this concentration was used in subsequent singlet oxygen quenching experiments.

Singlet oxygen quenching experiments were carried out in the presence of a mixture of HP (2.5 ppm) and NaN_3 (40 ppm) in distilled water. Control experiments with HP and azide alone were also set-up. Each solution was exposed to direct sunlight. Mortality of late 3rd instar larvae of *A. aegypti* under different conditions is summarized in Table 4.

In HP alone, the larval mortality was 90% in 3 h, and the value dropped to 40% in the mixture containing NaN_3/HP. This suggests that azide ions cause significant lowering of photodynamic effect of HP by quenching singlet oxygen.

Our results show that porphyrin derivatives, in the presence of light, exhibit a strong photodynamic effect on the 2nd and 3rd instar larvae of *A. aegypti* under laboratory conditions. In order to assess the toxicity effects of porphyrins against non-target, ecologically important aquatic fauna, the following were tested under laboratory conditions in glass tanks (12″ × 14″): Mayfly larvae (Ephemeroptera: Leptophebiidae; 10 larvae/50 ml); tadpoles (*Bufo melanostictus*; 10 tadpoles/100 ml) and guppies (*Poecilia retculata*; 10 guppies/1000 ml). Mayfly larvae and tadpoles did not show mortality even at 100 ppm levels, which is forty times higher than the concentration where mosquito larvae showed 100% mortality (2.5 ppm). Guppies survived for 30 days at 100 ppm in the presence of porphyrins. This apparent non-toxicity of porphyrins towards non-target freshwater fauna may be accounted for by the hard body cover of the organisms, which blocks the penetration of light. Thus, even if the particles are ingested in large quantities, they would remain inactive due to lack of light reaching them. Porphyrins show a high level of safety to humans and certain mammalian species. (The acute LC_{50} for systematically injected HPDHC is around 300–400 mg/kg body wt)[15].

Both HP and HPDHC are efficient candidates in photodynamic killing of *A. aegypti* larvae, which are the major vectors of dengue fever. The undetectable phototoxic effect against other aquatic fauna even at high concentration of porphyrins (100 ppm), reveals that the photodynamic approach for control of dengue mosquito would have no apparent impact on other non-target aquatic fauna.

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Studies on reproductive biology of a threatened tree fern, *Cyathea spinulosa* Wall. ex Hook

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***Cyathea spinulosa* Wall. ex Hook. is a highly-prized ornamental and economic tree fern and a significant component of tropical forests in southern, central and northern India. Currently, it is under threatened status and listed in the Red Data Books. Since the successful colonization of a fern in new habitats is dependent on gametophyte generation, the present study was conducted to observe the reproductive biology of the species. Sex ontogeny showed male gametophytes to hermaphrodite condition and the gametophytes remained bisexual for considerable period of time. Regeneration of gametophyte was common; the regenerated portions bore both sexes and produced sporophytes extensively. The reproductive behaviour revealed considerable success in sporophyte production through intragametophytic selfing. This shows that the species is of lesser genetic diversity and the gene pool is charged with lesser amount of genetic load, and is a good colonizer. In contrast, the taxon is under threatened status. The cause of this and the probable mode of conservation are also discussed here.**

ACCORDING to the literature, among the 11 species of *Cyathea* in India, *C. spinulosa* and *C. contaminans* are widely distributed and reported throughout mountainous regions[1]. *C. spinulosa* is a highly-prized ornamental and economic fern with an arborescent growth habit. This species is distinguished from other *Cyathea* species in having conspicuous brittle spines on the frond bases with shiny brown scales. Fronds are dark green and finely divided. This terrestrial

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species is distributed in subtropical regions of India, Thailand, China, Taiwan and Japan.

C. spinulosa is now under threatened status and listed in the Red Data Books. In central India, it is on the verge of extinction[2]. During a survey in Pithoragarh district, Kumaon Himalayas, this taxon has been collected from only two localities in a particular niche: Debbicheena (1360 m; 29°52′N, 80°15′E) with a population of less than 30 individuals and Pamtori (1250 m; 29°55′N, 80°8′E) with a population of less than 15 individuals. In these localities, the atmospheric humidity is quite high due to the presence of perennial water streams. The age of the population of plants in the above localities seems to be not very old, as the average plant height is 4–6 ft only and the populations are increasing every year[3].

Taking into consideration the occurrence of the population and its increasing tendency on the one hand and its threatened status on the other, the present investigation was conducted to study its reproductive biology.

Spores were collected from plants growing in Pamtori, Kumaon Himalayas. The Debbicheena population had no spore-producing plants. Collected spores were stored in a desiccator in the laboratory. After drying, the spores were surface-sterilized with 2% sodium hypochlorite solution for 2 min, followed by rinsing with double-distilled water thrice. These surface-sterilized spores were sown on Parker and Thompson's nutrient medium in glass petri dishes of 80 × 70 mm size. The pH of the medium was adjusted to 5.6. All the cultures were kept in a culture room at 22 ± 2°C and light intensity of 2500–3000 lux for 24 h. Spore germination, gametophyte differentiation and sex ontogeny were observed under Nikon trinocular microscope and photographs were taken using a Nikon camera UF-II.

Observations on spore germination, and growth and differentiation of gametophytic stages were recorded periodically. The spores were trilete, tetrahedral, aperinous and about 40 × 45 μm in size. After 7 days of sowing, more than 90% spores were able to germinate. The viability of the spores declined after three months of storage and was totally lost within six months.

On germination, the first wall was laid down parallel to the polar axis, and the spore divided into two unequal cells, the larger one prothallial initial and the smaller one rhizoidal initial. The rhizoidal initial elongated and developed into an elongated, hyaline, unicellular rhizoid. The first cell division and elongation of the prothallial initial was in a plane parallel to the equatorial plane of the spore. Such type of spore germination is termed as equatorial and *Cyathea*-type[4] and the prothallial development is of *Adiantum*-type[4]. The apical meristematic cells appeared at the spatulate stage and after repeated divisions these became a mature thallus, which was typically a heart-shaped structure (Figure 1 *a*). The spore took about 30–35 days after sowing to differentiate into a cordate thallus. The mature thallus consisted of a cushion in the central region with wings on both sides (Figure 1 *b*). These prothalli were long-lived and slightly elongated with age. Several rhizoids also developed from the lower portion of the ventral surface of the thallus. Pluricellular, elongated, thin-walled, chlorophyllous hair developed on both surfaces of the thallus (Figure 1 *c*). These hairs developed both before and after initiation of development of sex organs. Frequency of hairs was higher on ventral surface than on the dorsal side.

Before development of the sex organs, gametophytes from stock cultures were picked up randomly and separated as below, to test for intragametophytic and intergametophytic selfing: (i) Isolate (A): One prothallus per petri dish; 50 such replicates were raised. (ii) Composite cultures (A × A): Twenty prothalli per petri dish from one plant; 10 such replicates were raised.

The stock cultures were observed periodically and different ratios of gametophyte bearing male and female or bisexual conditions were recorded (Table 1). When the gametangial initiation was seen in stock culture then all the isolates and composite populations were watered from above with sterile distilled water, twice a week to facilitate fusion of gametes. Percentage of sporophytes produced at each level was recorded (Table 2). The stock cultures were kept unwatered to test the apogamous nature of the taxon.

Chronological sex ratio showed that after 35 days of spore-sowing, antheridia initiated first and after 40 days, all the gametophytes became bisexual. It was observed that all the gametophytes remain bisexual up to 70 days of spore-sowing. The antheridia were located towards the rhizoidal end as well as wings of the gametophytes and archegonia below the notch on the midrib. Both the sexes developed in succession.

In this taxon, it has been observed most significantly that the antheridium took at least 30 min to burst and release the antherozoids (Figure 1 *g*). Thus the species requires prolonged availability of water for releasing antherozoids for mating.

Table 1. Chronological changes in sex ratio in a composite culture of *Cyathea spinulosa*

Days after sowing	Sample size	Neuter	Male	Female	Bisexual
35	20	14	06	–	–
40	20	–	20	20	20
45	20	–	20	20	20
50	20	–	20	20	20
55	20	–	20	20	20

Table 2. Percentage sporophytes produced in different *in vitro* populations of *C. spinulosa*

Population	Gametophytes studied (number)	Sporophytes produced (number)	Sporophytes produced (%)
Isolate (A)	50	11	22
Composite (A × A)	200	159	79.5

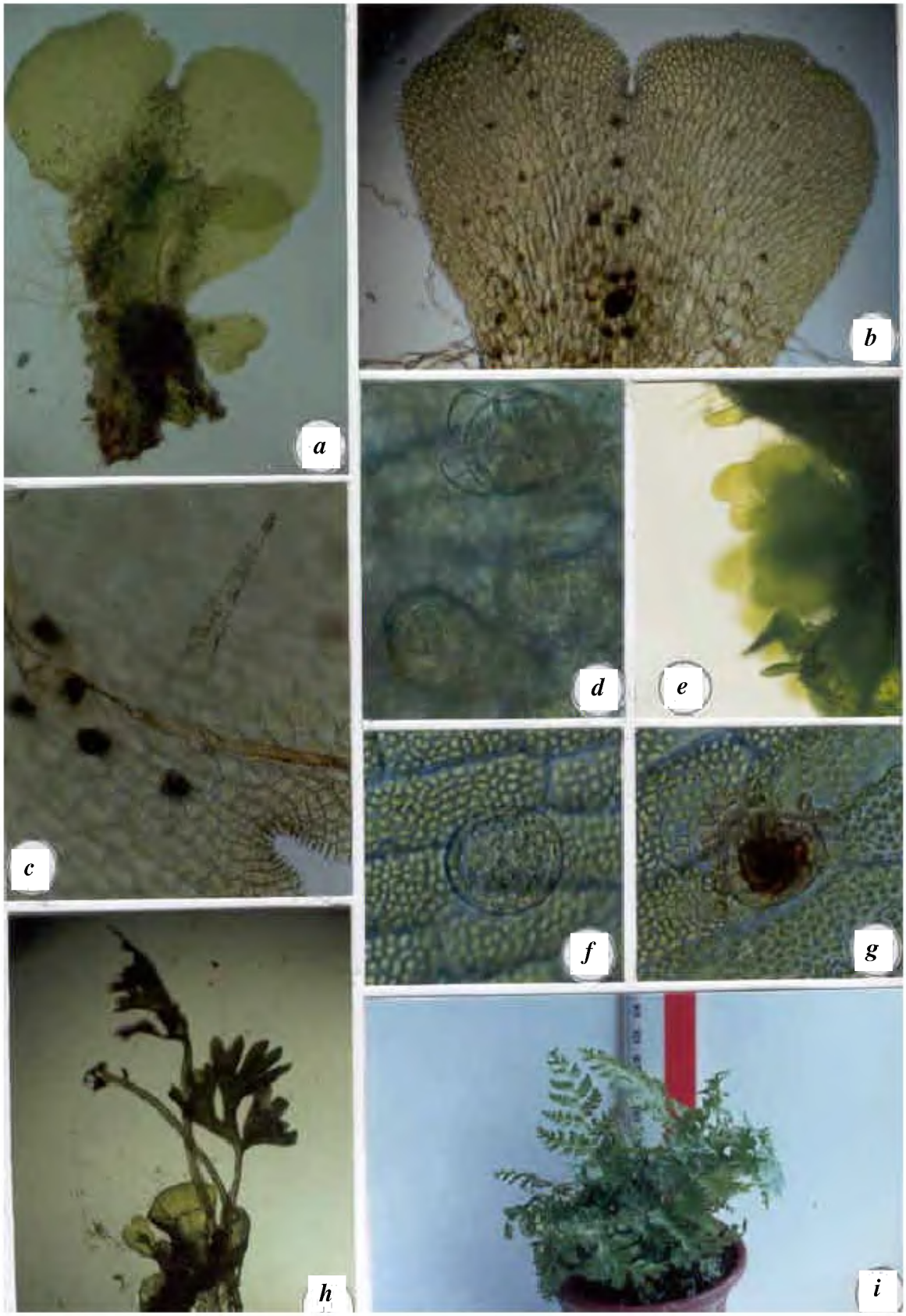

Figure 1. ***a***, Mature gametophyte; ***b***, Wings of gametophyte showing antheridia and archegonia; ***c***, Portion of gametophyte showing papillate hair and spent-off archegonia; ***d***, Close view of archegonia; ***e***, Regenerated gametophytes from lower portion of parent gametophyte; ***f***, Mature antheridium filled in antherozoids; ***g***, Antheridium showing release of anthrezoids; ***h***, Juvenile sporophytes arising from gametophytes. Regenerated gametophytes are also seen; ***j***, Acclimatized plantlet transferred in a pot.

Gametophytes which did not bear sporophytes were also observed for their sex ontogeny. It was observed that both the sexes are functional and formed in succession.

Gametophytes which did not form sporophytes after repeated watering were transferred to different petri dishes containing Parker and Thompson's medium. After 10–15 days, it was observed that several spatulate-shaped protuberances developed from the margin of gametophytes (Figure 1 *e*). Such outgrowths were also observed in gametophytes which had borne sporophytes. In due course, all these outgrowths developed into gametophytes as described earlier. In these regenerated gametophytes, sex organs initiated simultaneously. The antheridia were located towards the posterior side as well as the wings of the gametophytes, whereas the archegonia were situated below the apical notch (Figure 1 *b*, *d*, *f*). The multicellular hairs were lesser in frequency and number of cells was also meagre in comparison to the parent gametophytes. The thickness of the midrib was lesser than that in *C. contaminans*[5]. Thus, after complete regeneration, a single gametophyte became a composite culture consisting of parent gametophytes with 3–5 proliferated gametophytes. These composite gametophytes, after watering, yielded several sporophytes (Figure 1 *h*).

The mating behaviour of the taxon indicated that both intra- (fusion of antheridium and archegonium of the same gametophyte) as well as inter- (fusion of antheridium and archegonium of different gametophytes) gametophytic selfing prevailed. This is because through intra-gametophytic selfing, a considerable percentage (22) of sporophytes was produced and 80% sporophytes were produced in composite populations in which both intra- and inter-gametophytic selfing was possible. Production of considerable percentage of sporophytes through intra-gametophytic selfing indicated that the gene pool of this species had lesser genetic diversity and genetic load. Intra-gametophytic selfing may be advantageous for initiating new populations and long-distance spore dispersal, which may lead to colonizing new habitats/barren lands. Inter-gametophytic mating has the advantage of accumulating and increasing genetic diversity leading to the accumulation of high genetic load, generation after generation[6–9].

Attainment of bisexuality and synchronous maturation, indeterminate growth, clone-forming, perennial growth habit of gametophytes and lesser genetic load seem to be the primary factors for intra-gametophytic selfing, which may help in the wide occurrence of the species through long-distance spore dispersal[9]. The studied taxon showed all the favourable characteristics for the above breeding habit.

The significant barriers observed in this species for mating, were the viability of spores for only six months and prolonged availability of water at the time of antheridia maturation. Interestingly, it had been observed that the antheridia took at least 30 min to burst and release the antherozoids. This may be one of the reasons why this species is reported from well-watered regions of the tropics in India. At Pithoragarh also, the plants were collected from two localities where water was available round the year and the established populations were found increasing every year.

The regeneration and clone-forming ability of the gametophytes was another characteristic feature by which several sporophytes were borne in proliferated gametophytes[10–12]. In regenerated gametophytes, the antheridia and archegonia were produced continuously and their location on the gametophytes was the same as in the parent gametophyte, which increased the probability of selfing and crossing.

The present study showed that under favourable conditions, gametophytes grow perennially through vegetative proliferations which increased their life span, and both the sexes were produced profusely. Due to proliferations, a gametophyte became a composite gametophyte in due course of time, which increased the chances of production of sporophytes. The production of antheridia, in succession in the parent as well as in the proliferated gametophytes, may be due to antheridiogen activity. Due to the above-mentioned characteristics of gametophyte morphology, growth habit and antheridiogen production, in addition to environmental parameters, the species were found to be well suited for intra- as well as inter-gametophytic mating.

Thus, the reproductive biology indicated that the species is definitely a good colonizer. In both the cases, viz. in parent and regenerated gametophytes, fertilization success was solely dependent on the prolonged availability of water at the time of maturity of both the sexes.

In contrast, the species is categorized as threatened, and its populations are decreasing day by day in the areas of its occurrence in other parts of the country. This may be due to habitat destruction, unsuitable environment for gametophytic growth, fertilization and economic exploitation of this species. The genus *Cyathea* is economically important and the wood log is much in demand in orchid cultivation, especially in the south and northeast regions of India. Matured plants are being cut and tribals in these areas are using the trunk for starch extraction.

Taking into consideration the mode of sexuality and habitat preference of the species, it is of paramount importance to protect the habitat that will guarantee its reproductive success and colonization. As large-scale propagation is possible through spores, it is essential to establish some propagation centres of tree ferns for their conservation and mass production, so that these can be reclaimed from their endangered status.

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Isolation of endophytic plant growth promoting *Burkholderia* sp. MSSP from root nodules of *Mimosa pudica*

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Endophytic bacteria reside within plant tissues and have often been reported to promote plant growth. Rhizobia are particularly known for their symbiotic relationship with legumes. A bacterial strain MSSP was isolated from surface-sterilized root nodules of *Mimosa pudica*. MSSP was Gram-negative, capsulated, motile, non-endospore forming rod with free nitrogen (N) fixation ability. Unlike N-fixing bacteria forming symbiotic relationship with legumes that largely exist in α-subclass of proteobacteria, MSSP belongs to β-class of proteobacteria. Phylogenetic analysis of 16 S rDNA demonstrated that MSSP belongs to the genus *Burkholderia*. This isolate secretes phytohormone, ACC deaminase, solubilizes phosphate and is antagonistic against phytopathogens.

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PLANT growth promoting endophytic rhizobacteria are involved with host plants in mutual interaction. They promote plant growth directly or indirectly, via production of phytohormones, biocontrol of host plant diseases or improvement of plant nutritional status[1]. Rhizobia are perhaps the best-known beneficial plant-associated bacteria because of their importance in nitrogen fixation[2]. Forthcoming *Bergey's Manual of Systematic Bacteriology* identifies five genera of rhizobia, namely *Rhizobium*, *Sinorhizobium*, *Azorhizobium*, *Bradyrhizobium* and *Mesorhizobium* (www.cme.msu.edu/bergey's, p. 7). They all belong to the α-class of proteobacteria, where they are distributed in four distinct phylogenetic branches. However, during the last few years, bacteria from the β-class of proteobacteria, other than rhizobia have been reported from legumes[3]. Here we report a species of *Burkholderia* from the root nodules of *Mimosa pudica*, which belongs to the β-class of proteobacteria.

Plants of *M. pudica* were uprooted from the campus of Botanical Survey of India, Dehradun. Nodules were collected, washed several times in sterile water, surface-sterilized using 70% ethanol and 0.1% $HgCl_2$, and repeatedly washed with sterile water. Sterile nodules were crushed and the resulting suspension was streaked on yeast extract mannitol (YEM) agar plates[4], which were incubated at 28 ± 1°C. Pure isolates were subjected to phenotypic and biochemical characterization, wherein all isolates appeared similar. Effect of temperature and salinity was observed in 50 ml YEM broth in flasks. The flasks with 0.01, 0.1, 1.0 or 2.0% NaCl were inoculated with log phase culture to a final concentration of 10^3 cfu ml^{-1} and incubated at 28, 35 or 40°C at 150 rpm. Uninoculated broth served as control. Absorbance was measured after different time intervals and growth monitored at 610 nm. Acid production was tested in YEM agar plates supplemented with 25 ppm bromothymol blue indicator dye[5]; change in colour from green to blue indicated alkali reaction, and to yellow, acid production. Carbon source utilization was detected in basal liquid medium[6] (BLM) which contained K_2HPO_4, 1 g; KH_2PO_4,1 g; $FeCl_3.6H_2O$, 0.001 g; $MgSO_4.7H_2O$, 0.2 g; $CaCl_2$, 0.1 g; bromothymol blue indicator dye, 25 mg; DDW, 1000 ml with carbon source at a final concentration of 1 g l^{-1}. This was dispensed in tubes (20 mm) as 5 ml aliquot and inoculated by an exponentially growing bacterial culture (≈ 10^9 cfu ml^{-1}); tubes were incubated at 28 ± 1°C at 150 rpm for 4 days. Change in colour from green to yellow or blue indicated utilization of respective C-source. BLM supplemented with mannitol served as positive control, and that without any C-source as negative control.

Root nodulating ability of MSSP was determined. Surface sterile seeds of *M. pudica*, bacterized[7] with isolate MSSP were sown in pots with sterile soil. After four months,