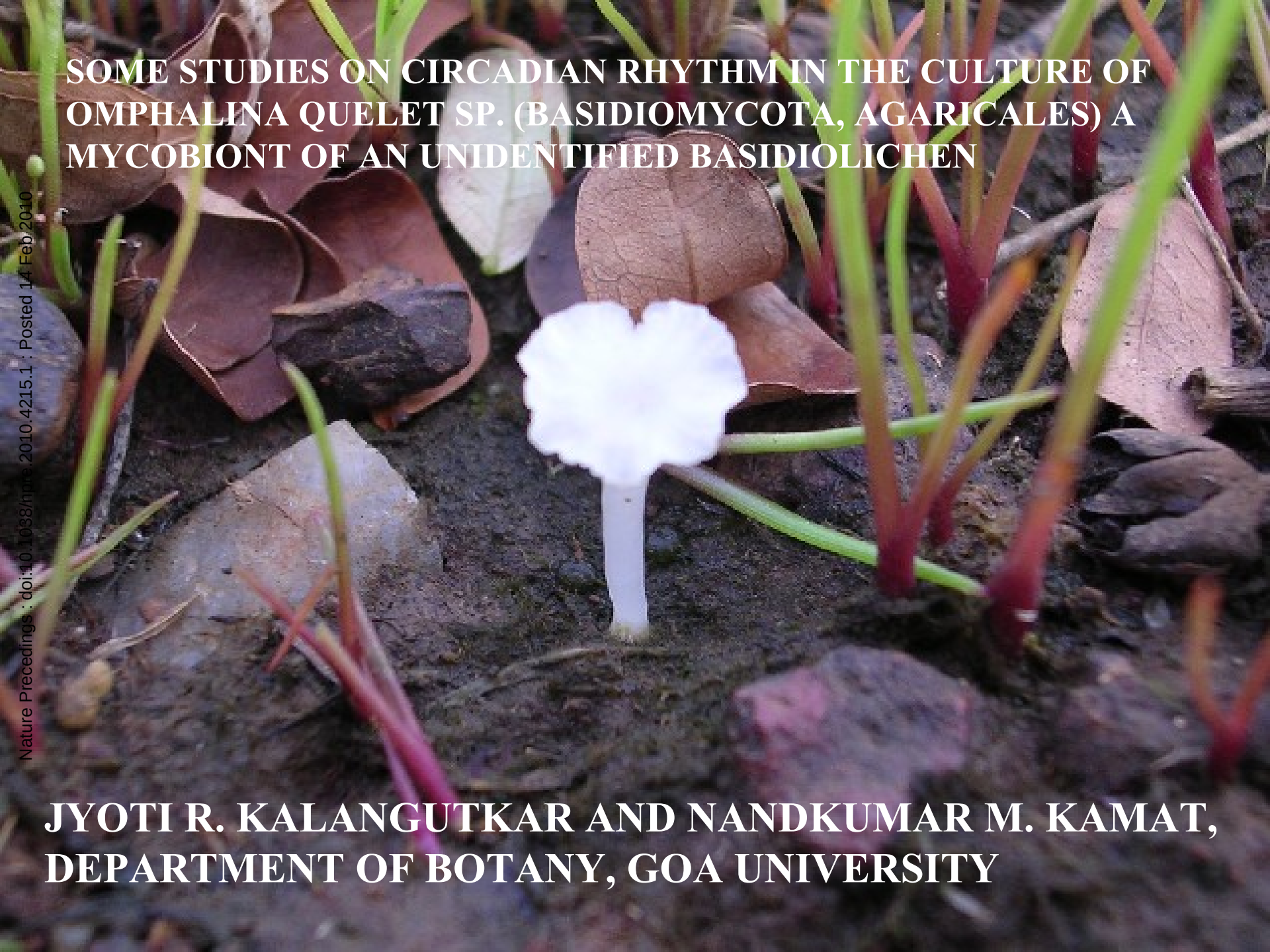
SOME STUDIES ON CIRCADIAN RHYTHM IN THE CULTURE OF OMPHALINA QUELET SP. (BASIDIOMYCOTA, AGARICALES) A MYCOBIONT OF AN UNIDENTIFIED BASIDIOLICHEN



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INTRODUCTION

- Circadian rhythms have been well studied in fungi. So far it has been reported only in two basidiomycetes, *Pellicularia filamentosa* and *Spaerobolus stellatus*.
- It has been reported that fungi exhibiting circadian rhythms do so to avoid injury from dessication and harmful UV radiations.
- This paper reports the discovery of circadian rhythm in *Omphalina* Quelet, in pure culture under laboratory conditions.
- Genus *Omphalina* includes approximately 40 species, eight of which are lichenized.
- Although lichen mycobiont are selective in choice of *Nostoc* symbionts, several fungi may often share identical cyanobiont strain.

Objectives

- **Collection and identification of the mushroom specimens associated with wet attached algal mats**
- **Confirmation of the phyco and mycobionts**
- **Tissue culture of *Omphalina* sp.**
- **Study of growth and colony morphology**
- **Assessment of the effect of light on the culture and the spectral characteristics of the pigment**

Materials and Methods

Fresh fruit bodies of *Omphalina* sp. collected (Goa University campus)

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Cleaned with wet cotton

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Specimens microscopically examined to identify the phycobiont associated with mycobiont

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Pileal tissue from mycobiont removed aseptically

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Washed with sterile distilled water

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Inoculated on MEA media plates

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Incubated at room temperature (28-30^0 C)

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Fresh growth from the tissues subcultured on fresh plates for purification and the cultures were maintained on MEA without antibiotics and subcultured every month. After screening the isolates, fast growing strains were used for further studies.

Studies on Colony morphology

Sections of 7 day old colonies from three different regions, i.e. central, middle and peripheral growth zones were asceptically dissected.

Slides were made in plain Lactophenol, Lactophenol with cotton blue and Ammoniacal congo red (glycerol as mounting medium) and observed microscopically.

Studies on the pigment:- The pink pigment from the colonies was extracted by macerating the agar blocks in Ethyl acetate and Methanol and the UV-Visible spectra was recorded using Shimadzu double UV-Visible Spectrophotometer in 190-750 nm range in Quartz cuvettes

Detection of circadian rhythm

The colonies were initially studied on MEA to see the response to changing light conditions. Plates were then kept under light and dark conditions to verify the growth form. Colonies which consistently showed concentric growth zones with thick and thin bands were scored as positive for circadian rhythm. This experiment was repeated 3-4 times using inoculum of different age and from different zones of the colony to verify "circadian response" as a dominant character of the *Omphalina* culture.

Results –1. The basidiolichen

Habit: single or groups of two-three

Habitat: Mycenoid. Found growing on greenish, black, soft, wet moss, under unidentified grasses and appear only between last week of June and first week of July.

Taxonomic diagnosis of *Omphalina* Quelet tax. sp.

PILEUS 1-2 cm, convex at first, with disc depressed and margin reflexed, expanding to broadly funnel-shaped, but often remaining with a spreading margin; hygrophanous, milky white, smooth, prominent striations remaining moist, context opaque, thin, pliant or fragile, whitish, fibrous

GILLS long decurrent, close to subdistant, rarely distant, narrow at mid-portion to moderately broad, forked at times, usually not intervened; white, decurrent, widely spaced; fade to creamy in age

STIPE 1-2 cm x 0.1-0.4cm, equal or nearly so, central or eccentric, slightly enlarged and bulbous at base, stuffed soon hollow, rather brittle, compressed at times; waxy white, faintly pruinose at first but soon naked and polished, base with slight white mycelium, smoothly transiting and embeded into a greenish slimy algal mat/moss

SPORE PRINT:- (ON GLASS)Thin, white

BASIDIOSPORES:- (6)7-9(11) x 4-5(6) µms, elliptic to broadly elliptic or oval, smooth, inamyloid,

BASIDIA :-mostly 4-spored but sometimes 1-, 2-, or 3-spored, 21-31(35) x 5-8 µms; clamp connections present, spores 7-10 x 4.5-6 µms, almond-shaped

Specimens examined:-GUMH-omph1/04,omph2/04,omph3/04, Coll. By N.Kamat, near ponds, Goa University campus, Taleigao, Goa, July 2004

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A mature specimen of *Omphalina* Quelet tax. Sp. with attached algal mat and soil

Results

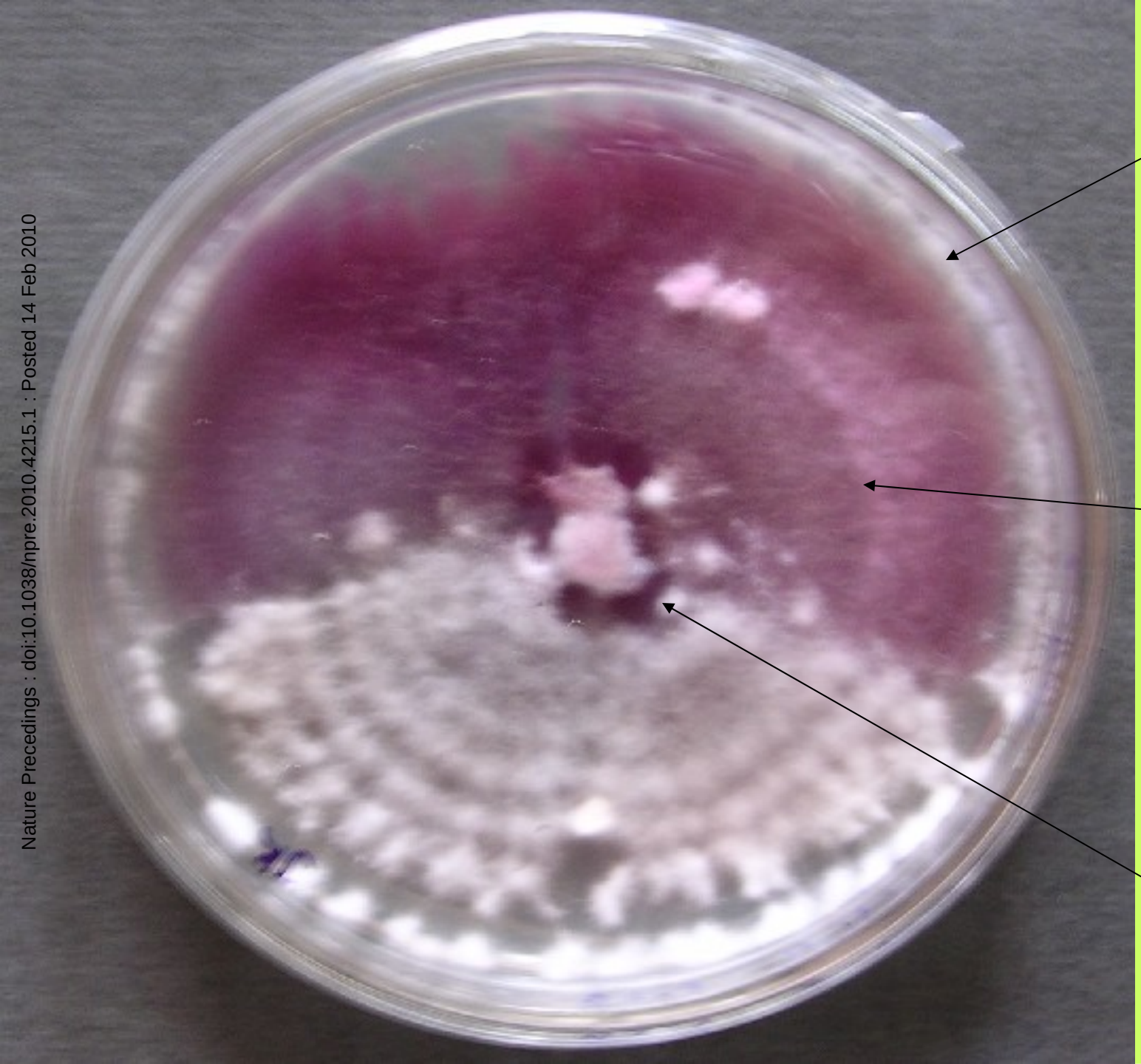
Micro-morphological features of *Omphalina* vegetative mycelial cultures

All the three zones central, middle and peripheral growth zones showed two types of hyaline hyphae, narrow and wide, with narrow being the dominant form.

1. Central zone: slow dense growth zone

Some hyphae with large and a few with short cells, associated with different types of chlamydospores such as intercalary and terminal. Septa with clamp connections. Some special modifications of hyphae are seen such as ringlets, spiral and knots.

2.The middle zone showed two growth forms, ”slow dense” and “fast effuse”



Results

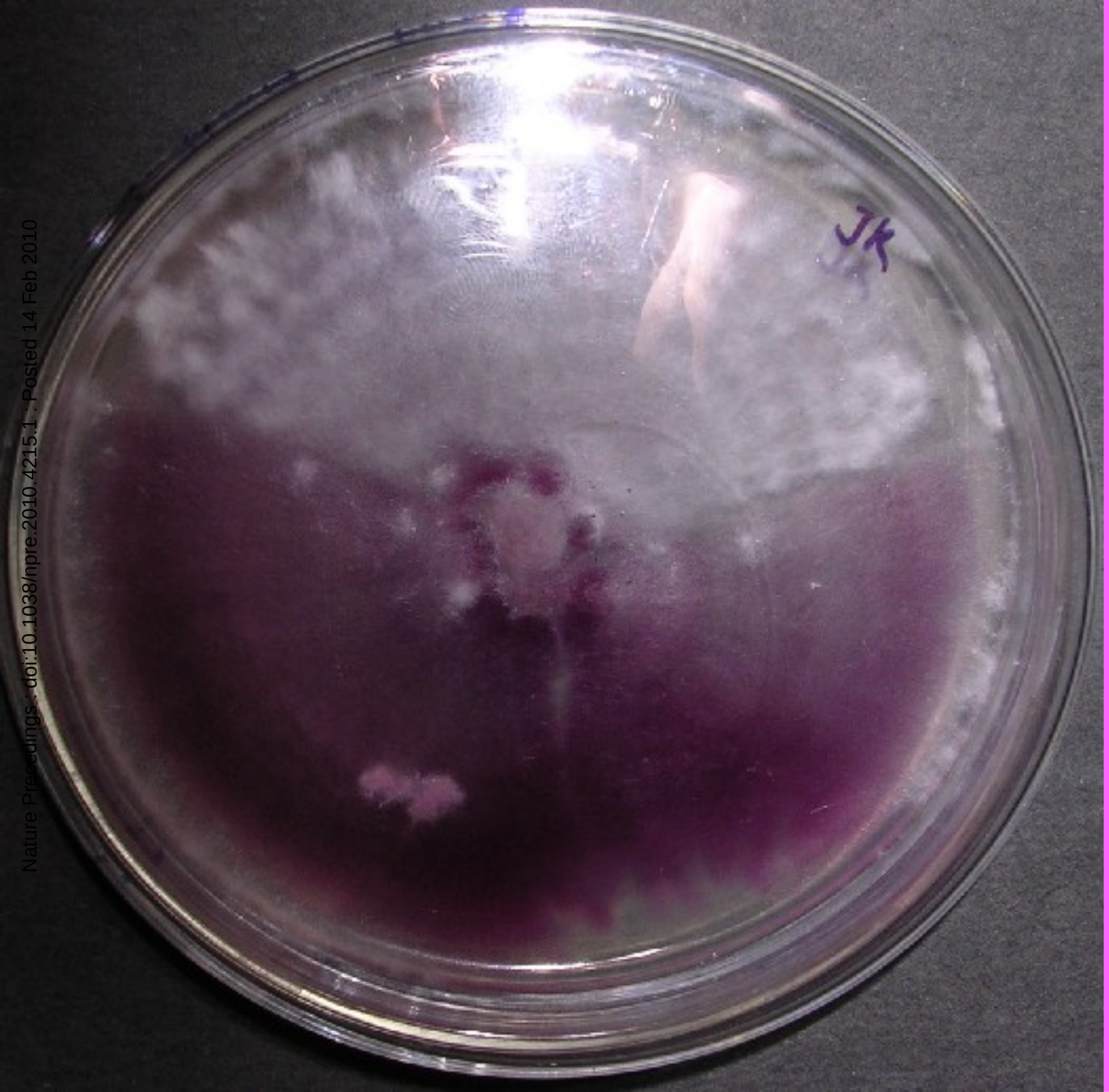
Middle slow dense zone:

Hyphae hyaline. Chlamydospores present, but number of chlamydospores are less as compared to central slow dense zone. Special modifications of hyphae are seen such as knots, ring, H-shape junctions etc.

Middle fast effuse zone: Chlamydospores are completely absent in this zone. Special modifications of hyphae such as rings, cords and various types of hyphal junctions are seen.

Peripheral slow dense zone:

Chlamydospores absent. Knots and cords are seen.

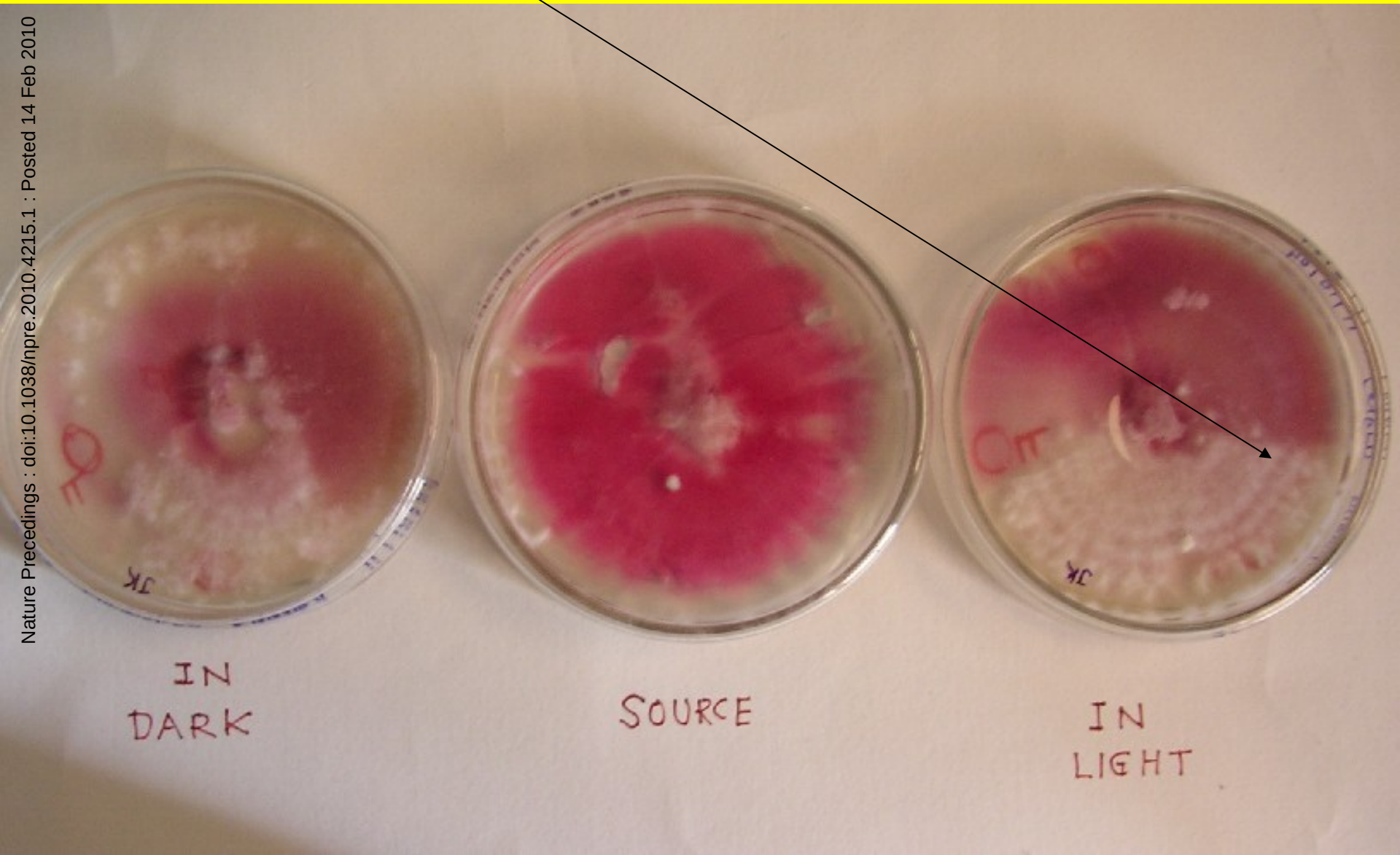


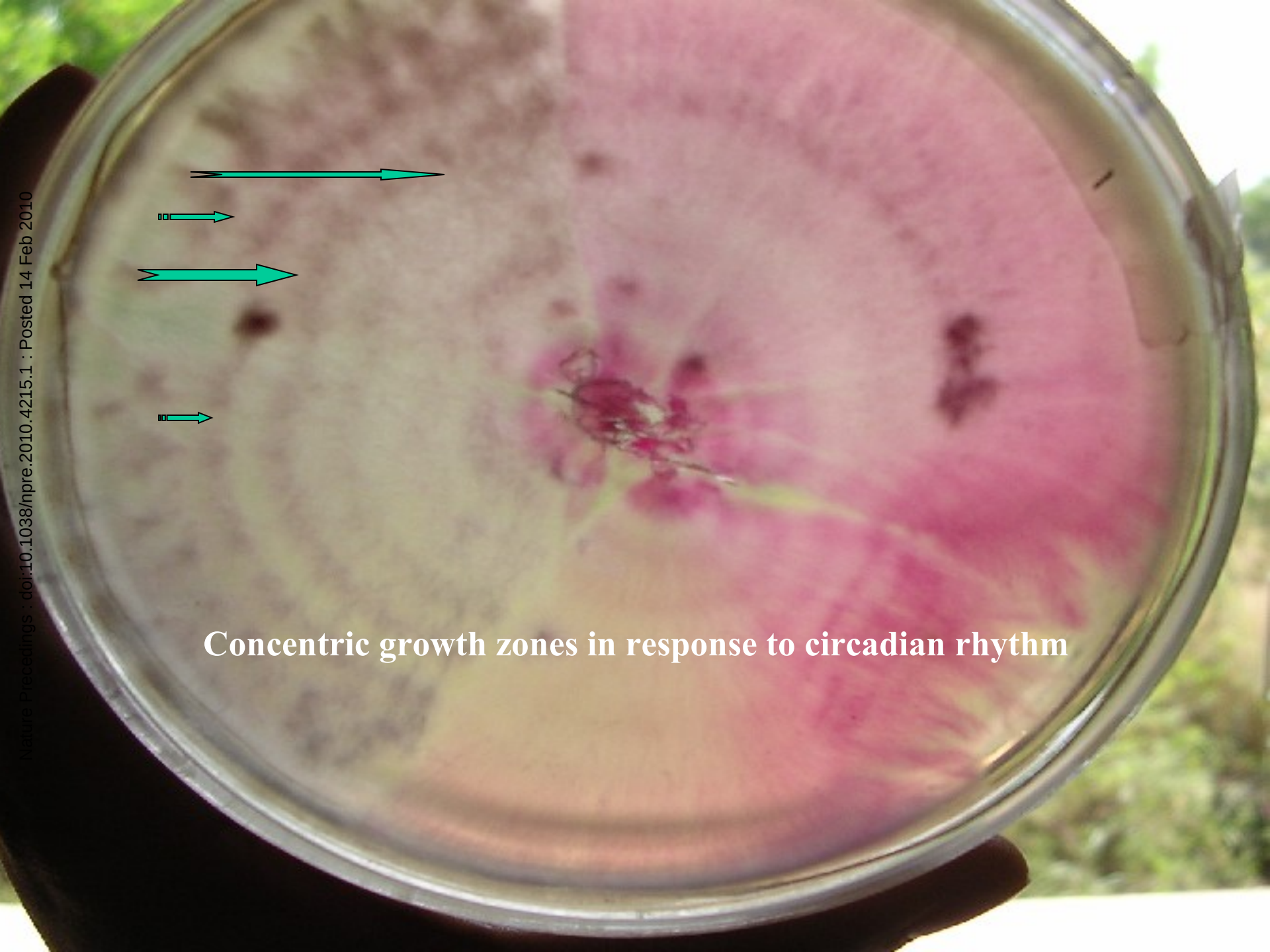
Certain sectors of Omphalina colonies produce a dark pink pigment after seven days on MEA, whereas the non productive or pigment less sectors do not show this property and remain so despite subculturing on fresh medium

Omphalina cultures respond to natural day-night cycle

- *Omphalina* colonies consistently incubated under ambient light showed typical concentric zones which corresponded to a 24 hours day-night rhythm
- The colonies seem photosensitive and produce pigment with higher intensity when exposed to light as compared to cultures grown in dark or in less light

The effect of light and darkness on *Omphalina* colonies. A distinct concentric growth pattern is seen in the plate incubated under ambient light





Concentric growth zones in response to circadian rhythm

A clear, distinct and remarkably stable and reproducible circadian growth pattern was identified in *Omphalina* colonies

RESULTS

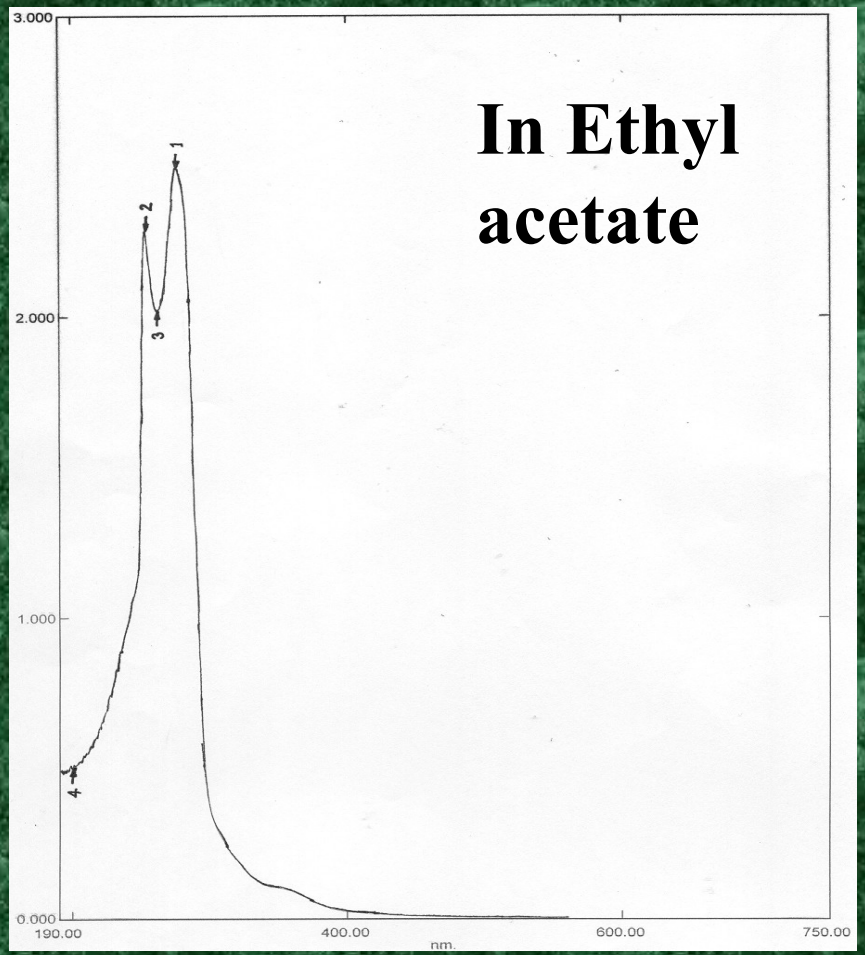
UV-Visible spectral absorption profile of the pigment in Ethyl Acetate and Methanol

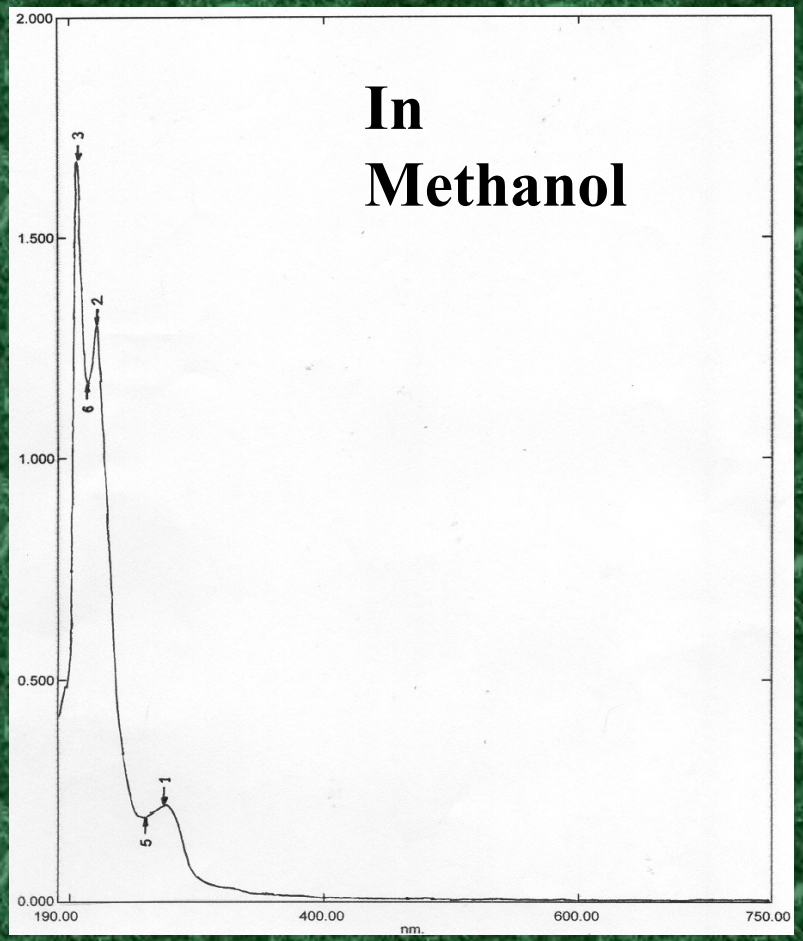
TABLE 1. WAVELENGTH AND ABSORPTION (ETHYL ACETATE)

Sr. No	Wavelength (nm)	Absorbance
1	275.50	2.494
2	253.00	2.288
3	261.50	2.018
4	199.50	0.498

TABLE 2. WAVELENGTH AND ABSORPTION (METHANOL)

Sr.No	Wavelength	Absorbance
1	274.00	0.216
2	220.50	1.302
3	205.50	1.677
4	531.50	0.003
5	258.50	0.187
6	231.50	1.171





UV-Visible spectral profile of the pink pigment from Omphalina sp.

Discussion

- **This is the first report of an Omphalaceous cyano-lichen from India and Goa**
- **Although specific name has not been assigned to the taxa the mycobiont shows close affinity to *Omphalina ericetorum* (Fr.) M.Lange Macromycetes Pt. II, Greenland Agaricales, Meddel. om Groenland 147: 25. 1955; Phytoconis ericetorum (Persoon:Fries) Redhead & Kuyper, Gerronema ericetorum (Pers.:Fr.) Singer**

The basidiolichen is a novel finding

- It has not been possible to establish the bi/tripartite nature of the basidiolichen. The basal mycelium of *Omphalina* fruit bodies was found to be interwoven and ramified in the matrix of an unidentified algal species *Nostoc*. We do not rule out the possibility of one more phycobiont. We have also noticed the ecological, evolutionary and mycogeographical significance of the basidiolichen and would plan more exhaustive studies in future.
- Very few basidiomycetous mycobionts have been cultured. This is first report in the world on a photosensitive *Omphalina* culture in which we have also observed mitospores as terminal and intercalary chlamydospores Whether such asexual propagules are formed in nature is an unanswerable question at present.

First report in the world on Circadian rhythm in a mycobiont basidiomycetes culture

This is also the first report on Circadian rhythm in vegetative stage of cultures of mycobiont basidiomycetes species

We postulate that *Omphalina* culture may have a dominant 'Clock gene' which regulates the circadian rhythm

The photosensitive cultures also produced a characteristic pink pigment which showed higher intensity after exposure to light. This process is biochemically and ecologically important.

Pigment synthesis is related to photosensitivity

- The UV absorption of pigment in ethyl acetate at 200, 253, 262 and 276 nms clearly showed that its synthesis may be a protective defence mechanism against natural UV radiation to which the mycobiont mycelium might be getting exposed in its' natural habitat
- The circadian clock in *Omphalina* sp. might exhibit an ecologically tuned response considering its symbiotic associationship with the phycobiont.

Thank You