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In vitro propagation of Sino-Himalayan liverwort *Solenostoma schaulianum* (Steph.) Váòa et D.G. Long

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ABSTRACT

An important leafy liverwort *Solenostoma schaulianum* (Steph.) Váòa et D.G. Long has been raised *in vitro* and successfully propagated under laboratory condition. This species is endemic to the Sino-Himalayan region.

Key-words: Axenic culture, conservation, endemic, liverworts.

INTRODUCTION

Axenic culture of bryophytes holds much importance as a tool for mass propagation and the stabilization of these plants *in vitro* that can effectively aid in their conservation and bio-prospection. Studies on axenic culturing, *in vitro* propagation and reproductive behaviour of Indian bryophytes have been undertaken since several decades with noteworthy accounts being those of Srinivasan (1940), Udar (1957a, 1957b, 1958), Kaul et al. (1962), Chopra & Sood (1973a, 1973b), Udar & Gupta (1977), Kumra & Chopra (1980), Chopra & Bhatla (1983), Bapna et al. (1984), Chopra & Vashistha (1993), Awasthi et al. (2010a, 2010b) and Awasthi et al. (2011). However, these studies have been limited to only a few selected thalloid liverworts, some mosses and with least emphasis on leafy liverworts (bryophytes) (Nehira 1966, Udar & Gupta 1977). Leafy liverworts in general are difficult to propagate *in vitro* or under artificially controlled conditions. However, very few attempts have been made for their successful propagation in axenic cultures (Kowlczyk et al. 1997, Matsuo et al. 1996, Nabeta et al. 1993, Rowentree et al. 2011). Basile (1967), Basile et al. (1985) and Basile & Basile (1994) have discussed (in detail) the regulatory role and effect of hydoxy-L-proline and 3,4-dehydroproline on the morphogenesis of leafy liverwort *Scapania nemorosa* Dumort. and *Plagiochila arctica* Bryhn & Kaal., respectively. Considering the enormity of this group of bryophytes, extensive accounts of leafy liverwort culture are still wanting. It is imperative to mention that some leafy liverworts that occur rarely or those which are encountered growing in association with other bryophytes exhibit very limited natural colonization and growth. Therefore, mass propagation of their pure population and hardening under controlled axenic conditions provides an important means for their conservation, restoration and bio-prospection.

The present contribution explicates the *in vitro* propagation of an important species of the genus *Solenostoma* that exhibits very limited distribution worldwide. *Solenostoma schaulianum* (Steph.) Váòa et D.G. Long belongs to the family Jungermanniaceae and endemic to the Sino-Himalayan region viz. eastern Himalaya (India, Bhutan and Nepal) and China, to the best of our knowledge (also refer Vana & Long 2009). The first record of *S. schaulianum* dates back to 1917 from the east Himalayan region (Darjeeling District, north India) (Stephani 1917-1925). Later, *S. schaulianum* was reported from Nepal (Amakawa

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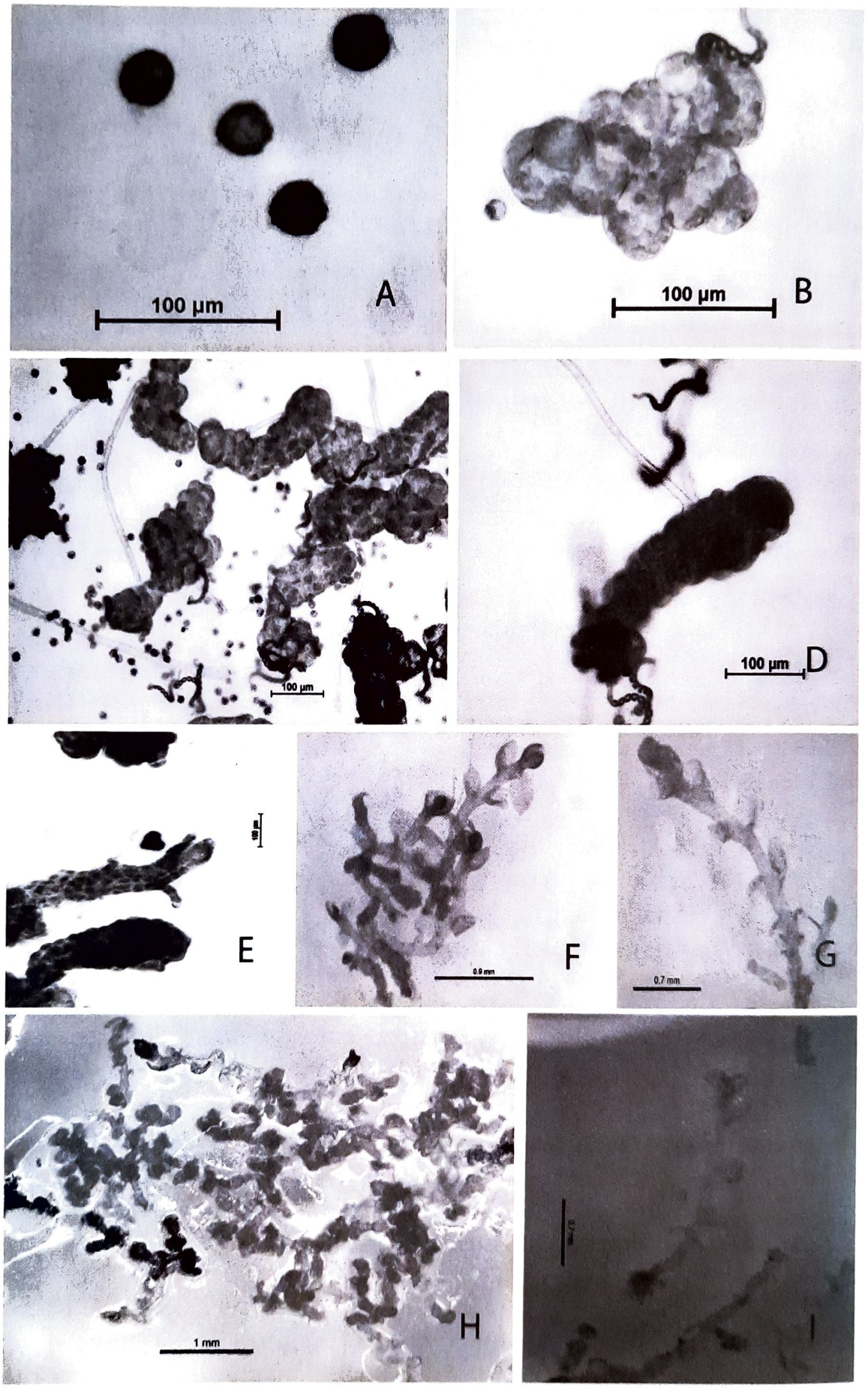

PLATE 1

S. Shaulianum (Steph.) Váòa et D.G. Long in half strength Knop's medium. **A.** Inoculated spores; **B.** Initiation of germination (21st day); **C.** Multicellular protonema with rhizoids (28th day); **D.** Nardia type protonemal growth (35 th day); **E.** shoot apex and leaf primordia formation (45th day); **F.** Leafy shoots with juvenile leaves (56th day); **G,H.** Formation of young plants (64th & 74th day); **I.** Mature plants (after100 days).

1967, Váòa 1973, Long & Grolle 1990), Bhutan (Váòa 1973, Long & Grolle 1990) and also from China (Gao & Cao 2000, Gao & Bai 2001). For undertaking the present investigation, the plants of *S. schaulianum* were freshly collected from the Darjeeling hills (the region of its initial record). The plant specimens were propagated successfully in axenic culture, and to the best of our knowledge the present study represents the first exultant effort to axenically multiply this restrictedly occurring liverwort.

MATERIAL AND METHODS

Spores were procured from mature sporophytes of *S. schaulianum* [India, eastern Himalaya, Darjeeling, Senchal Wildlife Sanctuary, ca. 2248 m; on soil covered rocks; 306512A (LWG)]. Mature capsules were surface sterilized with 4% sodium hypochlorite solution for one minute. After rupturing the capsules, spores were inoculated in half strength Knop's macronutrient medium (Knop 1865) and Hoagland basal medium (Hoagland & Arnon 1950) respectively. The pH was adjusted to 5.8 and media were gelled with 0.4% CleriGel. The cultures were maintained in constant light intensity of 2500-3000 lux and ± 22°C temperature.

RESULTS AND DISCUSSION

The spores of *S. schaulianum* germinated successfully on 21st day of inoculation in half Knop's medium (Plate 1B). The callus was formed by cell differentiation that got initiated on the 28th day (Plate 1C). The sporelings formed were *Nardia* type (Nehira 1983), that are known to be exhibited by several families of Jungermanniales. The typical *Nardia* type pattern shows multicellular massive protonema. Rhizoid formation (pale purplish to pinkish) was observed on the 28th day becoming extensive on the 35th day (Plate 1C, D), shoot apex and leaf primordia formation started on the 45th day (Plate 1E). Leafy shoots developed with juvinile leaves on the 56th day and from 64th to 74th day formation of small plants was observed (Plate 1F-H). Within 100 days, the fully grown pale yellow coloured plants were obtained (Plate 1I).

In the Hoagland medium, spores germinated earlier, i.e., on the 17th day (Plate 2B) giving rise to the characteristic Nardia type massive protonema by the 21st day (Plate 2 C). Rhizoid formation started on the 28th day (Plate 2D) whereas dense rhizoids along with shoot differentiation started on the 35th day (Plate 2E, F). Leaf primordia and leafy shoot with juvenile leaves were initiated on the 56th day (Plate 2G) whereas shoots with small leaves were developed on the 64th day (Plate 2G). Small plants were obtained on the 76th day (Plate 2H,I) while the fully grown plants were formed on the 101st day (Plate 2J, K). It should be noted here, that development of shoot and leaves set in early (i.e., on the 56th day; Plate 2G), by utilizing half Knop's medium as against the utilization of the Hoagland basal medium (i.e., on the 64th day; Plate 2G). However, the overall growth and well differentiated plant formation was observed to be more efficient in case of Hoagland medium as compared to the half strength Knop's medium. Further, the mature plants were observed to be greener and well-sized using the Hoagland medium and pale in colour and smaller in size using the half Knop's medium, even after full development. Continuous

Table 1. Morphogenesis and growth pattern of *Solenostoma schaulianum* (Steph.) Váòa et D.G. Long

S. No	Day	half Knop's media	Hoagland basal mixture
1.	17th	No Germination	Germination tube (green coloured) emerges out.
2.	21st	Germination tube emerges out (pale green color) a globose structure of cells formed.	Protuberance differentiates in many celled structure forming a globose structure or a green colour clump of cells.
3.	28th	Cells differentiate and forming elongated structure.	Rhizoids formation starts, pink or purple tinched rhizoids formed.
4.	35th	Rhizoids formation starts. Purple or pink)	Differentiation of cells starts. Numerous rhizoids.
5.	45th	Shoot apex and leaf primodia formation starts.	Differentiation occur shoot apex formed, rhizoids formed.
6.	56th	Shoot apex formed and leaves developed.	Shoot apex developed, leaf primodia formation starts.
7.	64th	Small plants developed.	Shoot and small leaves developed.
8.	76th	Shoot and leaves developed and small plants developed.	Shoot and leaves developed and small plants developed.
9.	101st	Fully grown plant developed. (pale colored).	Fully grown plant developed (green).

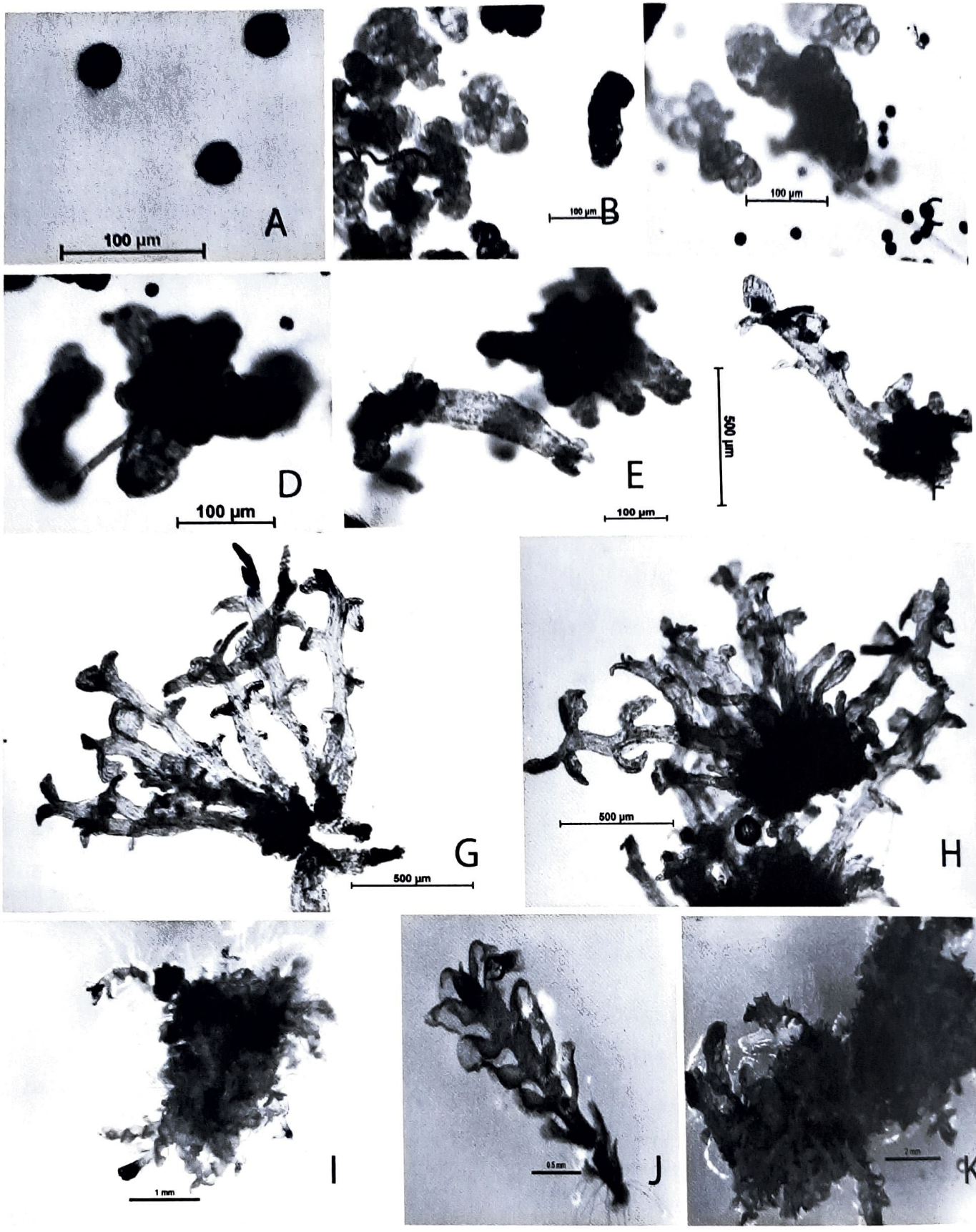

PLATE 2

S. Shaulianum (Steph.) Váòa et D.G. Long in Hoagland medium. **A.** Inoculated spores; **B.** Initiation of germination (17th day); **C.** Nardia type massive protonema formation (21st day); **D.** rhizoid formation initiated (28th day); **E.** shoot differentiation with dense rhizoids (35th to 45th day); **F,G.** shoot apex and leaf primordia formation (56th day); **H.** Leafy shoots with juvenile leaves formed (64th day); **I.** small plants formed (76th day); **J.** Mature plant (after 101 days); **K.** Dense Population of plants.

illumination of 2500-3000 lux and temperature of 22 ±1°C was found to be the optimal for the best growth of the plant.. Thus, evidently, Hoagland medium with continuous illumination should be considered as a better suited medium for axenic culture and multiplication of *S. Schaulianum* (Table 1). Finally, the plants were transferred to pots filled with sterilized soilrite after 120th day and successfully hardened.

It should also be noted that the growth media used in the present study are quite different in their nutrient constitutions. The half strength Knop's medium has macronutrients whereas the Hoagland basal medium has both macro- and micronutrients. This perhaps explains (to a certain extent) the better observed growth of *S. schaulianum* in the Hoagland medium. In an earlier study, Voth (1943) suggested that for the proper growth of *Marchantia polymorpha* L., the medium should contain potassium nitrate and phosphate, calcium nitrate and magnesium sulphate. Although, these nutrients are present in the half strength Knop's medium, however, in the present investigation, we observe that the half strength Knop's medium is not ideally suited for the proper growth of leafy liverworts (that need more nutrients for growth). Therefore, the Hoagland medium should be preferred over the half strength Knop's medium. Otha & Hirose (1982) proposed that for tissue culture of another leafy liverwort *Jungermannia subulata* A. Evans., medium with 4% glucose was sufficient, and hormone supplement was not a necessity. Nevertheless, in the present investigation, the utilization of sucrose was avoided to check fungal and algal contamination. In addition, in the present study, a media without sucrose and hormones was observed to be sufficiently viable for the growth of leafy liverworts.

To the best of our knowledge, limited data pertaining to the axenic culture and *in vitro* propagation of leafy liverworts is available in the literature. However, the effect of light and temperature on *in vitro* propagation of thalloid liverworts and mosses has been discussed sufficiently (Miller & Coldiance 1969, Courtoy 1972, Bostic 1981, Awasthi et al. 2011, Bopp 1983, Kumra & Chopra 1983). Previous workers have shown that temperature and photo period induce a variety of responses in case of bryophyte culture, highlighting the need to standardize the requirements of nutrients, light and temperature separately for each bryophyte taxon being studied for its reproductive behaviour. The present investigation clearly demonstrates that leafy liverwort *S. Schaulianum* can be successfully grown *in vitro* using the Hoagland or half strength Knop's media. The successful propagation of *S. Schaulianum* under *in vitro* conditions (as shown in the present study) certainly provides scope for further studies on other taxa of leafy liverworts for mass propagation and conservation.

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