. et Br.) Petch	0	0	0	0	**0**	4	4	0	4	**3**
Paecilomyces puntoni (Vuill.) Nannizzi	0	2	0	0	**0.5**	0	0	0	0	**0**
Paecilomyces sp.	0	4	0	0	**1**	0	20	0	0	**5**
Penicillium sp.	10	0	0	0	**2.5**	4	10	28	16	**14.5**
Phoma sp.	2	0	0	0	**0.5**	0	0	0	4	**1**
Pestalotiopsis sp.	2	0	0	4	**1.5**	0	0	0	0	**0**
Trichoderma sp.	0	0	0	0	**0**	0	0	6	0	**1.5**
Sterile mycelia (SM)										
SM 1	10	8	2	0	**5**	0	0	0	0	**0**
SM 2	10	0	0	8	**4.5**	0	0	0	0	**0**
Number of segments assessed	50	50	50	50		50	50	50	50	
Number of segments colonised	32	44	42	31		35	44	37	39	
Number of isolations	35	51	48	26		28	36	50	44	
Number of endophytes	11	12	8	9		10	8	7	11	
Mean number of endophytes per segment	0.7	1.02	0.96	0.52		0.56	0.72	1	0.88	

Table 2. Percent contribution by the dominant endophytes (% DE) to the assemblages in two halophytes (L – leaf; P – petiole; PR – prop root; R – root; RH – Rhizome; S – stem).

Dominant endophyte	% DE	Extent of colonisation
Acanthus ilicifolius		
Colletotrichum sp.	32.5	PR>S>L & R
Cytospora sp.	8.1	S
Acremonium sp.	7.5	S>PR & R>L
Yeast sp. 1	6.3	R>PR
Sterile fungus (SM 1)	6.3	L>S>PR
Acrostichum aureum		
Yeast sp. 1	36.8	RH>R>P>L
Penicillium sp.	25.4	RH>R>P>L
Acremonium sp.	20.2	P>RH & R>L
Aspergillus sp. 2	14.0	L>RH & R
Paecilomyces sp.	8.8	P

Table 3. Species richness, diversity and evenness of endophytic fungi in halophytes.

Host and tissue	Species richness	Diversity		Evenness	
		Simpson	Shannon	Simpson	Shannon
Acanthus ilicifolius					
Leaf	11	0.909	3.459	0.941	0.887
Stem	12	0.917	3.585	0.886	0.805
Prop root	8	0.875	3.000	0.602	0.567
Root	9	0.889	3.170	0.939	0.906
Acrostichum aureum					
Leaf	10	0.900	3.322	0.978	0.963
Petiole	8	0.875	3.000	0.938	0.897
Rhizome	7	0.857	2.807	0.872	0.814
Root	11	0.909	3.459	0.876	0.796

Table 4. Jaccard's similarity coefficients (JI, in %) of endophytes in tissues of halophytes (L – leaf; P – petiole; PR – prop root; R – root; RH – Rhizome; S – stem).

Acanthus ilicifolius		S	PR	R
	L	18.5	17.4	16.7
		S	23.1	16
			PR	25
Acrostichum aureum		P	RH	R
	L	21.7	22.7	25
		P	25	24
			RH	25

rhizomes; rhizomes and roots. The endophyte species composition between the two halophytes did not overlap more than 24.5 % even though the plants were growing in the same geographical area and exposed to similar environmental conditions.

DISCUSSION

Mitosporic fungi were more common than ascomycetes as endophytes in both halophytes studied, just as in foliar endophytes (Kumaresan and Suryanarayanan 2001, 2002; Suryanarayanan et al. 1998; Suryanarayanan and Kumaresan 2000) and root endophytes of mangroves (Ananda and Sridhar 2002) and seagrass (Devarajan et al. 2002). In decomposing mangrove litter, ascomycetes outnumbered mitosporic fungi (Kohlmeyer and Volkmann-Kohlmeyer 1991). Colonisation frequency of endophytes varies with altitude, humidity (Petrini and Carroll 1981), rainfall (Rajagopal and Suryanarayanan 2000, Suryanarayanan et al. 1998) and host susceptibility (Elamo et al. 1999, Petrini and Carroll 1981). In this study, although the overall colonisation (74.5 and 77.5 %) and mean number of endophytes per segment (0.8 and 0.79) were similar in *Acanthus ilicifolius* and *Acrostichum aureum*, about 75 % of the endophyte assemblage differed between them. Such difference between hosts from the same location reveals that endophyte colonisation depends on host species rather than edaphic or environmental factors.

In the present study, *Acanthus ilicifolius* was dominated by a single endophytic fungus *Colletotrichum* sp. (Table 1). The dominance of *Colletotrichum* sp. in prop roots of *Acanthus ilicifolius* drastically decreased the evenness indices (Table 3), whereas lack of dominance of single species in leaves and roots resulted in uniform evenness (Ludwig and Reynolds 1988). However, root endophytes of *Acanthus ilicifolius* showed multiple species dominance (*Cylindrocarpon* sp., *Phoma* sp., Sterile sp. 2 and 4; range 10–30 %). Multiple endophytic species dominance was also common in the roots of *Avicennia officinalis, Rhizophora mucronata* and *Sonneratia caseolaris* (Ananda and Sridhar 2002). Single species dominance in foliar endophytes is seen in many mangrove halophytes: *Avicennia marina* (*Phoma* sp., 15.3 %), *Bruguiera cylindrica* (*Colletotrichum gloeosporioides*, 34 %), *Rhizophora apiculata* (*Sporormiella minima*, 16.7 %), *Rhizophora mucronata* (*Sporormiella minima*, 15.7–19.3 %) and *Suaeda maritima* (*Camarosporium palliatum*, 11.7 %) (Kumaresan and Suryanarayanan 2001, Suryanarayanan et al. 1998, Suryanarayanan and Kumaresan 2000). In *Acrostichum aureum* multiple endophyte dominance was seen. Colonisation frequencies of *Acremonium* sp., *Penicillium* sp. and Yeast sp. 1 were above 10 %. These endophytes are common in all four tissues. Similarly, in leaves of *Lumnitzera racemosa* multiple endophyte dominance was seen (*Alternaria* sp., 8.3 %; *Phomopsis* sp., 10.3 % and *Phyllosticta* sp., 11.7 %) (Kumaresan and Suryanarayanan 2001). High rates of multiple endophyte colonisations have been previously recorded in roots of mangrove plant

species (Ananda and Sridhar 2002), leaves of palms (Fröhlich 2000) and temperate deciduous trees (Fisher and Petrini 1990). At the outset, although *Aspergillus* spp., *Cladosporium* sp., *Paecilomyces* spp. and *Penicillium* sp. were seem to be contaminants, they were recovered repeatedly from the surface sterilised segments of halophytes.

Acremonium sp., *Alternaria* sp., *Cladosporium* sp., *Colletotrichum* sp. and *Fusarium* sp. are common foliar endophytes of the beach halophyte *Suaeda fruticosa* (Fisher and Petrini 1987), mangrove plant species (Kumaresan and Suryanarayanan 2001, 2002; Suryanarayanan et al. 1998; Suryanarayanan and Kumaresan 2000) and the seagrass, *Halophila ovalis* (Devarajan et al. 2002). In this study, except for *Colletotrichum* sp. all the above species are common to both hosts and found in almost all tissues screened. In fact, *Acremonium, Alternaria* and *Cladosporium* are not host-specific and hence found in different tissues and hosts (Petrini et al. 1982, Suryanarayanan et al. 2000). Among endophytes, *Colletotrichum* spp. are most frequent in tropical plants, particularly in the *Musa acuminata* species complex in Hong Kong and Australia (Brown et al. 1998), and in mangrove plant communities of India: *Acanthus ilicifolius, Arthrocnemum indicum, Sesuvium portulacastrum, Avicennia marina, Bruguiera cylindrica, Ceriops decandra, Excoecaria agallocha* and *Lumnitzera racemosa* (Kumaresan and Suryanarayanan 2001, Suryanarayanan and Kumaresan 2000). In the present study, *Colletotrichum* sp. is most dominant in *Acanthus ilicifolius* prop roots and stems. This suggests a high adaptability of *Colletotrichum* spp. for an endophytic life style in mangrove halophyte plant communities too. In the present study, *Acremonium* sp. and *Colletotrichum* sp. were dominant endophytes in all tissues of *Acanthus ilicifolius*. Sadaba et al. (1995) observed increased occurrence of *Acremonium* sp. and *Colletotrichum gloeosporioides* in different parts of standing senescent *Acanthus ilicifolius* of Mai Po Mangrove of Hong Kong. Dominance of *Acremonium* and *Colletotrichum* in senescent standing wood of *Acanthus ilicifolius* indicates the role of these endophytic fungi in decomposition (Kumaresan and Suryanarayanan 2002). *Phomopsis* spp. and *Phyllosticta* spp. are common foliar endophytes in many mangrove plant species (Kumaresan and Suryanarayanan 2001, 2002; Suryanarayanan et al. 1998; Suryanarayanan and Kumaresan 2000). *Phomopsis* spp. were also common root endophytes of *Avicennia officinalis* and *Rhizophora mucronata* (Ananda and Sridhar 2002). But in our study, neither *Phyllosticta* nor *Phomopsis* sp. were recovered. Similarly, yeasts were not recorded in mangrove plant species so often as endophytes possibly due to the kind of media employed. Yeast sp. 1 is a dominant endophyte in both halophytes in the current study (Table 2) but we were unable to identify it in our laboratory.

According to Petrini (1986) a few endophytic fungi dominate a single host plant species. The dominant endophytes were different for each host plant species in a mangrove community (Kumaresan and Suryanarayanan 2001). In this study,

Colletotrichum sp. was dominant in prop roots (64 %) of *Acanthus ilicifolius*, which also colonised other tissues (leaf, stem and roots). Similarly, Yeast sp. 1 was dominant in rhizomes (38 %) followed by roots (32 %) although it was found in leaves and petioles of *Acrostichum aureum*. This shows the preference of specific tissue of host by *Colletotrichum* and Yeast sp. 1. Out of 25 endophytes in the two halophytes, 16 were isolated five or more times (Table 1). Differences in assemblage and frequencies of endophytes in different tissue types of a given host plant have been recorded (Rodrigues 1994, 1996). In fact, different tissues have been considered distinct microhabitats for endophytes (Petrini et al. 1992). The variations in endophytic fungal density in different tissues of the halophytes studied reveal that some selection operates in constituting the endophytic assemblages of tissues besides selection in each mangrove plant species (Kumaresan and Suryanarayanan 2001).

Among the endophytic fungi, the only known marine mitosporic fungus recovered was *Cumulospora marina*, which was isolated from the roots of *Acanthus ilicifolius*. Occurrence of *Cumulospora marina* accounts for 4 % of the total endophytes recovered. Although plant detritus on coastal sand dunes harbour several marine fungi, they were not dominant (13 %) root endophytes of coastal sand dune halophytes (Beena et al. 2000). Similarly, marine fungi were not dominant endophytes of roots of mangrove plant species (Ananda and Sridhar 2002). Due to paucity of information on endophytes of halophytes and mangrove plant species, based on the present pilot study and the available literature, definite conclusions cannot be drawn like that in the case of the palm endophytes (Fröhlich et al. 2000, Taylor et al. 1999).

In summary, root endophytes of 200 segments each of *Acanthus ilicifolius* and *Acrostichum aureum* of the west coast of India yielded 25 fungi in which terrestrial mitosporic fungi dominated. *Acanthus ilicifolius* showed single species dominance (*Colletotrichum* sp.), while *Acrostichum aureum* had multiple species dominance (*Acremonium* sp., *Aspergillus* sp. 2, *Penicillium* sp. and Yeast sp. 1). Except for *Cumulospora marina* (in roots of *Acanthus ilicifolius*) no marine fungi were recovered. It seems the endophytic fungal assemblage of herbaceous plants and tree species of mangroves differs. Mangroves are important forest ecosystems confined to tropics and subtropics. Future studies on different hosts in a wide geographic range, in different seasons, tissue types and age classes might reveal more on the endophytic fungal status and their significance.

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