Journal of Threatened Taxa | www.threatenedtaxa.org | 26 April 2016 | 8(4): 8719–8727

A report of hailstorm damage to two species of freshwater sponge (Demospongiae: Haplosclerida: Spongillidae) populations of West Bengal, India

ISSN 0974-7907 (Online)
ISSN 0974-7893 (Print)

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Abstract: Selected freshwater bodies in Jagaddal of North 24 Parganas in West Bengal, India are inhabited by two species of sponge, *Eunapius carteri* (Bowerbank, 1863) and *Spongilla alba* (Carter, 1849) (Porifera: Demospongiae: Spongillidae). Most of these wetlands are perennial ponds without a history of aquaculture and toxin contamination. On 22 March 2014, the entire area of Jagaddal experienced an unprecedented hailstorm associated with a sharp decline of environmental temperature from 35 ºC to 21 ºC within 10–15 minutes. The hailstorm associated with torrential rain lasted for about 30 minutes. The natural habitat of the sponge was visited after six hours of the hailstorm in open day light conditions. During the field investigation, we recorded large-scale damage to the populations of *E. carteri* and *S. alba*. Macroscopic observation revealed that the fragmentation of body masses were also associated with cellular disintegration of the external surface. Sponge cells were experimentally dissociated from the sponge fragments and subjected to dye exclusion assay. A vital dye (trypan blue) exclusion assay of sponge fragments confirmed a high degree of mortality of the cells of *E. carteri* and *S. alba.* Hailstorm associated with the decline of environmental temperature down to 21 ºC resulted in the mass destruction of these two species of sponges in their natural habitat. Any unprecedented and acute change in the climatic and hydrological parameter may lead to physiological adversity in the freshwater sponge. Subsequently, recovery from cold shock and mechanical stresses was overcome and a regeneration of sponge specimens was recorded within a period of around six months from the date of the hailstorm.

Keywords: Bio-indicator, *Eunapius carteri,* Hailstorm, Natural calamity, Poikilotherm, Scanning electron microscopy, *Spongilla alba*, Spongillidae, Temperature fluctuation.

Sponges, the representatives of the Phylum Porifera, are considered to be the earliest extant metazoans (Adamska et al. 2011). Phylogenetically, sponges have survived before the Cambrian (Erpenbeck et al. 2011) and overcame varied degrees of environmental challenges and stressors (Saby et al. 2009) including the extreme environmental hypothermia of the Varanger-Marinoan ice age of about 605–585 million years ago (Müller et al. 2007). Sponges are filter feeding aquatic invertebrates, which evolved an advanced grade of canal system. Freshwater sponges, belonging to the family Spongillidae, are the important members of the aquatic ecosystem and are considered as sources of bioactive and biomimetic molecules (Manconi et al. 2013). *Eunapius carteri* and *Spongilla alba* are common inhabitants of the freshwater ecosystem in India, and are distributed in the perennial and seasonal ponds and lakes. There is inadequate information available regarding their survival and propagation under the influence of environmental calamities. Sponges bear the potentiality to function as efficient bio-indicators (Cebrian et al. 2007) and are promising indicators of water quality (Mahaut et al. 2013). Sponges are

DOI: http://dx.doi.org/10.11609/jott.1739.8.4.8719-8727

Editor: Anonymity requested.

Date of publication: 26 April 2016 (online & print)

Manuscript details: Ms # 1739 | Received 01 February 2015 | Final received 16 March 2016 | Finally accepted 21 March 2016

Citation: Mukherjee, S., M. Ray & S. Ray (2016). A report of hailstorm damage to two species of freshwater sponge (Demospongiae: Haplosclerida: Spongillidae) populations of West Bengal, India. *Journal of Threatened Taxa* 8(4): 8719–8727; http://dx.doi.org/10.11609/jott.1739.8.4.8719-8727

Funding: DST INSPIRE fellowship and UGC - SAP DRS II research programme.

Conflict of Interest: The authors declare no competing interests.

inspire University of Calcutta UGC

Acknowledgements: The Department of Science and Technology, Government of India is thankfully acknowledged for the INSPIRE fellowship grant support to S. Mukherjee. The authors thankfully acknowledge the Center for Research in Nanoscience and Nanotechnology of the University of Calcutta for scanning electron microscopy facility. The UGC SAP DRS II research programme of the department of Zoology of University of Calcutta is acknowledged too.

considered “ecological hotspots” (Mukherjee et al. 2015b) and may act as sites for reproduction, parental care and nesting for other aquatic organisms including crabs, shrimps, insect larvae, mites, nematodes, oligochaetes, molluscs, fishes (Soota 1991) and isopods (Roy & Mitra 2014).

A field survey carried out by us in the district of North 24 Parganas of the state of West Bengal revealed the distribution of *E. carteri* and *S. alba* in the 14 selected waterbodies. The normal temperature of the wetlands in the district of West Bengal generally varies from 8 ^{0}C to 45 ^{0}C throughout the year.

Materials and Methods

Field observation and manual collection of sponges

A field survey was carried out during the premonsoon (February–April), monsoon (May–July) and postmonsoon (August–January) periods in the freshwater bodies of selected regions of the North 24 Parganas District of West Bengal State of India to identify the distribution and abundance of spongillid sponges. We identified one medium sized freshwater pond (Image 1A) located 3 km away from Kankinara railway station (22^{0}86’N & 88^{0}40’E). The waterbody was approximately 80 m in diameter and 8-9 m in depth. Submerged plant twigs and emergent aquatic weeds were present in sufficient numbers in the aquifer on which the sponges developed. Anthropogenic activities including bathing of humans and cattle, netting, application of washing soda and pesticides were not in practice. We have been observing and monitoring the ecological condition and health status of spongillid sponges in this pond routinely for a period of 4-5 years since 2011. Sponges were visually located in the peripheral shallow margins of the waterbody at a depth of 5 to 7 cm (Images 1B, C, E). The pieces of collected freshwater sponges were dissected using a scalpel. During the collection procedure, any mechanical damage to the sponge specimen was avoided. Live sponges were photographed in situ with a digital camera (Nikon coolpix L820).

Taxonomical identification of sponge specimens

Sponges were identified according to the morphology of their skeletal structures including megascleres, microscleres, gemmoscleres and the architecture of the gemmules, as seen under a SEM (Penney & Racek 1968; Jakhalekar & Ghate 2013) (Images 2 & 3). For identification, pieces of freshly collected live sponges were dissolved in concentrated nitric acid and washed with distilled water for isolation of the siliceous spicules (Annandale 1911; Soota 1991). The gemmules were removed from the sponge body masses with precision and washed in distilled water twice to remove debris and other adhered particles. For ultra structural analyses with SEM, isolated spicules were thoroughly washed with distilled water and air dried. Longitudinal sections of the gemmules were made using a sharp stainless steel blade (Jakhalekar & Ghate 2013). Gemmules, both intact and sectioned, were fixed in 3% glutaraldehyde (Sigma, USA) in 0.1 M sodium cacodylate buffer (pH 7.8) for 2 hours at 4 ^{0}C (Willenz & Van de Vyver 1982; Mukherjee et al. 2015c), washed with cacodylate buffer 3 times and fixed in 1% osmium tetroxide (Sigma, USA) for 1 hour. Finally, the processed gemmules were dehydrated with graded ethanol. The samples were allowed to dry and mounted on the stub of the electron microscope with the help of double-sided carbon tape, sputter coated with gold in an ionic coater and examined under a scanning electron microscope (Zeiss EVO 18 special edition, Germany). Morphometry of different spicules and gemmules were estimated by using the software attached with the scanning electron microscope. For measurement, 10 gemmules and 25 spicules of each type were taken into consideration.

Documentation of the freshwater sponges before and after the hailstorm

Hailstorm was documented in the open field by eye observation and digital photography (Nikon Coolpix L820). The temperature of water was measured using water thermometer. A visual examination of the sponge specimens were carried out to estimate the morphological damage and destruction of the sponge due to the hailstorm. The extent of damage was photographed the next morning (Image 4C–F). Pieces of body mass with an approximate dimension of 0.5 cm^3 were excised with a scalpel and examined for viability of sponge specimens by smearing the trace amount of cells over glass slides and stained with 0.4% trypan blue following the principle of dye exclusion (Mussino et al. 2013; Mukherjee et al. 2015a).

Result

Taxonomically two species of sponges were identified on the basis of the anatomy of the spicules and gemmules in the area. They were *E. carteri* and *S. alba*. Both were found to be morphologically affected by the hailstorm associated with a sharp decline of environmental temperature from 35 ^{0}C to 21 ^{0}C within 10–15 minutes.

Eunapius carteri (Bowerbank, 1863)

Habitat and morphological description: *E. carteri*

Image 1. The natural habitat of *Eunapius carteri* and *Spongilla alba* before the reported hailstorm (A); Healthy specimens of *E. carteri* lifted above the water surface for photodocumentation (B–C); Gemmules of *E. carteri* attached to body mass (D); Healthy and live specimen of *S. alba* in its natural habitat (E); Live *S. alba* bearing the surface attached gemmules shortly after collection (F).

were found attached with submerged vegetation. The sponges were massive, forming irregular masses with varying dimension (Images 1B–C). Live and fresh animals varied from grayish to yellowish-brown whereas the dried specimens were dark brown in colouration. The external surface of *E. carteri* was mostly irregular and hispid without rounded or lobose projections. Oscula were large, rounded and conspicuous but not elevated above the surface. The texture of live sponges varied from moderately soft to fragile and had a peculiarly strong and pungent smell. There were some dry sponges on exposed vegetation suggesting that the water level of the pond had declined in the recent past. The dry gemmules were collected and later identified as *E. carteri*.

Diagnostic characters: Spicules of two types: megascleres and gemmoscleres. Megascleres (Image 2A) entirely smooth, amphioxea, stout, fusiform and

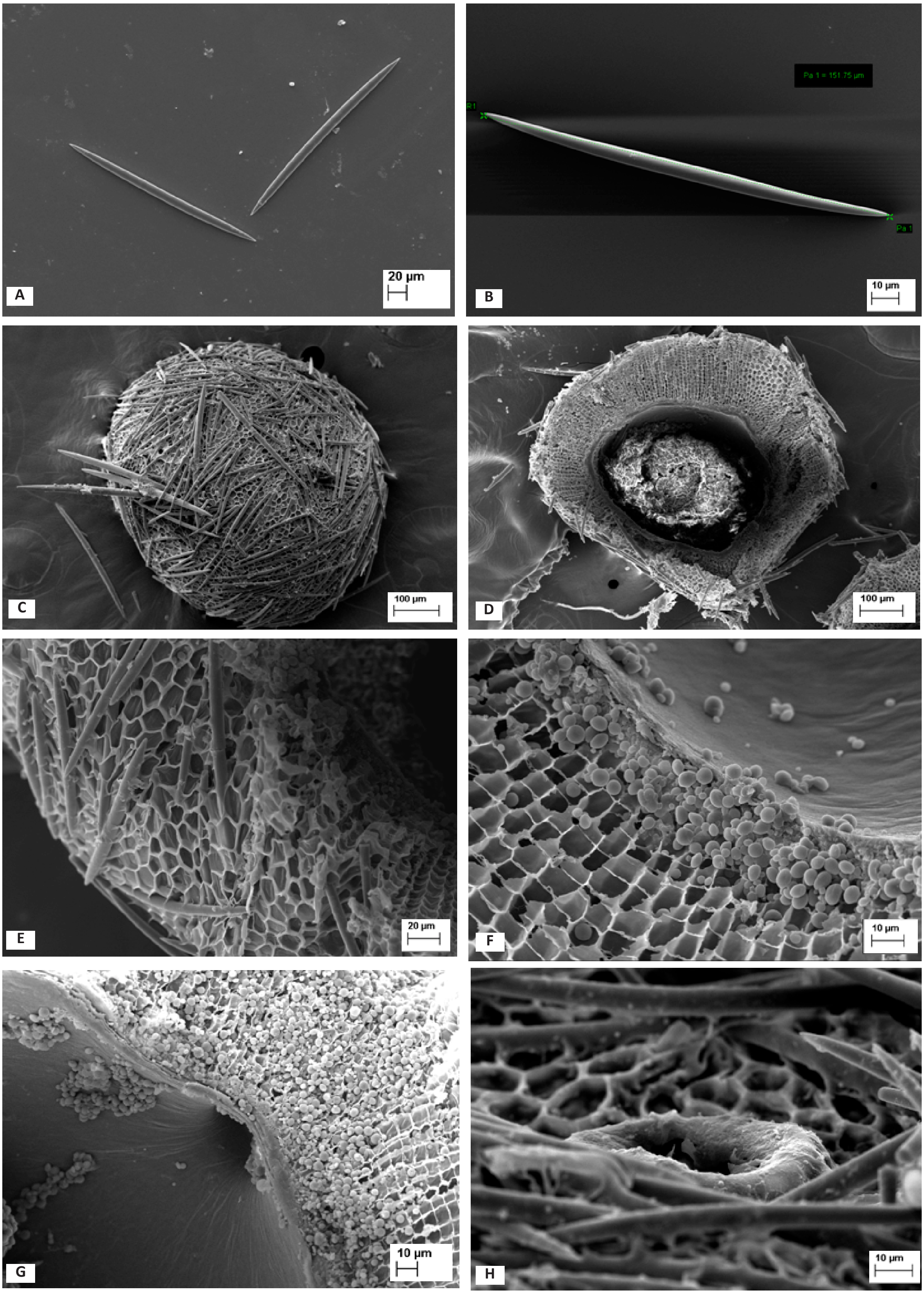

Image 2. Scanning electron microscographs of the spicules (A, B) and gemmule (C–H) of *E.carteri*. Megascleres (A) and gemmosclere (B); intact gemmule (C); cross section of the gemmule with mass of thesocytes at the centre (D); arrangement of gemmoscleres within the external surface of the gemmule (E); well developed cellular pneumatic layer (F); foraminal tube (G) and foramen of the gemmule (H).

slightly curved; 321.25 ± 47.67 µm long and 19.50 ± 5.80 µm wide. Microscleres absent. Morphologically, gemmoscleres (Image 2B) resemble megascleres but relatively smaller in size (176.25 ± 33.50 µm long and 6.50 ± 1.73 µm wide), slender, more curved with smooth oxeas and sharply pointed at both ends. Gemmules numerous and scattered singly throughout the skeletal meshes of the sponge (Image 1D), condensed towards the base, i.e., area of attachment to the substratum. Gemmules of *E. carteri* spherical (Image 2C–D), comparatively large (537.50 ± 81.80 µm in diameter) and flattened at the base. Pneumatic layer thick and well constructed (Image 2F–G); gemmoscleres tangentically or horizontally embedded within the periphery of the thick multilayered pneumatic layer (Image 2E) with several layers of regularly arranged polygonal air spaces.

Spongilla alba **(Carter, 1849)**

Habitat and morphological description: This sponge formed bulky growths with large encrustation (Image 1E–F). The external body surface was moderately smooth with irregular outwards projections and generally green in colouration. Oscula were moderate in size but inconspicuous. The sponge attached mostly to submerged vegetation and the consistency of the live sponges was firm but brittle.

Diagnostic characters: Spicules of megascleres, microscleres and gemmoscleres. Megascleres (Image 3A) amphioxea, feebly curved and slender with a completely smooth surface; 292.02 ± 64.33 µm long and 18.67 ± 5.64 µm wide. Microscleres (Image 3B) slender with slightly curved amphioxea and covered entirely with erect spines. Spines much longer and more distinct in the central region of the microscleres with knob like inflations at their tips. Microscleres 101.12 ± 26.56 µm in length and 2.62 ± 0.74 µm in width. Gemmoscleres (Image 3C) with slender, feebly curved, cylindrical amphistrongyla covered with large erect spines. Spines more numerous at the tips forming several annular groupings (Image 3D); length 99.62 ± 21.67 µm and width 6.75 ± 2.18 µm. Gemmules large (Image 3E–F), spherical, abundant and scattered throughout the sponge body (Image 1F), 550 ± 64.08 µm in diameter. The pneumatic layer distinctly granular and moderately thick (Image 3F & G) in which the gemmoscleres embedded at irregular angles with their tips protruding beyond the outer surface. Foramen cup-shaped (Image 3G & H) but not tubular, and slightly elevated above the surface.

During the midnight of 22 March 2014, selected areas of the district of North 24 Parganas including Jagaddal experienced a strong hailstorm (Images 4A & B) from 23:20–23:50 hr. During the hailstorm of around 30 minutes, hailstones of varied weight and sizes with a maximum of more than one kilogram of weight hit the ground and pond water continuously along with heavy rainfall and a strong flow of cold wind at the speed of 60 km/h (The Telegraph, March 24, 2014). Severe morphological damage and destruction took place in the sponge specimens due to the hailstorm. This is a first record of destruction of freshwater sponges due to a natural calamity.

Mortality in some parts and severe morphological damage of all the *E. carteri* and *S. alba* specimens were recorded due to hailstorm. After the hailstorm and associated sponge damage, the same habitat was under scientific surveillance for regeneration of the sponges, which overcame the stress (Image 5).

Discussion

The pioneering investigations on Indian freshwater sponges were carried out by Annandale (1911) and Soota (1991). Khera & Chaturvedi (1976) published a checklist of freshwater sponges of the Indian subcontinent. Soota (1991) demonstrated a consolidated and comprehensive description of these lower invertebrates. However, only 16 freshwater sponge species including *E. carteri* and *S. alba* were found in West Bengal (Pattanayak 1998). Occurrence of the freshwater sponge, *E. carteri* was reported by various workers from India (Devarshi 2006; Kekavipure & Yeragi 2008), with insufficient information available in West Bengal in the current scientific literature. Most of the previous research on Indian sponges dealt with taxonomy with almost no information on the biology of these specimens.

It was assumed that the heavy mortality of *E. carteri* and *S. alba* might have been resulted due to an undesirable shift in the physical parameters like temperature, rain and hailstorm. These sudden and abrupt changes in the physical parameters of environment are assumed to affect the cellular physiological profile of the freshwater sponges.

Indian freshwater sponges, in general, are a neglected group of aquatic invertebrates, which receive inadequate scientific attention. The structural and functional uniqueness of sponges and the ability to regenerate their body parts, provide a scope to study the internal defense mechanism in metazoans. Being poikilotherms, spongillids evolved adaptability against a wide range of fluctuation of water temperature. The physiological ability of sponges to survive in different environmental regimes ensures the survival success

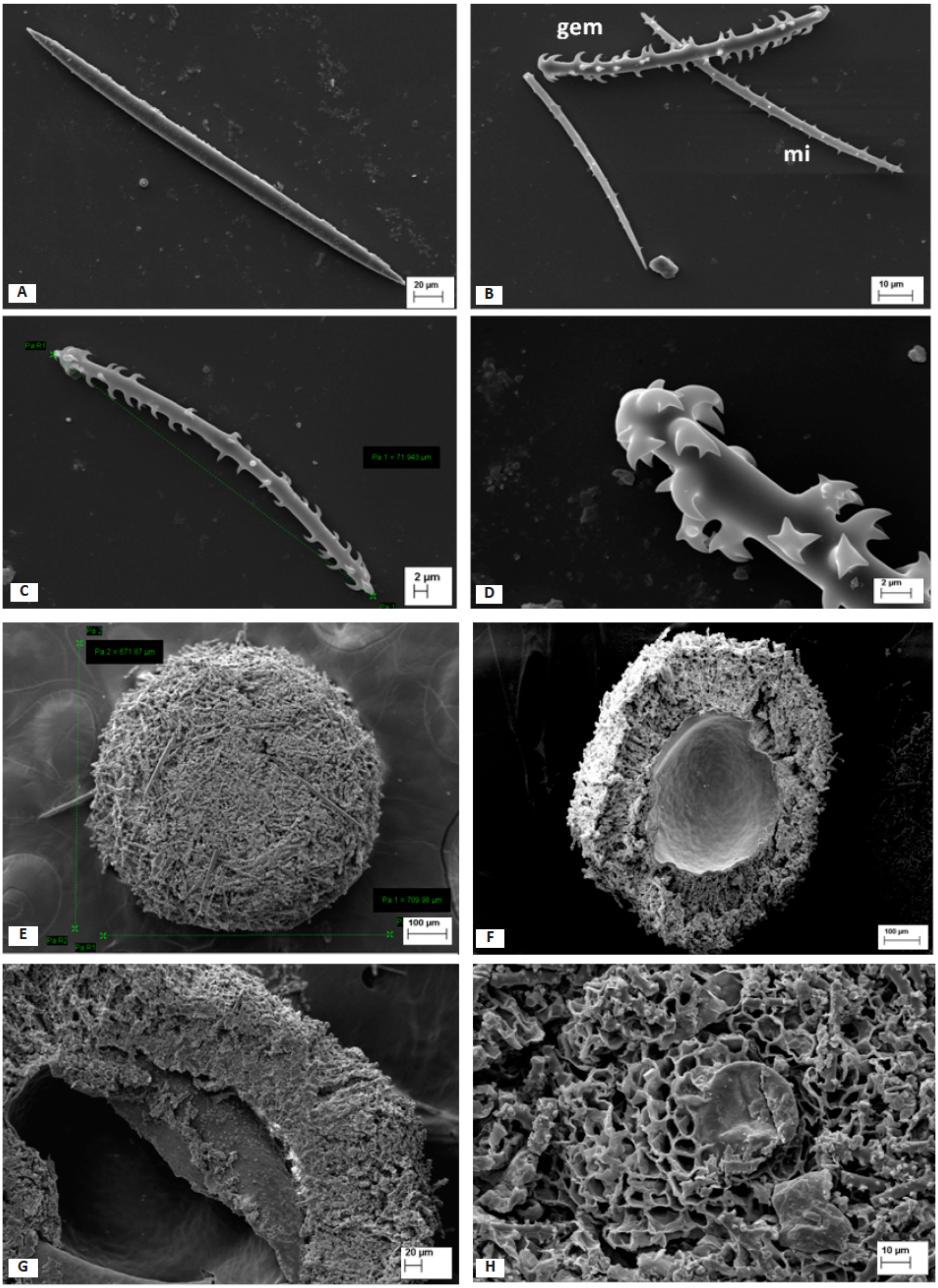

Image 3. Scanning electron microscographs of the spicules (A–D) and gemmule (E–H) of *S. alba*. Megascleres (A), microsclere (mi) and gemmosclere (gem) (B); gemmosclere (C); tip of the gemmosclere with spines (D); Intact gemmule (E); cross section of the gemmule (F); cellular pneumatic layer with foraminal tube (G) and foramen of the gemmule (H).

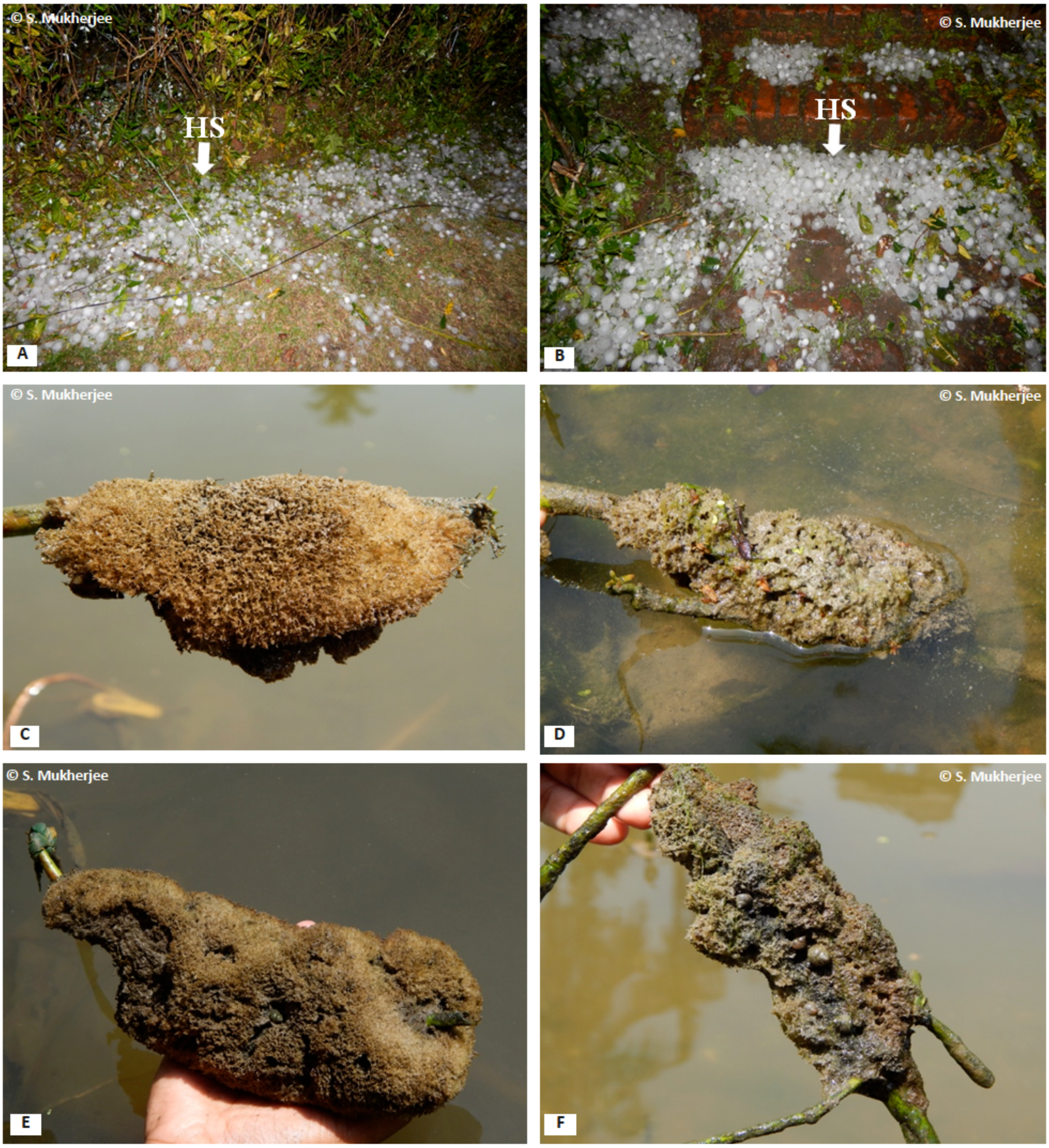

Image 4. Hailstones (HS) scattered around the natural habitat of sponge after one hour of the hailstorm and rain (A, B). Dead and damaged specimens of freshwater sponges photodocumented after six hours of the hailstorm (C–F). Skeletal mass of spicules were left without any live cells in both species.

of sponge. There is no report available regarding its survival under the face of drastic and sudden changes of common environmental parameters. Post calamity recovery of these species took almost six months of time (Image 5). A substantial growth of *E. carteri* and *S. alba*was recorded in the middle of September 2014. The sponges in general, follow asexual and sexual mode of reproduction. It is reported (Funayama 2008) that sponges prefer the asexual mode of reproduction during unfavourable environmental condition. However, a detailed study of the effect of environmental parameters on the survival and propagation of sponge is needed to understand the mode of reproduction during post calamity recovery.

Image 5. Early (A) and late (B) stages of regeneration in situ of *E. carteri* during post-hailstorm recovery. Early (C) and late (D) stages of regeneration in situ of *S.alba* during post hailstorm recovery.

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