Biotechnological Communication

Bioactivity, Chemical Profiling and 16S rRNA-based Phylogeny of Haloalkaliphilic *Nocardiopsis* sp. GhM-HA-6 Isolated from the Gulf of Khambhat, Gujarat, India

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ABSTRACT

Actinomycetes are well known sources of antibiotics, however; recently the focus of antimicrobial research has been turning towards actinomycetes of extreme environments. Therefore, present work would highlight the isolation, identification and characterization of antimicrobial metabolites produced by marine haloalkaliphilic actinomycetes. Saline soil sample was collected from Ghogha coast (Gulf of Khambhat), Bhavnagar, Western India. Isolation was carried out using selective media while identification was done based on morphological, cultural and molecular characterization. The antimicrobial potential was checked by spot inoculation method. Optimization was carried out by the one variable at a time (OVAT) method. The antimicrobial compounds were extracted using ethyl acetate and characterized by GC-MS. The haloalkaliphilic actinomycetes *Nocardiopsis* sp. GhM-HA-6 was isolated from saline soil of Ghogha coast using starch agar with 10% w/v NaCl and pH 9 and was identified as *Nocardiopsis* sp. based on morphology, cultural characteristics and 16S rRNA phylogenetic analysis (NCBI Genbank Accession number: KF384492). The organism showed antimicrobial activity against five Gram positive and three Gram negative bacteria while the isolate didn't show any antifungal activity. Results of optimization showed that the highest production of antimicrobial compounds was obtained using starch broth with 0.5% w/v starch, 1% w/v yeast extract, 10% w/v NaCl and pH 9. GC-MS analysis of ethyl acetate extract of the isolate showed presence of a total 18 compounds including various antimicrobial compounds like 2, 4-bis (1, 1-dimethylethyl)-Phenol, various types of alkanes and their derivatives. Haloalkaliphilic actinomycete *Nocardiopsis* sp. *GhM-HA-6*, from a rarely explored marine habitat, can be a source of antimicrobial compounds with the novel biotechnological applications.

KEY WORDS: ANTIMICROBIAL ACTIVITY; HALOALKALIPHILIC; GAS CHROMATOGRAPHY-MASS SPECTROSCOPY; MARINE ACTINOMYCETES; 2, 4-BIS (1, 1-DIMETHYLETHYL)-PHENOL.

INTRODUCTION

Actinobacteria are well known for their ability to produce valuable secondary metabolites such as antibiotics and antimicrobial substances. Amongst genera of actinobacteria, *Nocardiopsis* genus was primarily described by Meyer in 1976 and was placed in the class of Actinobacteria; subclass Actinobacteridae; order Actinomycetales and family Nocardiopsaceae (Ibrahim et al. 2018). Rainey et al. (1996) defined a new family referred as Nocardiopsaceae considering phylogenetic position, morphological features, and chemotaxonomic properties of *Nocardiopsis* sp. This genus includes Gram positive, aerobes, non-acid fast with catalase positive properties (Cook and Meyers 2003; Bennur et al. 2015; Ibrahim et al. 2018). Studies by Kroppenstedt and Evtushenko (2006) showed various features of genus *Nocardiopsis* which includes the presence of meso-2, 6-diaminopimelic acid, but lack of diagnostically important carbohydrates in cell wall structure, absence of madurose or nocardomycolic acids in whole cell hydrolysates and high GC content in their genomes (Dhakal et al. 2017; Subramani and Sipkema 2019).

Actinobacteria are widely found in soil and physiologically extreme environments such as desert, saline, hyper saline, and alkaline origins. Particularly, *Nocardiopsis species* are often

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Response of Occurrence of Winter Weeds to Physico-Chemical Characteristics of Soil of Gandhinagar District, Gujarat, India

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ABSTRACT

Gandhinagar is the capital city of Gujarat State. The paper deals with the estimation of nutrients (pH, EC, N, P, K, Zn) in soil of different talukas of Gandhinagar district. This district has four talukas with 290 villages and 10 urban habitations in the district. For the study purpose, the entire district was divided into 20 sites. Five sites of each talukas were selected. Total 20 sampling sites were selected to collect samples. The study was carried for a period of 2 year (2011 to 2013). Soil samples were colleted and analysed for their parameters like pH, EC, Nitrate, Phosphorus, Potassium and Zinc (Zn) at monthly. Zn was recorded critical in Mansa and Dehgam taluka and quite low in Kalol taluka. The occurrence types and distribution of Amaranthus viridis L., Achyranthus aspera L., Trianthema portulacastrum L, Boerhavia diffusa L., Cyperus rotundus L., Cynodon dactylon (L.) Pers., Tridex procumbens L., Parthenium hysterophorus L., Euphorbia hirta L., Vernonia cinera (L.) Less., Digera muriata (L.) Mart., Cassia tora L., Chenopodium album L., Portulaca oleracea L. in the study area of Gandhinagar showed relations with the soil in which they occur.

Keywords : Weeds, Physico-chemical characteristics

I. INTRODUCTION

A good knowledge of the soil properties and their relationships with weeds distribution is said to be highly essential for integrated weed management programs (Akobundu, 1993). Fertile soil is the most important source for the entire living world. Apart from providing a solid substratum on which we live, the soil provides us most of our necessities through the plant and animals communities which develop on it (Asthana and Asthana, 2003). Soil testing is one of the best available tools to ascertain the physical characteristics and nutrient status of a field so as to assess the fertilizer requirement (Singh, 2007). Different plants are known to have different requirements. Differences in the distribution of weed flora could be an indication of the variation in soil properties. Understanding the relationship between

certain soil properties and specific weed species could act as a guide to the farmer to understand the likely soil conditions that could suitable for a particular purpose. Such knowledge may also aid in mineral prospecting (Veeranjaneyulu and Dhanaraju, 1990).

II. MATERIAL AND METHODS

The present study was undertaken for the period of 2 years. The collection was made with repeated field trips. Soil samples were collected monthly from selected sites and analyzed for their parameters like pH, EC, Nitrate (N), Phosphorus (P), Potassium (K) and Zinc (Zn). The sampling was done by method of Piper (1950). Samples were analyzed as per methods suggested by Trivedy and Goel (1986). Micronutrient Zinc (Zn) was estimated in Atomic Absorption Spectrophotometer (AAS).

III. RESULTS AND DISCUSSION

The standard values of parameters by District Agriculture Plan (DAP) in soil of Gandhinagar district are given in Table-01 and standard values of requirement of different nutrients of weeds by Anand Agriculture University (AAU) are given in Table–02. The values of parameters like pH, EC, Nitrate (N), Phosphorus (P), Potassium (K) and Zinc (Zn) analyzed in soil in present study are given in Table-03. In present study the soil properties of 20 selected sites were observed with reference to winter season weeds. Results showed that among the families, Poaceae was dominant, followed by Asteraceae and Amaranthaceae. pH was recorded lowest (7.71) and highest (8.34) . EC was ranged between 0.38 to 0.73 m mho/cm in present study. The requirement of N by selected weeds is high in percentage (Table – 01). Nitrogen (N) was ranged between 0.038 to 0.06 in present study. Because of the high N value in soils which indicate the contribution of N for the occurrence of weeds. So the requirements of nitrogen by weeds show the association between soil and weeds. Acrachne racemosa (Heyne ex R. & S.) Ohwi, Aristida funiculata Trin. & Rupr. Sp. Gram., Avena sterilis L., Brachiaria setigera (Retz.) Hubb, Cenchrus pennisetiformis Hochst. & Steud., Chloris montana Roxb. Hort. Beng., Chloris virgata Sw. Fl. Ind. Occ., Cynodon dactylon (L.) Pers. Syn., Dactyloctenium aegyptium (L.) P. Beauv., Dichanthium annulatum (Forsk.) Stapf., Digitaria adscendens (H.B. & K.) Henrard, Digitaria ciliaris Prain, Dinebra retroflexa (Vahi) Panz., Echinochloa colonum (L.) Link. Hort. Chenopodium album L. Chenopodium murale L. Amaranthus spinosus L. Amaranthus viridis L. were recorded dominant during winter season.

Poaceae is the largest family among the monocotyledon recorded during winter season and Amaranthaceae, Asteracae, convolvulaceae and chenopodiaceae are main families of dicotyledon recorded during the same season. Poaceae family represented the highest number of species. In dicotyledone, Aateraceae family represented the highest number of genera and species.

The present study shows that maximum numbers of weed species (73 species) were found in winter season. In Gandhinagar district, winter weeds are most dominant represented growth with 39.67% species of all recorded species in all the three seasons.

The value of Phosphorus was recorded lower in winter. Phosphorus is limiting factor for living organisms. Due to the high density of weeds the phosphorus was absorbed by weeds so the P values in soils were lower in winter season. The value of Potassium (K) was recorded more than 20 as per the DAP standard. Results showed that presence of high value of K may be due to leaching and gradual decrease occurs due to uptake by plants. Because of the requirement of K by weeds, Portulaca oleracea L., Vernonia cinera (L.) Less., Digera muriata (L.) Mart., Cassia tora L., Chenopodium album L., Solanum sp., Amaranthus viridis L., Amaranthus lividis L. were strictly associated with the soils of study area. Zinc (Zn) is essential for the transformation of carbohydrates and regulates consumption of sugars. It

is the part of the enzyme systems which regulate plant growth. In present study Zn values were recorded high in some soils and medium in remain soil samples. The highest weed density was observed for Achyranthus aspera L. The requirement of zinc for Achyranthus aspera L. is higher among the recorded weeds (Table – 02). Decreased Zn value in monsoon is may be due to contribution of Zn to weeds and high density of Achyranthus aspera L. A Weed species in the study area showed responses to the soil properties and nutrients in which they occurred.

Woo et al. (1991), Malik and Born (1988) and Frick (1984), in their various studies observed that weed species distribution was influenced by soil series.

TABLE – 01 Standards (DAP Gandhinagar)

Source: Soil fertility indices (DAP) – Gandhinagar

Taluka	Parameters				
	pH	**EC** (m mho/cm)	**Available N** (kg/ha)	**Available P** (ppm)	**Available K** (ppm)
Gandhinagar	6.5 to 7.5	0.25 to 0.75	> 250	1.36 to 2.73	> 20
Dehgam	6.5 to 7.5	0.25 to 0.75	> 250	1.36 to 2.73	> 20
Kalol	6.5 to 7.5	0.25 to 0.75	> 500	1.36 to 2.73	> 20
Mansa	6.5 to 7.5	0.25 to 0.75	> 500	1.36 to 2.73	> 20

TABLE – 02 Requirements of nutrients by selected weeds (in %)

No.	Weed Species	Nitrate (N) %	Phosphorus (P) %	Potassium (K) %	Zinc (Zn) %
1.	Digitaria sp.	1.90	0.55	1.08	-
2.	*Cynodon dactylon* (L.) Pers.	2.08	1.01	1.22	0.50
3.	*Cyperus rotundus* L.	1.61	1.52	1.13	0.54
4.	*Argemon maxiana* L.	1.01	1.36	1.33	0.53
5.	*Portulaca oleracea* L.	1.26	1.51	2.21	0.52
6.	*Vernonia cinera* (L.) Less.	2.56	1.53	3.12	0.54
7.	*Eclipta alba* L.	1.61	1.49	1.52	0.55
8.	*Digera muricata* (L.) Mart.	3.24	1.63	3.15	0.55
9.	*Amaranthus lividis* L.	1.86	1.56	3.13	0.51
10.	Achyranthus aspera L.	2.21	1.63	1.32	0.60
11.	*Chenopodium album* L.	2.59	1.51	4.34	0.51
12.	*Phyllanthus fraternus* L.	2.43	1.53	1.85	0.53
13.	Solanum sp.	2.56	1.63	2.12	0.56
14.	*Boerhavia diffusa* L.	2.01	1.54	1.12	0.50
15.	*Trianthemaportulacastrum* L.	2.64	0.43	1.30	-
16.	*Euphorbia hirta* L.	1.91	1.53	1.22	0.49
17.	*Tridex procumbens* L.	2.24	0.73	1.08	-
18.	*Amaranthus viridis* L.	2.16	0.60	4.51	-
19.	*Cassia tora* L.	3.08	1.56	2.31	-
20.	*Parthenium hysterophorus* L.	2.68	0.60	1.45	-

Source: Krishigovidhya, Anand Agriculture University (AAU)

TABLE – 03 Recorded parameters in soil in present study (Year 2011 to 2013)

Parameters	Samples									
	1	2	3	4	5	6	7	8	9	10
pH	7.98	7.78	8.11	8.04	7.96	8.16	7.94	7.85	8.12	7.92
EC	0.64	0.43	0.46	0.47	0.64	0.41	0.55	0.38	0.38	0.47
N	0.045	0.042	0.050	0.044	0.050	0.040	0.038	0.046	0.042	0.043
P	2.88	3.62	4.16	4.44	4.56	3.13	3.20	5.46	1.86	2.58
K	30.8	31.6	29.4	31.5	26.7	43.9	42.1	38.0	20.9	27.1
Zn	1.42	2.01	1.76	1.46	2.48	0.81	2.49	2.23	0.95	1.83
	11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
pH	8.34	7.92	8.28	7.71	7.96	7.79	7.79	7.78	7.77	7.96
EC	0.73	0.44	0.31	0.52	0.40	0.42	0.45	0.41	0.60	0.59
N	0.047	0.052	0.061	0.039	0.042	0.051	0.049	0.050	0.060	0.060
P	2.13	4.23	3.92	5.04	4.38	3.34	2.28	2.12	3.75	1.19
K	29.2	20.7	46.4	32.8	30	28.9	27.6	30.6	33.5	31.3
Zn	1.26	0.85	0.93	1.21	1.92	0.83	1.05	0.88	1.24	1.24

Parameters in ppm, except pH, EC = m mho/cm.

TABLE - 04 Physico-chemical characteristics and nutrient status of soil of different talukas of Gandhinagar District (Year 2011 to 2013)

No.	Parameters	Taluka			
		Dehgam	Mansa	Gandhinagar	Kalol
1.	pH	7.97	7.99	8.04	7.81
2.	EC	0.52	0.43	0.48	0.49
3.	Nitrogen [N]	0.046	0.041	0.048	0.054
4.	Phosphorus [P]	3.93	3.24	3.94	2.53
5.	Potassium [K]	30	34.4	31.8	30.3
6.	Zinc	1.82	1.66	1.23	1.04

IV. CONCLUSION

This study has shown that weed species in the study area of Gandhinagar showed relations with the soil in which they occur. 73 species (39.67%) were recorded during winter season. Also, the occurrence types and distribution of specific weed species influenced by certain soil properties in the study area.

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AMALGAMATION OF TECHNOLOGIES & SCIENTREPRENEUR PROSPECTIVE OF ELECTROCHEMICALLY ACTIVE HALOPHILIC MICROBUGS - A REVIEW PAPER

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Abstract

Turning opportunity to advantage is mandatory in this competitive land for survival. Climate changes and the rapid depletion of natural resources forms of energy, like coal and petrochemicals, have sparked human civilization; this problem needs feasible and effective sustainable energy sources. Energy is a vital need of any living organism to accomplish diverse physiological and physical processes. Consequently, coal and gas sources have been exploited to greater verge. Now a day's emphasis is on alternative energy sources in order to fulfill the need for sustainable energy generation. Scientific research in pollution reduction and environmental management of

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Antimicrobial Metabolites from Halophilic Actinomycete *Nocardiopsis sp. Al-H10-1* (KF384482) Isolated from Alang, Gulf of Khambhat, India

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Keywords

Marine actinomycetes; halophilic; 16S rRNA gene sequencing; antimicrobial activity; spot inoculation method; optimization; OVAT method

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ABSTRACT

The overuse of antibiotics has resulted in the development of drug resistant, a major problem in disease curing processes i.e. development of drug resistance. The World Health Organization (WHO) released its first list of the most concerning pathogens for human health in 2017 which suggested that there are total 12 bacterial families which have developed multiple drug resistance and for which novel antibiotics are required immediately (WHO 2017). There is a requirement to explore some novel compounds to overcome this issue. Thus our study aimed at exploration of marine actinomycetes as a valuable resource for novel products with antimicrobial properties. The halophilic actinomycete *Nocardiopsis sp. Al-H10-1* (KF384482) was isolated from saline water (20 m away from shore) of Alang coast (Gulf of Khambhat), Bhavnagar, Gujarat, India. The isolate Al-H10-1 was identified as *Nocardiopsis sp.* through rigorous morphological and cultural characteristics; the species was confirmed through 16s rRNA phylogenetic analysis. The antimicrobial potential of *Nocardiopsis sp. Al-H10-1* was assessed against a range of Gram-positive and Gram-negative bacteria as well as three fungi, there it demonstrated antimicrobial activity against four Gram negative bacteria and one Gram positive bacteria. The isolate didn't show any antifungal activity. The results of optimization showed that the highest antimicrobial compound production was obtained in the presence of 0.5 % starch, 10% NaCl and pH 9.

Introduction

The World Health Organization (WHO) released its first list of the most concerning pathogens for human health in 2017 which suggested that there are total 12 bacterial families who have developed multiple drug resistance and for which novel antibiotics are required immediately (WHO, 2017). The study by O'Neill suggests that every year approximately 7,00,000 people die because of drug resistant infections globally and if current trends continue, it will be increased by 10 million people per year by 2050 (O'Neill,

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Protective effect of *Nigella sativa* against diethyl phthalate- induced changes in mitochondrial enzymatic activities in liver of mice

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Abstract: *Present study focuses on the evaluation of Diethyl phthalate (DEP) exerted hepatotoxicity in mice by measuring mitochondrial activities (Succinate dehydrogenase, Adenosine triphosphatase and phosphorylase) and its alleviation by Nigella sativa seed extract. Colony inbred Swiss strain adult female albino mice were orally administered with 310, 620 and 1240 mg/kg body weight/day (low, mid, high dose respectively) for 30 days. Treatment caused, as compared with the control, significant ($p < 0.05$) and dose – dependent decrease SDH, ATPase and Phosphorylase activities. Nigella sativa seed extract (150 and 300 mg/kg body weight/day) treatment along with HD of DEP, caused significant ($p < 0.05$) restoration in mitochondrial activities in liver as compared to DEP alone treated mice. It is concluded from the present study that supplementation of Nigella sativa extract can be beneficial in positively modulating DEP - induced alterations in liver.*

Key Words: *Diethyl phthalate, Nigella sativa, Succinate dehydrogenase, Adenosine triphosphatase, liver, Phosphorylase.*

1. INTRODUCTION:

Animal including humans beings are regularly exposed to toxic chemicals through food, water, air or from direct contact with a variety of consumer products. Many of these chemicals are toxic at some dose and under certain conditions of exposure. Through various route of exposure can significantly influence a chemical's toxicity. Diethyl phthalate is a member of esters of phthalatic acid known as phthalates, used ubiquitously as solvents and plasticisers worldwide (ECB (European Chemicals Bureau) substance ID (2006) and Godwin *et al*.,(2010)). Its release into the environment occurs primarily as a result of production, use and disposal of products containing DEP. (Giam *et al.*, (1987) and Joblins *et al.*, (1995)). Diethyl Phthalate was found to be one of the more toxic phthalates (hauser *et al*.,2005). DEP, an endocrine disrupter chemical, has been found to have diverse acute and chronic toxic effects in several species at different trophic levels (Staples *et al.*,2000). It caused mitochondrial swelling, focal dilation and vesiculation of smooth endoplasmic reticulum and increased interstitial macrophage activity associated with the surface of Leyding cells of rats (Zou *et al.*, 1997).

Medicinal plants are also used in the preparation of herbal medicines as they are considered to be safe as compared to modern allopathic medicines. Amongst the various medicinal plants, *Nigella sativa* (family Ranunalaceaes) was selected to evaluate its potency in ameliorationg the DEP – induced toxicity. *Nigella sativa* seeds contain other ingredients, including nutritional components such as carbohydrates, fats, vitamins, mineral elements, and proteins, including eight of the nine essential amino acids (Bhatia et al., 1972; Correa et al., 1986; Jassir et al., 1992; Omar et al., 1999; Chun et al., 2002).

Therefore, the aim of the present study was investigate the possible protective effect of *Nigella sativa* seed extract against DEP – induced toxicity in mice.

2. MATERIALS AND METHODS:

Chemicals: Diethyl phthalate was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India and was of analytical grade. All the other chemicals used in the present study were of analytical grade and purchased from Hi Media Laboratories Pvt. Ltd., Mumbai, India, Sisco Research Laboratories Pvt. Ltd., Mumbai, India and Sigma Aldrich, St. Louis, MO, USA. Olive oil was obtained from Figaro, Madrid, Spain.

Experimental animals: All animal studies were sanctioned by Institutional Animal Ethics Committee of Gujarat University, Ahmedabad and approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India. Healthy young female albino mice of Swiss strain weighing 30-35 gm were obtained from Zydus Research Centre, Ahmedabad, India. The animals were kept in the Animal House of Zoology Department of

Gujarat University, Ahmedabad, India under controlled conditions (Temperature 25±2˚C, relative humidity 50-55% and 12h light/dark cycle). They were fed with certified pelleted rodent feed supplied by Amrut Feeds, Pranav Agro Industries Ltd., Pune, India and potable water ad libitum. Animals were handled according to the guidelines published by the Indian National Science Academy, New Delhi, India (1991).

***Nigella sativa* extract preparation:** Seeds of *Nigella sativa* were purchased from local market and hydro - alcoholic extract was prepared according to Bhargava and Singh with slight modification. Finely ground *Nigella sativa* seeds powder was mixed with 50% methanol and allowed to stand overnight for maximum extraction of polyphenols. Percolation of the extract was performed at room temperature in two stages. Collected filtrate was evaporated below 50˚C to obtain a final product in the form of residues which was stored under refrigerated conditions. Extract was dissolved in double distilled water and used for studies.

Experimental Design: Eighty animals were randomly divided into eight groups. Animals of Group 1 were without any treatment. Animals of Group 2 received 0.2ml olive oil/animal/day (olive oil was used to dissolved DEP) for 30 days and marked as vehicle control. Antidote control group (Group 3) animals were given oral treatment of *Nigella sativa* (300 mg/kg body weight/day). Group 4, 5 and 6 animals were given oral treatment of low dose (310 mg/kg body weight /day), mid dose (620 mg/kg body weight /day), and high dose (1240 mg/kg body weight/ day) of DEP. Animals of Group 7 and 8 were treated with DEP (1240 mg/kg body weight/ day) along with 150 and 300 mg/kg body weight/ day of *Nigella sativa* extract. Dosages of DEP treatment were based on the LD50 value i.e. 8600 mg/kg (National Toxicology Program, 2006). Animals were given treatment for 30 days and autopsied on 31st day. Liver was quickly isolated, blotted free of blood and used for determination of biochemical parameters.

Adenosine triphosphatase activity: The adenosine triphosphatase (ATPase) activity in the liver and kidney was assayed by the method of Quinn and White (1968). ATPase causes hydrolysis of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and inorganic phosphate (i.p.). The liberated inorganic phosphate was estimated by the method of Fiske and Subbarow (1925). The optical density was read at 660 nm. The enzyme activity was expressed as µmoles inorganic phosphate released/mg protein/30 min.

Succinic dehydrogenase activity: The succinic dehydrogenase (SDH) activity in the liver and kidney was assayed by the method of Beatty et al. (1966) using 2-(4-iodophenyl)-3-(4-nitrophenyl)-5- phenyl- 2H – tetrazolium chloride (INT) as an electron acceptor. The electrons released by the enzyme SDH from the substrate are taken up by INT, which was reduced to a red coloured formazon. This was extracted in ethyl acetate and the absorbance was read at 420 nm. The enzyme activity was expressed as µg formazon formed/mg protein/15 min.

Phosphorylase activity: The liver phosphorylase activity was assayed by the method of Cori et al. (1943). The inorganic phosphate (i.p.) formed at the end of the reactions was estimated by the method of Fiske and Subbarow (1925). The enzyme phosphorylase hydrolyses the substrate glucose -1-phosphate. The inorganic phosphate formed at the end was treated with an acidic molybdate solution; it forms phosphomolybdic acid which on addition of 1-amino-2-nephthol-4-sulphonic acid (ANSA) is quantitatively reduced to a blue coloured complex which is measured spectrophotometrically at 660 nm. The enzyme activity is expressed as µg phosphorus released/100 mg fresh tissue/15 min.

Hepatoprotective index (HP index): Hepatoprotective index (HP index) The liver protecting activity of the Nigella sativa seed extract was expressed as hepatoprotective percentage (H) (Prakash *et al.* 2008) which was calculated using the formula:

$$H = 1 - \left(\frac{T - V}{C - V} \right) X\ 100$$

Where T is the mean value of plant extracts along with the DEP, C is the mean value of DEP alone, and V is the mean value of vehicle control animals.

Statistical analysis: All the data are expressed as the means ± standard error mean (SEM). Statistical analysis anwas performed using Graphpad Instat, software, version 5.03. The data were statistically analyzed using one - way Analysis of Variance (ANOVA) followed by Tukey's test. The level of significance was accepted with $p < 0.05$.

3. RESULT AND DISCUSSION:

Table 1: Showing effect of diethyl phthalate on succinic dehydrogenase, adenosine triphosphatase and phosphorylase activities in liver of mice

Parameters	Experimental groups				
	Untreated 1	Vehicle control 2	Low dose of DEP 3	Mid dose of DEP 4	High dose of DEP 5
SDH	55.87 ± 2.03	54.29 ± 1.92	41.60 ± 1.12 [a]	31.12 ± 1.01 [a]	26.44 ± 0.76 [a]
ATPase	1.51 ± 0.06	1.54 ± 0.03	1.34 ± 0.07	0.92 ± 0.12 [a]	0.50 ± 0.02 [a]
Phosphorylase	1.39 ± 0.04	1.37 ± 0.02	1.15 ± 0.02 [a]	0.89 ± 0.04 [a]	0.52 ± 0.05 [a]

Values are mean ± S.E.M.; n = 10
Significant at the level
[a]p < 0.05 verses vehicle control group (Group 2)
No significant difference between control groups (Group 1 and 2)
Units: SDH - µg formazon formed/mg protein/15 min; ATPase - µmoles i.p. released/mg protein/30 min; phosphorylase - µg phosphorus released/100 mg fresh tissue/15 min.

Table: 1 depicts the results of various doses of DEP caused changes in energy metabolism. No significant changes were observed in the activities of SDH, ATPase and phosphorylase in the liver of untreated and vehicle control group (group 1 and 2). However, in all three doses of DEP treatment (Group 3, 4 and 5) significant ($p<0.05$) reduction was observed in the activity of SDH (LD: 23.38%, MD: 42.68%, HD: 51.30%). However, significant reduction was in mid and high doses DEP -treated groups. The reduction in both SDH and ATPase activities were dose-dependent (r^2 =0.963 and 0.977 respectively). Similarly, oral administration of three different doses (LD, MD and HD) of DEP caused decrease phosphorylase activity significantly ($p<0.05$) and dose – dependently (r^2 = 0.985) in liver (16.06%, 35.04% and 62.05% respectively).

Oral administration of DEP to mice for 30 days had significantly altered the energy status. DEP treatment resulted in reduction in SDH activity – an enzyme bound to inner mitochondrial membrane, which could be due to structural and functional disorganization of the mitochondrial assembly. Srivastava et al. (1978 and 1977) reported that di (2-ethyl hexyl) phthalate (DEHP) also found to inhibit the activity of total and Mg+ - stimulated ATPase activity in rat liver. Beside liver, the activity of SDH and ATPse was also inhibited in rat heart, kidney (Srivastava et al., 1977), lung and gonads (Seth et al., 1976), indicating that suppression of energy- linked reactions may be a generalized effect of DEHP. Alteration in mitochondrial potential decreases the rate of cellular ATP synthesis and, thus nucleotide synthesis which may cause the reduction in DNA and RNA contents. Energy deficiency of the cell characterised by reduced activity of SDH and ATPase could be well correlated with reduction in protein content (Panet and Altan, 1979). Mitochondria contains biochemical machinery for oxidation of various biomolecules and produced energy is captured in the form of ATP. Phthalates inhibited the respiration of isolated mitochondria from rat liver primarily by uncoupling oxidative phosphorylation (Inouye et al., 1978; Melnick et al., 1982). Other researchers have suggested that the phthalates inhibited electron transport or energy transport (Ohyama et al., 1976). Dibutyl phthalate and dimethyl phthalate inhibited the activities of SDH and ATPase, enzymes of the rat liver inner mitochondrial membrane (Srivastava et al., 1977; Tanaka et al., 1978; Melnick et al., 1982).

Experimental Groups	SDH	ATPase	Phosphorylase
(I)Control			
1. Vehicle	54.29 ± 1.92	1.54 ± 0.03	1.37 ± 0.02
2. Antidote(NS300)	54.19 ± 1.78	1.60 ± 0.05	1.39 ± 0.04
(II) Diethyl phthalate – Treated			
3. DEP1240 ; HD	26.44 ± 0.76 [a]	0.50 ± 0.02 [a]	0.52 ± 0.05
(III) DEP1240(HD)+ Nigella sativa extract – Treated			
4. HD DEP + NS150	35.06 ± 1.56 [a] (31.00)	0.78 ± 0.02 [a] (27.00)	0.83 ± 0.02 [ab] (37.00)
5. HD DEP + NS300	49.27 ± 2.45 [b] (82.00)	1.07 ± 0.06 [ab] (58.50)	1.0 ± 0.02 [ab] (57.00)

Values are mean ± S.E.M.; n = 10
Significant at the level

[a]p < 0.05 verses vehicle control group (Group 2)
[b]p < 0.05 verses high dose DEP - treated group (Group 3)
No significant difference between control groups (Group 1 and 2)
Values in parenthesis indicate hepatoprotective index (HPI)
Units: SDH - µg formazone formed/mg protein/15 min; ATPase - µmoles i.p. released/mg protein/30 min

Oral administration of 300 mg/kg bw *Nigella sativa* seed extract did not cause any significant change than that of vehicle control (Group 2) (Table 2). High dose of DEP treatment reduced activities of hepatic SDH, ATPase and phosphorylase to 48.70% and 32.46% respectively as compared to vehicle control. Cotreatment of *Nigella sativa* seed extract along with HD of DEP for 30 days caused significant amelioration in all parameters as compared to HD of DEP alone treated groups (Table: 2). Percent protection in SDH and ATPase activities were NS150: 31.00, NS300: 82.00 and NS150: 27.00, NS300: 58.50 respectively as indicated by HPI. Cotreatment of *Nigella sativa* seed extract along with high dose of DEP for 30 days caused significant amelioration in phosphorylase activity as compared to HD of DEP alone treated groups. Percent protection in phosphorylase activity was NS150: 37.00, NS300: 57.00 as indicated by HPI. All three doses of DEP were found to reduce the activities of SDH and ATPase activities in liver of the animals resulting in altered status. Treatment with *Nigella sativa* seed extract along with DEP significantly ameliorates DEP caused changes in the activities of SDH and ATPase in liver and kidney of mice (Table 1). Erşahin et al. (2011) reported that *Nigella sativa* with its potent free radical scavenging properties, inhibited subarachnoid-haemorrhage-(SAH-) induced lipid peroxidation in the brain tissue of rat against the reactive hydroxyl, peroxyl, and superoxide radicals. In addition, the level of antioxidant glutathione (GSH) was preserved, thereby ameliorating oxidative damage. The SAH-induced reduction of Na+ /K+ -ATPase activity indicated the presence of membrane damage. The Na+ /K+ - ATPase is involved in the generation of the membrane potential through the active transport of sodium and potassium ions in cellular membrane. It maintains neuronal excitability and controls cellular volume in the central nervous system. Treatment with *Nigella sativa* was able to restore Na+ /K+ -ATPase activity back to normal levels. Hamed et al. (2013) reported that treatment with black seed alleviated the elevation of SDH and Na+ /k + ATPase. The restoration of ATPase activity suggest the ability of *Nigella sativa* to protect the sulphyhydryl group from oxidative damage through inhibition of lipid peroxidation. Normalised metabolism of protein, carbohydrates and lipid as well as free radical scavenging effect of plant improves integrity and oxidative phosphorylation in mitochondria which was highly disturbed in case of energy deficient state-induced by DEP.

4. CONCLUSION:

In conclusion, oral administration of DEP caused alteration in Succinate dehydrogenase, Adenosine triphosphatase and phosphorylase activities in liver. *Nigella sativa* seed extract reduced DEP induced mitochondrial enzymatic changes due to its phytochemicals having antioxidative properties.

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Taxonomy of three allied *Tectaria* species (Tectariaceae) from North-East India and Western Ghats

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Abstract

The detailed taxonomic account of three allied taxa, *Tectaria polymorpha* ((Wall. ex Hook.) Copel., *T. pseudosiifolia* Fraser-Jenk. & Wangdi and *T. wightii* (C.B.Clarke) Ching (Tectariaceae) are studied which are often reported erroneously or misidentified as either *T. herpetocaulos* Holttum or *T. polymorpha*. Therefore, present study was aimed to find the taxonomic differences to discriminate the species.

Key words: Tectaroid fern, Exindusiate sori, *Tectaria pseudosiifolia*.

INTRODUCTION

The treatment of tectaroid ferns has un-orthodoxically undergone several changes in their names time to time (Ding *et al.* 2013; Ding *et al.* 2014). The pantropical genus *Tectaria* Cavanilles (1799: 115) of Tectariaceae is comprised of 200 species worldwide, 27 of which are distributed in India (PPG-I 2016; Fraser-Jenkins 2020). These are terrestrial plants, medium to large herbs, growing along the cut edges of hills and hillocks or edges of river, having erect, suberect, short creeping or long creeping rhizomes, fronds either monomorphic, dimorphic or subdimorphic, lamina decurrens, simple pinnate with or without lower pinnae lobe and hairy on both sides, lamina hairy on lower side only, lamina sparsely scaly on both sides, lamina glabrous, *veins* visible on lower side, either anastomosing with branched included veinlets, simple anastomosing with or without veinlets free veins or free but areole along the costa and costule, sori many, indusiate or exinduciate, indusia either persistent or shaded, *spores* monolete or trilete (Lindsey & Middleton 2012; Patil *et al.* 2019a, 2020). Venation pattern is an important criterion in delimiting tectaroid ferns has been shown in Philippines and Indian members (Salgado 1982; Banerjee (Mukherjee) & Mukhopadhyay 2008). Species like *Tectaria herpetocaulos* Holttum (1965: 241), *T. polymorpha* (Wall. ex Hook.) Copel. (1907: 413), *T. pseudosiifolia* Fraser-Jenk. & Wangdi (2015: 31) and *T. wightii* (C.B. Clarke) Ching (1931: 20) of this genus are conventionally defined based on the morphological features and identification of species within the genus *Tectaria* is often difficult due to the high level of morphological similarities among the species. Until recently, the *T. polymorpha* from south India is treated as either *T. wightii* or *T. herpetocaulos* and a novel Eastern Himalayan species *T. pseudosiifolia* was misidentified as *T. polymorpha* (Patil *et al.* 2019b). However, present study will help to avoid/rectify the confusion in identification of these species in future.

MATERIALS AND METHODS

Collection of plant specimens: Field survey were carried out during June 2017 to January 2020 from different biogeographic regions of India. Specimens were collected in sterile polyethylene bags and brought to the laboratory for further processing.

Literature survey and identification of taxon: A critical examination of the related literature has been used for the confirmation of the identity of the taxa under investigation. The detailed information of the *Tectaria* species were gathered from national as well as regional floras, books, journals, periodicals and research publications. Further, the characteristics of each specimen were compared with the literature (Manickam & Irudayaraj 1992; Sing & Panigrahi 2005; Fraser-Jenkins *et al.* 2015, 2018; Patil *et al.* 2019b) and identified as *Tectaria polymorpha, T. pseudosiifolia* and *T. wightii*. The range of variations were studied by examining characters of 5 specimens collected from each geographic zone.

Documentation: Voucher specimens has been deposited in BARO Herbarium of the Department of Botany, The Maharaja Sayajirao University of Baroda, Vadodara (Gujarat).

Phenology: Phenology of the species was studied in field by observing the life phases (vegetative and reproductive) of each species. If the number of sterile fronds is more at a time, then that was considered as vegetative phase and if the number of fertile fronds is more at that time was considered as reproductive phase. Also, studied the cultivated plants in botanical garden.

Ecology: The prediction of the studied species on rainfall, temperature and humidity was based on monthly analysis of the meteorological data from respective regions of India. Whereas, altitude was determined with a Garmin Oregon 750 GPS.

Conservation status: It was analysed by using the criteria given in IUCN Red list of Threatened Species (Version 2020-1).

RESULTS

Key to the species

1a. Rhizome long creeping, sori exindusiate or indusiate ……..……...…… 2
1b. Rhizome short creeping or suberect, sori strictly indusiate …… 3

2a. Lower pinnae bipartite, sori indusiate ……...……..…...…….. *T. herpetocaulos*
2b. Lower pinnae not bipartite, sori exindusiate ...……...…….. *T. wightii*

3a. Rhizome suberect, stipe, rachis and lamina densely hairy ……...…... *T. pseudosiifolia*
3b. Rhizome short creeping-suberect, stipe, rachis and lamina sparsely hairy ... *T. polymorpha*

Taxonomy

The taxonomic analysis of three species, *T. polymorpha* (Wall. ex Hook.) Copel.*, T. pseudosiifolia* Fraser-Jenk. & Wangdi *and T. wightii* (C. B. Clarke) Ching were studied from India. A detailed morphological description, synonyms, distribution, ecology, phenology and conservation status of three species viz., *Tectaria polymorpha, T. pseudosiifolia T. wightii* is provided herewith. However, taxonomic analysis of *T. herpetocaulos* Holttum is based on available literature and photographs/ illustrations provided in Lindsay & Middleton (2012), Xing *et al.* (2013) and Fraser-Jenkins *et al.* (2018) and herbarium specimens examined (K , CAL), provided comparative analysis with other species (Table 1).

Tectaria polymorpha (Wall. ex Hook.) Copel. in Philipp. J. Sci., C. 2: 413. 1907; Hoitt., Kew Bull. 27 (3): 419. 1972; Sledge, Kew Bull. 27: 419. 1972 & Bot. J. Linn. Soc. 84: 18. 1982; Nayar & Kaur, Comp. Bedd., Handb. 51. 1974; Dixit, Census 144. 1984; Kuo, Taiwania 30: 29. 1985; Chandra & Kaur, Norn en. Guide I–4. 1987; Punetha & Kholia New Bot. 14: 124. 1989; Dixit, Indian Fern J. 6: 158. 1989; Rajagopal & Bhat, Indian Fern J. 15: 21. 1998; Fraser-Jenkins, Taxon. Revis. Indian Subcontinental Pteridophytes 314–315. 2008; Fraser-Jenkins *et al.*, Annot. Checkl. Ind. Pterid. II. 379–380. 2018; Patil *el al.*, Pl. Sci. Today, 6 (2): 170–182. 2019.

Table 1. Comparative morphology of closely allied species of *Tectaria*

Characters /Species	***Tectaria herpetocaulos***	***Tectaria polymorpha***	***Tectaria pseudosiifolia***	***Tectaria wightii***
Rhizome	Long creeping, thin	Short creeping or suberect	Suberect	Long creeping, thick
Scale	Margin ciliate, subentire, base cordate	Margin smooth, entire, base round	Margin smooth, subentire, base round	Margin ciliate, base round
Pinnae	5-11, lower pinnae bipartite	5-9, lower pinnae, bipartite	5-7 lower pinnae, bipartite	7-11, lower pinnae, not bipartite
lamina	Sparsely hairy, hairs shorts, 1-3 celled	Sparsely-densely hairy, hairs short, 2-4 celled	Densely hairy, hairs long, 3-8 celled	Sparsely hairy, short, 1-2 celled
Sori	Arranged in two rows	Arranged in two rows or scattered	Arranged in two rows	Arranged in two rows
Indusia	Indusiate	Indusiate	Indusiate	Exindusiate
Spores	Reticulate, margin wavy	Reticulate, margin sharp spinulose	Reticulate, margin wavy	Smooth, margin consists of blunt spines

Lectotype (designated by Holttum)**:** NEPAL, *N. Wallich Num. List no. 382*, (K); Fl. Malesiana, 2, 2(1): 87. 1991.

Basionym: *Aspidium polymorphum* Wall. ex Hook., Sp. Fil. 4:54. 1862.

Other synonyms: *Aspidium nantoense* Hayata, Icon. Pl. Formosan. 8: 139-140, t. 63, 64. 1919. *Dryopteris polymorpha* (Wall. ex Hook.) Kuntze, Revis. Gen. Pl. 2: 813. 1891. *Nephrodium polymorphum* (Wall. ex Hook.) Baker, in Hooker & Baker, Syn. Fil. 297. 1867. *Nephrodium subpedatum* Harr., J. Linn. Soc., Bot., 16: 30. 1877. *Tectaria khonsaensis* Sarn. Singh & Panigrahi, Ferns Fern-Allies Arunachal Pradesh 2: 646. 2005. *Tectaria subpedata* (Harr.) Ching, Sinensia 2: 23. 1931.

Plants medium-large herbs, terricolous, 30 – 100 cm (rarely >100 cm) in height; *rhizome* 1 – 2 cm in diameter, suberect-short creeping, stout, densely scaly; *scales* 4 – 6 mm long, 1 – 2 mm broad, linear to lanceolate, concolorous, pale brown, glossy, apex long acuminate, base broad, round, margins entire, wavy; *fronds* 49 – 98 cm long, 20 – 35 cm broad, simple pinnate, clustered, dimorphic (sometimes subdimorphic); *sterile fronds* 42 – 90 cm long, 22 – 35 cm broad, shorter than fertile one; *stipe* 18 – 32 long, scaly at base only, dark brown, grooved, glabrescent; *lamina* 24 – 56 cm long, 25 – 35 cm broad, simple, imparipinnate, dark green or brown when dried, ovate or deltoid to oblong, sparsely hairy rachis, costa and veins; *hairs* ctenitoid type, 1 – 2 celled, reddish, articulate, also present along the margin; *pinnae* 11 – 16 cm long, 2 – 6 cm broad, 3 – 9 (rarely 11) per frond, ovate-oblong or ovate-lanceolate, upper pinnae sessile, lower pinnae petiolate (short), apex acute-acuminate, margin entire or undulate (rarely segmented), bases round, lower pinnae bipartite; *venation* highly reticulate, raised on both sides, veinlets forming conspicuous subhexagonal areoles with included branched veinlets; *fertile fronds* 49 – 98 cm long, 15 – 28 cm broad, longer but narrower than sterile one; *stipe* 21 – 35 cm long, scaly at base, dark brown, grooved, glabrescent; *lamina* 28 – 63 cm long, 15 – 28 cm broad, ovate-lanceolate, sparsely hairy, rachis costa and costule; *pinnae* 10 – 15 cm long, 2 – 3 cm broad, 3 – 9 (rarely 11) per frond, ovate-lanceolate, upper pinnae sessile, lower pinnae petiolate (short), apex long acute-acuminate, margin entire or undulate, lower pinnae trifurcate; *sori* 1 – 2 mm in diameter, indusiate, orbicular, on veinlets or anastomosing veins, in 2 rows along the lateral veins or in irregular rows between lateral veins; *indusia* greenish white (at young)-brown (at maturity), orbicular-reniform, thin, membranous, entire, caducous; *sporangia*

many, brown, 2 – 3 celled stalk, annulus 10 – 14 celled; *spores* 28 – 30 ìm in diameter, monolete, oval, yellow, exine thick, dark brown, dentate with sharp apices, perine smooth, wrinkled into elongated ridge like folds.

Distribution: World: **Asia** – Bangladesh, Bhutan, Burma, China, Cambodia Java, Indonesia, Malaya Peninsula, Malaya Islands Myanmar, Nepal, Philippines, Sri Lanka, Sumatra, Taiwan, Thailand, Tibet.

India: **Himalaya's:** Arunachal Pradesh, Himachal Pradesh, Sikkim, Uttarakhand, West Bengal (Darjeeling); **North East India**: Assam, Manipur, Meghalaya, Mizoram Nagaland, Tripura; **Gangetic Plains**: West Bengal; **Peninsular India**: Andhra Pradesh, Maharashtra, Madhya Pradesh, Tamil Nadu; **Western Ghats**: Karnataka, Kerala; **Andaman and Nicobar Islands**: North and South Andaman.

Phenology: The rhizomes of *T. polymorpha* (in triplicate) was cultivated in the garden produced initial frond after 10 – 12 days. Initial frond was having 1–3 pinnae, of which lower pinnae is forked and at mature stages possessed 5 – 9 sterile pinnae. After three months of cultivation from the same rhizome new fertile frond was arisen. The development of fertile frond is similar to sterile on and having sori in 2 rows or in irregular rows between lateral veins. It took 2 – 4 months to become mature and shade the spores.

The field observations revealed that vegetative phase (maximum vegetative fronds) was observed during July to September (three months) whereas reproductive phase (maximum reproductive fronds per individual) was observed during October to February (four months) and March to June is considered as resting phase (except North East and Eastern Himalaya's). It also observed that in high humidity region, it was growing as perennial species. However, it also observed that population size (50 individuals per sp. km.), height of plant (30 cm) and number of pinnae (03 per fronds) with considerably reduced. In the month of March and April due to raining at North East and Eastern Himalayan regions the reproductive phase is extended up to May.

Ecology: Terricolous or saxicolous species observed along cut surfaces of hills especially in the Ghat sections. In general, the *T. polymorpha* was found growing in the climatic conditions where annual rainfall ranges from 500 – 3000 mm, altitude in between 100 – 1800 m, temperature 15 – 32° C and atmospheric humidity > 30 %. However, the maximum population was observed in the regions where annual rainfall was measured from 1000 – 2500 mm, altitude in between 500 – 1200 m, temperature 20 – 28° C and atmospheric humidity >50 %. With the increase in the altitude (1200), temperature above 28°C) and decreased (<50) then the population of species was decline considerably.

Conservation status: *T. polymorpha* was collected from different biogeographic regions of India. A population of 250 – 350 per sq. km was observed at each locality. The area of occupancy is 700 – 1200 sp. km. Therefore, as per IUCN categories and criteria (IUCN ver. 2017-1), it was assessed as least concerned (LC) for India. However, globally this species was not assessed i.e., data deficient (DD) species (IUCN ver. 2017-1).

Taxonomic Note: *Tectaria polymorpha* is having suberect or short creeping rhizome (Figure 1b), scales long acuminate, margin smooth (Figure 1c), frond unipinnate, sparsely hairy with lower bipartite pinnae (Figure 2a-c), sori either in two rows or scattered (Figure 3a), sporangia 2-3 celled stalk, annulus 10 – 14 celled (Figure 3d), spores monolete, exine thick, dark brown, dentate with sharp apices, perine smooth, wrinkled into elongated ridge like folds (Figure 3e).

Specimens Examined: INDIA: Karnataka, Dt., North Kananda, Castle Rock alt., 1100 m, 13.08.2018, S.M. Patil & K. S. Rajput 3041, BARO; On the way of Anmode to Goa, 10.07.2015,

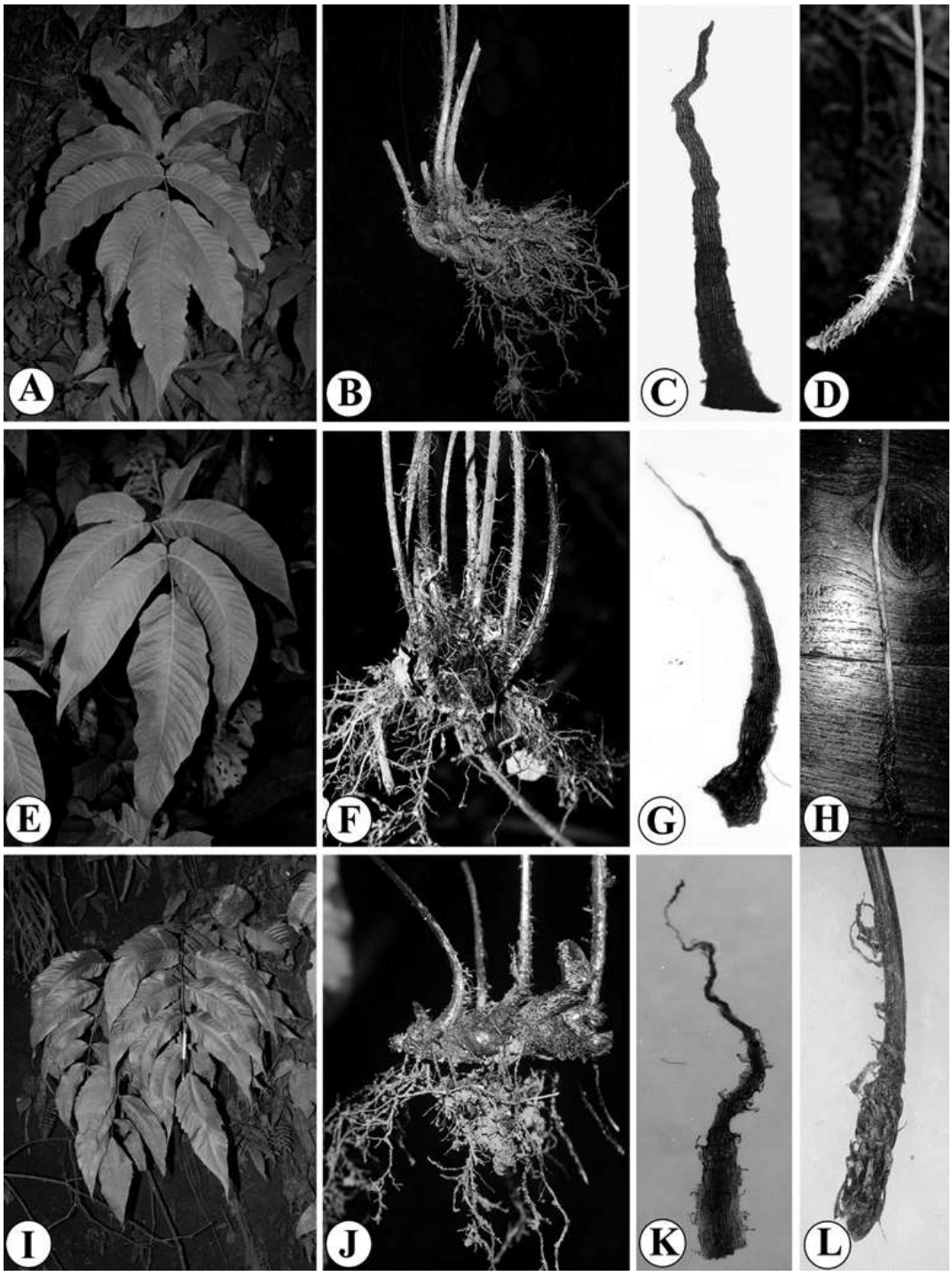

Figure 1: A - D. *Tectaria polymorpha*: **A**. habit; **B**. rhizome; **C**. scale; **D**. stipe base; **E - H**. *Tectaria pseudosiifolia*: **E**. habit; **F**. rhizome; **G.** scale; **H**. stipe base; **I - L**. *Tectaria wightii*: **I**. habit; **J.** rhizome; **K**. scale; **L**. stipe base.

Dubal and Kale, 103 (SUK); Arunachal Pradesh, Dt., Papam Pare, Itanagar, Sanki Park, 18.02.2018, S.M. Patil & K. S. Rajput 3043, BARO; Ganga Lake, 31.02.2009, Fraser-Jenkins & A. Benniamin 12858 (ARUN); Dibang valley Dt., on the way to mehao sanctuary lake, 06.09.1990, A. Pramanik, 24826 (ARUN); Mehao lake to Roing, 27.11.200, A.R.K. Sastry, (ARUN); Tirap Dt., Nignu to Niusa,13.08.1958, G. Panigrahi, 14886 (CNH) Meghalaya, Jowai Hills, 26.02.2018, S.M. Patil & K.S. Rajput 3043, BARO; Assam (NEFA), Foothill Camp, alt., 800 m, G. Panigrahi, 5830A (ASSAM); Tripura, Dt., North Tripura, Jampui Hills, alt., 900 m, 26.06.2011, A. Benniamin, 28249 (ARUN); Meghalaya, Jowai hills, Sohmynting SG, D. Verma 730 (ASSAM!); Mizoram, Dampa Tiger reserve, alt., 900 m, 03.11.2011, A. Benniamin, 28441 (ARUN).

Tectaria pseudosiifolia Fraser-Jenk. & Wangdi, Fraser-Jenkins *et al.,* Ferns Fern-allies Nepal 1:31. 2015; Fraser-Jenkins *et al*., Annot. Checkl. Ind. Pterid. II. 380 – 381. 2018.

Holotype: BHUTAN: 3 km N. of Samdrup Jongkhar, 2 km S. of Pinchinang Check-post, *C.R. Fraser-Jenkins*, *T. Wangdi*, *S. Lungten* & *T. Dorji* 33930 (FN 16), 20.5.2009, TAIF.

Plants small-medium sized herb, terricolous 30 – 80 cm (rarely >80 cm) in height; *rhizome* 1 – 3 cm in long, erect-suberect, stout, densely scaly at apex and stipe bases; *scales* 5 – 8 mm long, 1 – 2 mm broad, linear to lanceolate, concolorous, pale brown, glossy, apex long acuminate, base broad, round, margins entire, wavy; *fronds* 29 – 77 cm long, 25 – 35 cm broad, simple pinnate, clustered, dimorphic (sometimes subdimorphic), densely hairy; *sterile fronds* 20 – 71 cm long, 20 – 30 cm broad, shorter than fertile one; *stipe* 8 – 30 cm long, densely scaly at base, sparsely above, green at young, brown at maturity, grooved, densely pubescent; *lamina* 12 – 41 cm long, 25 – 35 cm broad, simple, imparipinnate, pale green or brown when dried, ovate or deltoid to oblong, densely hairy rachis, costa and veins; *hairs* 2 – 7 mm long, ctenitoid type, 3 – 8 celled, pale brown, articulate, also present along the margin; *pinnae* 10 – 15 cm long, 3 – 5 cm broad, 3 – 7 per fronds (rarely more than 7), oblong-elliptic, apex long acuminate, base cuneate, margin entire-wavy, opposite- subopposite, basal 2 – 3 pairs petiolate, upper 3 – 5 pairs sessile, lower pinnae bipartite, costa and costules raised beneath, pubescent (densely hairy lower side); *venation* highly reticulate, raised on both sides, veinlets forming conspicuous subhexagonal areoles with branched included veinlets; *texture* sub-coriaceous; *fertile fronds* 29 – 77 cm long, 25 – 35 cm broad, longer but narrower than sterile one; *stipe* 12 – 35 cm long, scaly at base, sparely above dark brown, grooved, densely pubescent; *hairs* ctenitoid types similar to sterile one; *lamina* 17 – 42 cm long, 20 – 28 cm broad, oblong-ovate, densely hairy, rachis costa and costule; *pinnae* 10 – 15 cm long, 2 – 3 cm broad, 3 – 7 (rarely 09) per frond, oblong-ovate or ovate lanceolate, upper pinnae sessile, lower pinnae petiolate (short), apex long acute-acuminate, margin entire or undulate, lower pinnae bifurcate; *venation* similar to sterile one; *sori* 1–2 mm in diameter, indusiate, orbicular, on veinlets or anastomosing veins, in 2 rows along the lateral veins; *indusia* greenish white (at young)-brown (at maturity), orbicular-reniform, thin, membranous, entire, caducous; *sporangia* many, brown, 2 – 3 celled stalk, annulus 10 – 16 celled; *spores* 20 – 28 ìm in diameter, oval, yellow, exine thick with dark brown, crenate, perine reticulate.

Distribution: World: **Asia** – Bhutan, India and Nepal; endemic to Eastern Himalaya.

India: Himalaya: Arunachal Pradesh, Sikkim.

Phenology: The rhizomes of *T. pseudosiifolia* (in triplicate) was cultivated in the garden produced initial frond after 7 – 9 days. Initial frond was having 5 – 7 pinnae, of which lower pinnae is forked at maturity each frond having 7 – 9 pinnae. After two months of cultivation from same rhizome, fertile frond was arisen which is longer but narrower than sterile. The development of fertile frond is similar to sterile on and having sori in 2 rows along the lateral veins. It took withing 2 – 3 months become mature and shade the spores.

The field observations revealed that vegetative phase (maximum vegetative fronds) was observed during July to September (three months) whereas reproductive phase (maximum reproductive fronds) was observed during October to February (four months) and March to June is considered as resting phase. It also observed that in high humidity region, it was growing as perennial species. It also observed, in high humid regions of Arunachal Pradesh it was found growing up to April however, the population size (<50 individuals per sp. km.), height of plant (15 cm) and number of pinnae (1 or 3 per fronds) were considerably reduced.

Ecology: Terricolous species collected along the roadside or cut surfaces of hills. In general, the *T. pseudosiifolia* growing in the region were annual rainfall ranges from 500 – 3000 mm, altitude from 100 – 800 m, temperature 13 – 28° C and atmospheric humidity >40 %. However, the maximum population was observed in the regions where annual rainfall was measured from 1000 – 2000 mm, altitude between 300 – 600 m, temperature 18 – 25° C and atmospheric humidity is >50 %. With the increase in the altitude (600 m) temperature above (25° C) and decrease in humidity (<50 %) then the population of species was declined considerably.

Conservation status: *T. pseudosiifolia* was collected from Arunachal Pradesh, India. A population of about 100 – 150 individuals was found at each locality. The area of occupancy (AOO) is 50 – 100 sq. km. However, other similar wildlife areas of the country are yet to be explored wholly and it presumes that the species might be spread in similar ecological conditions. Thus, more floristic surveys are required to determine and document the full range of distribution of *T. pseudosiifolia*. Therefore, according to IUCN (27) criteria, at present this species is considered as data deficient (DD). Globally, also this species was not assessed i.e., data deficient (DD) species (IUCN ver. 2017-1).

Taxonomic Note: *T. pseudosiifolia* is having erect-suberect rhizome (Figure 1f), scales long acuminate, margin smooth (Figure 1g), fronds unipinnate, densely hairy with lower bipartite pinnae (Figure 2d-f)), sporangia *sporangia* 2 – 3 celled stalk, annulus 10 – 16 celled (Figure 3i), spores monolete, exine thick, dark brown, crenate, perine reticulate (Figure 3j).

Specimens examined: INDIA: Arunachal Pradesh, Dt., Papam Pare, Itanagar, Ganga Lake, 18.02.2018, S.M. Patil & K.S. Rajput 3034 (BARO); Sanki Park, 21.02.2018, S.M. Patil & K.S. Rajput 3035 (BARO); On the way of BSI, 16.2.2009, CRFJ 33726 (ARUN); Naharlagun to Ziro road, 02.12.2018, S.M. Patil & K.S. Rajput 3035 (BARO); Subansiri dt., Ziro, 31.05.2008, A. Benni. 12861 (ARUN); West Siang dt., On the way of Mechuka-tato, 20.11.2008, A. Benni. 12864 (ARUN); Upper siang dt., Megging to tuting road, 18.01.2013, A. Benni., 20252 (ARUN); Kameng, Foot Hill camp, alt., 800 m, 12.03.1957, G. Panigrahi 6425 (ASSAM); BHUTAN: 3 km N. of Samdrup Jongkhar, 2 km S. of Pinchinang Check-post, 20.5.2009, *C.R. Fraser-Jenkins*, *T. Wangdi*, *S. Lungten* & *T. Dorji* 33930 (TAIF).

Tectaria wightii (C.B.Clarke) Ching, Sinensia 2: 28, t. 10. 1931; Manickum and Irudayaraj, Nayar & Kaur, Comp. Bedd., Handb. 51. 1974; Dixit, Census 145. 1984; Manickam & Irudayaraj, Pterid. Fl. West. Ghats 258. 1992; Fraser-Jenkins, New Sp. Syndr. Indian Pterid. 243. 1997; Fraser-Jenkins *et al.*, Annot. Checkl. Ind. Pterid. II. 384–385. 2018.

Lectotype (designated by Fraser-Jenkins)**:** INDIA: South India, Tamil Nadu, *Herbarium Hookerianum* 1867 (K).

Basionym: *Nephrodium wightii* Clarke, Trans. Linn. Soc. London 11, Bot. 1: 538. 1880.

Other synonyms: *Aspidium polymorphum* var. *macrocarpum* Bedd., FSI t. 117. 1865. *Aspidium polymorphum* sensu Bedd., Handb. 218. 1883, *pro parte*. & Suppl. 45. 1892. *Tectaria macrocarpa* B.K. Nayar & Geev., Bull. Bot. Surv. India 28 (1-4): 134. 1988 ("1986"), *nom. nud.* (Description not in Latin).

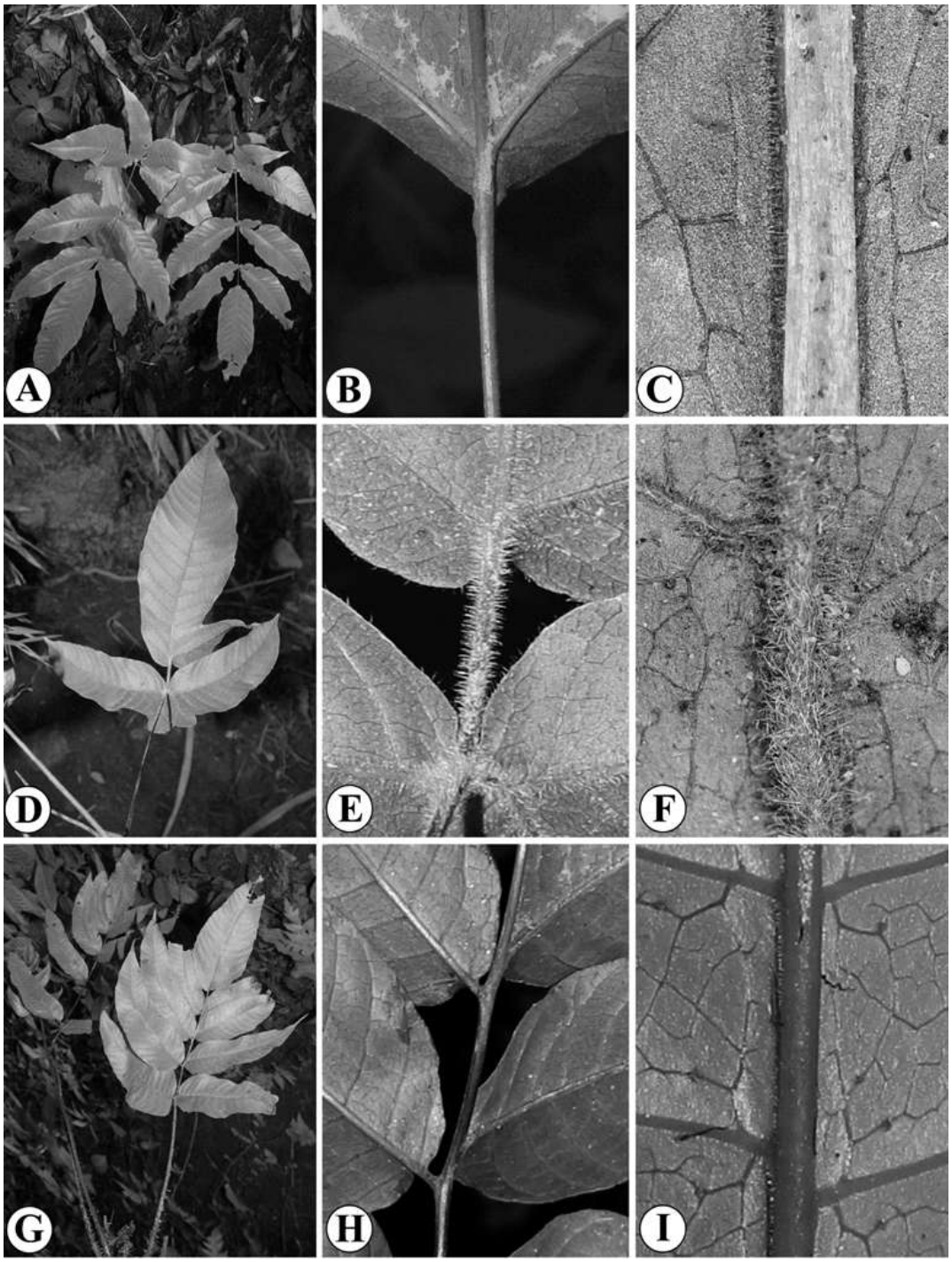

Figure 2: **A - C**. *Tectaria polymorpha*: **A**. Lamina; **B**. enlarge portion of sparsely hairy rachis; **C**. densely hairy costa, hairs short; **D - F**. *Tectaria pseudosiifolia*: **D**. Lamina; **E**. enlarge portion of densely hairy rachis; **F.** densely hairy costa, hairs long; **G - I**. *Tectaria wightii*: **G**. Lamina, **H** enlarge portion of sparsely hairy rachis; **I**. sparsely hairy costa, hairs short.

Plants medium-large sized herb, terricolous or saxicolous, 60 – 140 cm (rarely >140 cm) in height; *rhizome* 0.5 – 1 cm in diameter, long creeping, branched, stout, densely scaly at apex and stipe bases; *scales* 6 – 9 mm long, 1 – 3 mm broad, linear to lanceolate, bicolorous, brown at center, pale at periphery, glossy, apex long acuminate, base broad, round, margin ciliate (uniseriate, multicellular hairs), wavy; *fronds* 49 – 139 cm long, 30 – 45 cm broad, simple pinnate, clustered, dimorphic (sometimes subdimorphic), hairy; *sterile fronds* 49 – 128 cm long, 30 – 46 cm broad, shorter than fertile one; *stipe* 22 – 60 cm long, densely scaly at base, sparsely above, green at young, brown at maturity, grooved, glossy; *lamina* 27 – 68 cm long, 30 – 45 cm broad, simple, imparipinnate, dark green at young, brown when dried, ovate-deltoid or elliptic-oblong, sparsely hairy rachis, costa and veins; *hairs* ctenitoid type, 1 – 3 celled, pale brown, articulate; *pinnae* 15 – 23 cm long, 4 – 8 cm broad, 5 – 11 per fronds (rarely more than 11), oblong-elliptic or ovate or boat shaped, apex long acuminate, base cuneate-cordate, margin entire-wavy (sometimes serrate also) opposite-subopposite, petiolate, lower pinnae not bipartite, costa and costules raised beneath, sparsely pubescent; *venation* highly reticulate, raised on both sides, veinlets forming conspicuous subhexagonal areoles with included branched veinlets; *texture* coriaceous; *fertile fronds* 55 – 139 cm long, 25 – 40 cm broad, longer but narrower than sterile one; *stipe* 25 – 65 cm long, scaly at base, sparely above dark brown, grooved; *hairs* ctenitoid types similar to sterile one; *lamina* 30 – 74 cm long, 25 – 40 cm broad, oblong-ovate, rachis, costa and costule hairy; *pinnae* 12 – 20 cm long, 2 – 5 cm broad, 3 – 11 (rarely more than 11) per frond, oblong-ovate or ovate or boat shaped, opposite-subopposite, petiolate, apex long acuminate, margin entire or undulate, lower pinnae not bifurcate; *venation* similar to sterile one; *sori* 1 – 2 mm in diameter, exindusiate, orbicular, on veinlets or anastomosing veins, in 2 rows along the lateral veins; *sporangia* many, brown, 2 – 3 celled stalk, annulus 10 – 16 celled; *spores* 30 – 45 ìm in diameter, oval-oblong, yellow, exine thick, dark brown, sinuate, perine smooth.

Distribution: World: **Asia** – India; endemic

India: Western Ghats: Karnataka and Kerala; **Deccan Peninsula**: Andhra Pradesh and Tamil Nadu.

Phenology: The rhizomes of *T. wightii* (in triplicate) was cultivated in the garden produced initial frond after 12 – 15 days. Initial frond was having 1 – 3 pinnae and lower pinnae is not forked. At maturity each frond having 5 – 11 pinnae per frond. After three months of cultivation, fertile frond was arisen from same rhizome, which is longer but narrower than sterile. The development of fertile frond is similar to sterile frond and having sori in 2 rows along the lateral veins. It took withing 2 – 3 months become mature and shade the spores.

The field observations revealed that vegetative phase (maximum vegetative fronds) was observed during July to September (three months) whereas reproductive phase (maximum reproductive fronds) was observed from October to February (four months) and March to June is considered as resting phase. However, in high humid regions of Western Ghats it was found growing up to April and the population size (<50 individuals per sq km), height of plant (30 – 45 cm) and number of pinnae (5 – 7 per frond) were considerably reduced.

Ecology: Terricolous species, observed along the roadside or cut surfaces of hills or rocky surface. In general, the *T. wightii* was found growing in the climatic conditions where the annual rainfall ranges between 500 – 3000 mm, altitude from 100 – 1000 m, temperature 18 – 28°C and atmospheric humidity >50 %. However, the maximum population was observed in the regions where annual rainfall was measured from 1000–2500 mm, altitude between 300–800 m, temperature in between 18–25° C and atmospheric humidity is >60 %. With the increase in the altitude (i.e., above 800 m), temperature (25° C) and decrease in humidity (i.e., <60), it was observed that the population of species was declined considerably.

Conservation status: *T. wightii* was collected from Arunachal Pradesh India. A population of about 100–150 individuals was found at each locality. The area of occupancy (AOO) is 200–300 sp. km. However, it is an endemic species hence, according to IUCN (27) criteria, at present this species is considered as Endangered (EN) species. Globally, this species was not assessed i.e., data deficient (DD) species (IUCN ver. 2017-1).

Taxonomic Note: *T. wightii* is having long creeping rhizome (Figure 1j), scales long acuminate, margin ciliate (Figure 1k), present up to rachis, fronds unipinnate, sparsely hairy, without lower bipartite pinnae (Figure 2g-i), sporangia *sporangia* 1–2 celled stalk, annulus 10–16 celled (Figure 3o), spores monolete, exine thick, dark brown, sinuate, perine smooth (Figure 3p).

Specimens examined: INDIA: Karnataka, Chikkamagaluru Dt., on the way of Shringeri to Hornadu, 08.10.2018, S.M. Patil & K.S. Rajput, 3056 (BARO); Kudremukh National Park, 13.01.2014, S. Morajkar & Team 009 (FSI); Dakshina Kannada Dt., Sitanadi, 19.11.1962, R.K. Arora 2776 (CAL); Uttara Kannada Dt.: Tinighat, 12.1917, without collector's name, Acc. No. 3362 (BLAT); Jog, 10.1919, without collector's name, Acc. No. 7049 (BLAT); Katlekan, 08.05.1969, M.R. Almeida 1216 (BLAT); Hassan Dt., Kempuhole, Shiradi Ghat, 13.04.1971, T.P. Ramamoorthy, 1544 (HFP); Kerala, Waynad, 07.01.2018, S.M. Patil & K.S. Rajput 3057 (BARO); Kasaragod Dt., Mananthavady, Periya, 06.04.1989, P. Madhu. & P.J. Sivichan 297337 (CALI); Palakkad Dt., Silent Valley, Kummattanthode, 850–950 m, 20.03.1981, B.K. Nayar & Party 10020 (CALI).

DISCUSSION

Specimens collected from different location from southern part of India and their comparison with the literature revealed that *T. polymorpha* occurring in this region was misidentified or misreported as *T. wightii* or *vice versa* by earlier researchers (Kumar *et al.* 2016; Deepa *et al.* 2013, 2016). Recently, *T. wightii* (long creeping rhizome, frond unipinnate without bipartite lower pinnae) was wrongly identified as *T. herpetocaulos* (Rajagopala & Bhatt 2018). Their results may be verified by referring their herbarium specimens submitted in JCB Herbaria (http://florakarnataka.ces.iisc.ernet.in.). They also synonymised the species viz. *T. polymorpha* (indusiate sori) and *T. wightii* (exindusiate sori) under *T. herpetocaulos* (indusiate sori). On the basis of results obtained in the present study, authors disagree with merging of these species with *T. herpetocaulos* because the later species is characterised by the presence of thin, long creeping rhizome simple pinnate frond with bipartite lower pinnae and indusiate sori (Xing *et al.* 2013; Fraser-Jenkins *et al.* 2018). As per authors opinion, all these three species are distinct and are in agreement with Fraser Jenkins *et al.* (2018). Further, these species may be very well separated from each other on distinct morphology *e.g. T. polymorpha* (suberect-short creeping rhizome, unipinnate fronds with bipartite lower pinnae) and *T. wightii* are most common in Central and south western Ghats whereas *T. herpetocaulos* is found in Andaman Islands.

Conclusion

On the basis of critical analysis of morphological features indicates that *Tectaria herpetocaulos, T. polymorpha*, *T. pseudosiifolia* and *T. wightii* are distinct from each other. Authors disagree with the merging of *T. polymorpha* and *T. wightii* with *T. herpetocaulos* by earlier reports and all three species should be treated as distinct species

Acknowledgments

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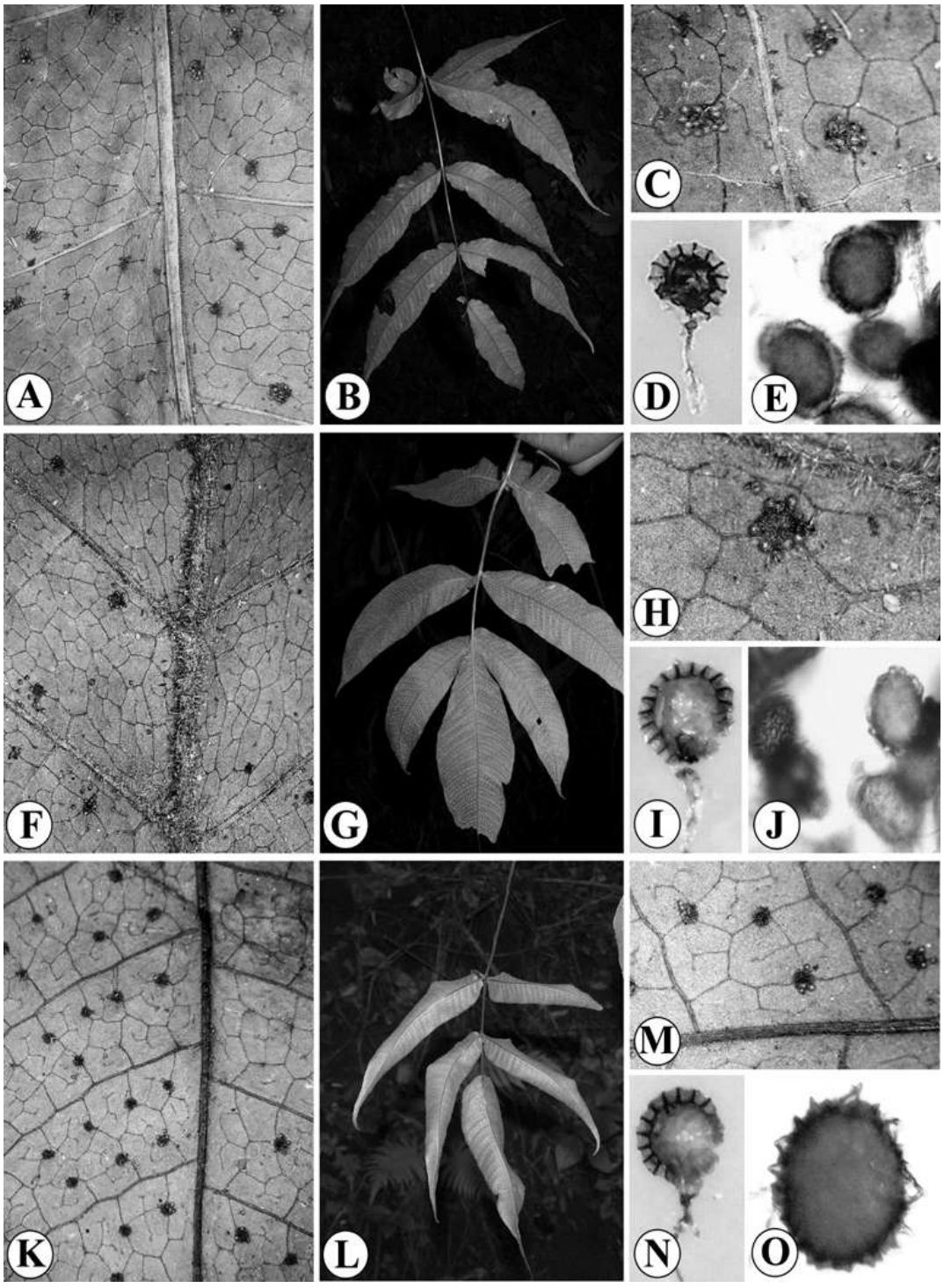

Figure 3: A - E. *Tectaria polymorpha*: **A**. venation; **B**. fertile frond; **C**. sori arrangement, **D**. sporangia; **E**. spore; **F - J**. *Tectaria pseudosiifolia*: **F**. venation; **G**. fertile frond; **H**. sori arrangement; **I**. sporangia; **J**. spore; **K - O**. *Tectaria wightii*: **K.** venation; **L**. fertile frond; **M**. sori arrangement; **N**. sporangia; **O**. spore.

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Rediscovery, resurrection and lectotypification of endemic Isoetes sampathkumarnii L. N. Rao from India

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RESEARCH COMMUNICATION

Rediscovery, resurrection and lectotypification of endemic *Isoetes sampathkumarnii* L. N. Rao from India

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ABSTRACT
An interesting species of *Isoetes* was collected from Jambughoda, Wildlife Sanctuary, Gujarat. After a review of literature and comparison of the morphological characters with type specimens, it was identified as *I. sampathkumarnii* L. N. Rao. It is endemic species of south India and rediscovered after a lapse of 63 years. The species shows several features that make it unique in the genus. Earlier, *I. sampathkumarnii* was also treated as synonym of *I. coromandelina* L.f. and *I. sahyadrii* Mahabala. However, it has an idiosyncratic velum character and spore ornamentation that makes it different from other species. Hence, the authors resurrected it as a distinct species. The original material is ambiguous hence, a lectotype of *I. sampathkumarnii* has been designated here.

Introduction

Isoetes is an interesting and unique pteridophyte, popularly known as 'quillworts' or 'Merlin's grass'. Available literature indicates that nearly 300–350 species are distributed worldwide, of which 19 species, one subspecies and four varieties have been documented from India (1). Among these, only four species have been recognized *viz*., *I. coromandelina* L.f., *I. dixitii* Shende, *I. sahyadrii* Mahabale and *I. udupiensis* (2). All the Indian taxa are described based on velum characters, megaspore ornamentation and chromosome counts. However, several species are published by earlier researchers that are yet to be recognized (1, 2) and accepted as distinct species, of which the *I. sampathkumarnii* is one of them. The status of *I. sampathkumarnii* is changing from time to time because after its description as a new species, no reports were found in other parts of the state or country. Initially, it was (3, 4) merged under *I. coromandelina*. Later, lectotypification and epitypification of *I. sahyadriensis* Mahabale (=*I. sahayadrii*) was proposed and merged all the species having reticulate spores including *I. sampathkumarnii* (5). Further, it was mentioned that, uncertainty about the status of *I. sampathkumarnii*, which is characterized by the presence of disconnected ridges on the megaspores. The lectotypification and epitypification proposed (5) were later rejected (6). The authors of the present study agree with this (6) and concluded that *I. sampathkumarnii* stands as a distinct species in the reticulate complex of *Isoetes* (Fig. 1).

During the survey of pteridophytes from Gujarat, an interesting specimen of *Isoetes* was collected in September and October 2017 for the first time and subsequently observed regularly till date. After comparing the characters, type specimens and spore characteristics, it was identified as *I. sampathkumarnii* L. N. Rao. Therefore, in the present communication, the authors report it as a rediscovery of *I. sampathkumarnii* and also proposed the resurrection of the species. It was found that typification of *I. sampathkumarnii* was not designated earlier. Hence, a lectotype has been designated here.

Materials and Methods

Collection of plant materials

Isoetes sampathkumarnii was collected from Jambughoda Wildlife Sanctuary during 2017–20. For comparative study, *I. panchganiensis* was collected from Panhala Fort, Kolhapur during 2018–19.

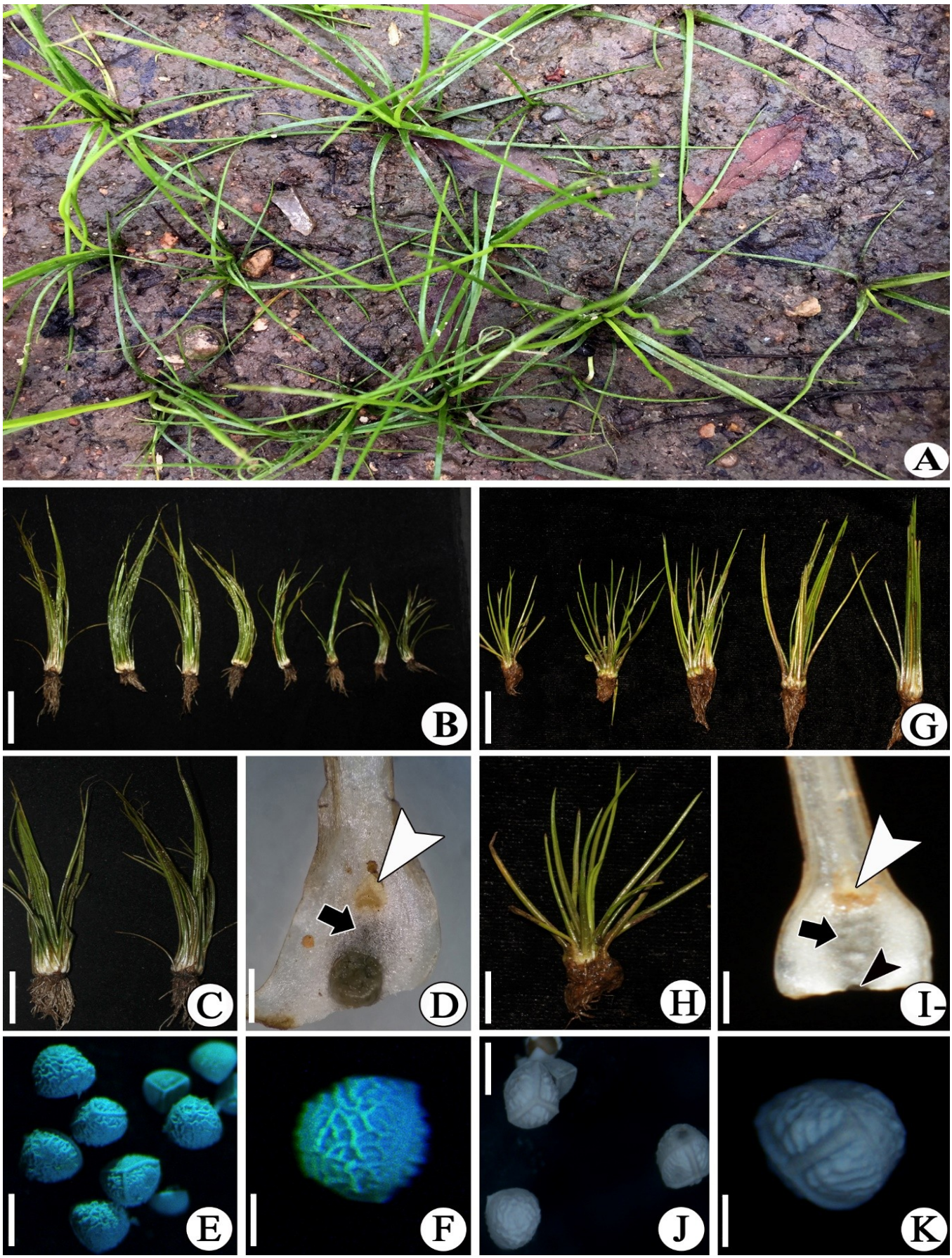

Fig. 1A. Habit of *Isoetes sampathkumarnii*, **B.** Range of variations, **C.** Enlarged view, **D.** Sporophyll showing ligule (arrowhead) and velum covering half of the sporangium (arrow), **E.** Spores, **F**. Enlarged single spore, **G.** Range of variations in *Isoetes panchganiensis*, **H.** Enlarge view, **I.** Sporophyll showing ligule (arrowhead) and velum covering the entire sporangium (black arrow). Note the slit-like opening (small, black arrowhead) at the base of the sporangium, **J.** Spores, **K**. Enlarged single spore. **Scale bar**: **B & G**= 3 cm, **C & H** = 5 cm, **D & I** = 5 mm, **E & J** = 250 µm, **F & K** = 200 µm

Identification and Voucher specimens

The collected specimens were identified with the help of available literature (1, 7-11). Voucher specimens are deposited at BARO, the herbarium of the Department of Botany, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat. The authors also had a personal consultation with Prof. S. P. Khullar for the confirmation of the identity of the species.

Conservation status

It was analysed by using the criteria given by IUCN (12) Red list criteria (Version 2020-2).

Results and Discussion

Taxonomic account

Isoetes sampathkumarnii L.N. Rao, Curr. Sci. 13(11): 286. 1944.

Lectotype (designated here), India, Karnataka, Bangalore, Govt. Bot. Gard., 06 August 1944, L.N.Rao, s.n. K000518076, image!; *isolectotype*, Bangalore, South India, Govt. Bot. Gard., L.N.Rao s.n. CAL0000063267!

Plant submerged, erect; corm two-three lobed, subterranean, covered by traces; sporophylls 5–16 per plant, 4–16 cm, dark green, base white, spirally arranged, linear, tapering towards apex, broader at the base, margin membranaceus; ligule 1.3–2.5 mm wide, and 0.8–2 mm present, thin, membranaceus, apex acute, margin ciliate, base cordate, triangular, yellow-brown; peripheral strands absents; air chambers presents, 4; velum present, 1/2 to 3/4 covering the sporangia; sporangium 5–7 × 1– 3 mm, longer than broad, dimorphic, ovate, white and at maturity it turns reddish-brown, covered with traces; megaspores 270–350 µm in diameter, trilete, reticulate, grey-black when wet, turns white after drying; microsporangia and microspores are not found.

Distribution: India

India: Karnataka and Gujarat (Jambughoda Wildlife Sanctuary).

Ecology: The species is growing along the periphery of shallow water streams and reservoirs. Specimens are collected in the late monsoon when the water level was low.

Specimens examined: Lalbagh Bot. Gard., 17/10/1958, Subramanyam 7078 (CAL); Gujarat, Shivrajpura, Jambughoda Wildlife Sanctuary, 19/09/2019, SMP & KSR 1050 (BARO).

Conservation Status: *Isoetes sampathkumarnii* is rediscovered from Jambughoda Wildlife Sanctuary. This species is luxuriously growing along the periphery of wetlands, on flat surfaces along the seasonal streams. A population of about 1000 individuals were found and the Area of Occupancy (AOO) is 50 km^2. However, other forest areas of the state are yet to be explored completely. Additionally, we assume that the species might be distributed in similar ecological conditions. Therefore, more floristic explorations are needed to determine and document the full range of distribution. Hence, according to IUCN (12) criteria, at present, *I. sampathkumarnii* is considered data deficient (DD) species.

Habitat dominance in late monsoon season

Like all other quillwort species, *I. sampathkumarnii* is a submerged hydrophytic herb inhabiting the periphery of the wetlands or seasonal streams. In the early or mid-monsoon, this species could not be noticed during several excursions from 2014–2017. During our visit in 2017 at the late monsoon to Jambughoda Wildlife Sanctuary, authors recorded more than 1000 small-sized *Isoetes* individuals growing along the periphery of reservoirs and seasonal streams. After a detailed study and consultation at CAL, this population was identified as *I. sampathkumarnii*. In the early monsoon season, the periphery region of the lake, slow running small streams and open adjacent land flourish with *I. coromandelina* (having tuberculate spores), whereas in the late monsoon season a population of small individuals flourishing with *I. sampathkumarnii* (reticulate spores).

The general structure of the velum and spore morphology

Velum is a thin, membranaceus outer covering of sporangium present in some *Isoetes* species. It is either rudimentary (*I. dixitii* Shende), half to 1/3 of sporangium (*I. sampathkumarnii*) or fully cover the sporangium with a slit opening at the base (*I. panchganiensis*). It is a constant character that is used to segregate the species from each other. The species in which velum is absent, such species are having tuberculate spores (except *I. dixitii* and *I. sahyadrii* Mahabale) whereas the species in which velum is present such species having reticulate spores (except *I. rajasthanensis*). Therefore, the majority of Indian species are identified based on the presence or absence of velum, tuberculate or reticulate spores and chromosome numbers (1). The sporangium of *I. sampathkumarnii* is covered by half to 1/3 velum and encloses reticulate spores (Fig. 1).

Rediscovery and resurrection of Isoetes sampathkumarnii

When working on *Ophioglossum* from Jambughoda Wildlife Sanctuary, the authors came across small-sized *Isoetes* in the late monsoon. During the early monsoon, authors collected *I. coromandelina*, from the same location which was 35–50 cm in height, without velum covering on the sporangium and having tuberculate spores. However, the species which was collected in the late monsoon was less than 15 cm, and sporangium was covered with half to 1/3 velum. Further, by comparing other morphological characters with the type description and spore, it was identified as endemic species *I. sampathkumarnii* that was described by Rao (4) from Lalbagh Garden, Bangalore. After the discovery, a single collection was made by Subramanyam in 1958. Since then, it was not collected from the type locality and any other places from India. Thus, after the lapse of 63 years, the species was rediscovered. This species was merged under *Isoetes coromandelina* and *I. sahyadrii*, however, both the species are having tuberculate spore ornamentation whereas *I. sampathkumarnii* is having reticulate

spores. Therefore, it stands as distinct species in the reticulate complex and thus, at present authors resurrected the species.

Lectotyfication

Rao (8) mentioned that the type specimens deposited at K, CAL and in the Central College, University of Mysore, Bangalore. It seems to be of the same gathering deposited in three different herbaria. So, they are to be treated as syntypes (13). The citation of a type before 1990 cannot be considered that of a holotype unless one particular herbarium was indicated in the protologue (and only one specimen of the gathering was deposited there) or if it were made clear that only a single specimen of the gathering existed, or if there is evidence that only one particular specimen was used. More commonly there will be duplicates, often housed in more than one institution, and these must all be treated as syntypes (Art. 40 Note 1). Marsden, C.R. on 20/04/1977 annotated the Kew specimen as the lectotype but it was not formally published. Hence, the Kew specimen (K000518076) has been designated here as the lectotype.

Comparative study

The comparative account of *Isoetes coromandelina, I. panchganiensis, I. sahyadrii* and *I. sampathkumarnii* is provided herewith in Table 1. In the available recent literature, *I. sampathkumarnii* was misidentified and merged under the species *I. coromandelina* (6). Later, it was merged under the species *I. sahyadrii* (2, 8). However, both the species, *Isoetes coromandelina* and *I. sahyadrii* are having tuberculate spore ornamentation whereas reticulate spore ornamentation was observed in *I. sampathkumarnii*. Therefore, *I. sampathkumarnii* stood as distinct species and was resurrected here. Due to the presence of velum and reticulate spores, the present specimen was also compared with *I. panchganiensis*. This comparison showed that in *I. panchganiensis* the velum completely covered the sporangia with slit at the base and possess reticulate spores whereas in *I. sampathkumarnii* the velum covers half to one-third of sporangia that enclose reticulate spores (Table 1). Therefore, both species showed morphological differences and stand as distinct species.

Conclusion

The present study concludes the rediscovery of I. *sampathkumarnii* from a new locality and has resurrected the species. A lectotype has been designated here. This study also suggests that at present there are two confirmed species of *Isoetes* in the reticulate complex *i.e., I. sampatkumarnii* and *I. panchganiensis*. Further, studies are warranted to fully understand the reticulate complex by re-examining the morphological characters, their growing season, elevation, cytological and molecular studies.

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Authors' contributions

SMP collected the plant material, identification, photography of *Isoetes sampathkumarnii* and a preliminary draft of the manuscript was written;

SKP helped during the collection plant material, processing of the plant material and preliminary draft writing; **SSP** collected *Isoetes panchganiensis* and its microphotography;

KSR provided administrative support, laboratory facilities, arrange field visit, preparation of figures and overall compilation of the manuscript.

Table 1. Comparative account of some *Isoetes* species

Attributes/ Name of Species	***I. coromandelina***	***I. panchganiensis***	***I. sahyadrii* (*I. sahyadriensis*)**	***I. sampathkumarnii***
Type Locality	Coromandel coast, Tamil Nadu	Panchgani, Maharashtra	Panchgani Maharashtra	Bangalore, Karnataka
Plant size	More than 40 cm	9–15 cm	Up to 20 cm	Up to 11 cm
Rhizomorph	Tri-lobed (rarely tetra or penta lobed)	Tri-lobed	Tri-lobed	Bi-lobed
Sporophylls per plant	17–9 (in triploid) 8–23 (in tetraploid)	9–20	4–32	3–16
Peripheral strands	4-main, several subsidiary strands	Absent	Absent	Absent
Velum	Absent	Completely covered for the sporangia except for a base arched slit	Completely covered the sporangia except for a base arched slit	½ to ¾ covered the sporangia
Ligule	Triangular-cordate	Triangular	Triangular with armed	Triangular
Megaspores	Tuberculate, tubercles even	Reticulate	Tuberculate	Reticulate
Distribution	Throughout India	Maharashtra	Maharashtra	Karnataka, Gujarat

Compliance with ethical standards

Conflict of interest: No research conflicts.

Ethical issues: None.

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Taxonomic notes on *Astragalus vogelii* subsp. *fatimensis* (Galegeae, Fabaceae)

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Abstract

The correct authority of *Astragalus vogelii* subsp. *fatimensis* and its distribution range in India are discussed. The taxon, which is considered rare in India, has been recollected from Gujarat State from new localities in the Banaskantha District. The description of the taxon along with photographs and other taxonomic details are provided here for easy identification.

Keywords: *Astragalus vogelii* subsp. *fatimensis*, nomenclature, authority, recollection, India.

Introduction

The genus *Astragalus* L. (1753: 755), distributed throughout the world chiefly in cold to warm arid and semiarid mountainous regions, comprises about 3156 species in 255 sections (Podlech & Zarre 2013, Zarre & Azani 2013, Maassoumi 2020). *Astragalus vogelii* (Webb) Bornm. (1915: 233) has been accepted in the section *Astragalus* sect. *Herpocaulos* Bunge (1868: 9) with two subspecies, *Astragalus vogelii* subsp. *vogelii* and *Astragalus vogelii* subsp. *fatimensis* Maire (1933: 126) (Podlech 1984). Based on molecular analyses (Kazempour Osaloo *et al*. 2003), *Astragalus vogelii* was transferred from *Astragalus* to a new genus *Podlechiella* Maassoumi & Kazempour Osaloo (2003: 22). However, in gross morphology it resembles *Astragalu*s and, therefore, again it was restored in *Astragalus* by Chaudhary (2018). Although Podlech & Zarre (2013) treated the taxon in *Podlechiella*, they also clearly mentioned that it is closely similar to *Astragalus. Astragalus vogelii* subsp. *fatimensis* has been treated in Podlech (1984), Podlech & Zarre (2013) and Chaudhary (2018), but problems related to authorship and orthography of the taxon continue to exist. In addition, some new observations on the distribution of the taxon in India have also been noticed.

Distribution of *Astragalus vogelii* subsp. *fatimensis* in India

Astragalus is chiefly a Himalayan genus in India with 79 species (Chaudhary 2018). However, a few annual species have also been reported from the plains of some States, such as Punjab [*A. ophiocarpus* Bunge (1868: 10), *A. scorpiurus* Bunge (1847: 249), *A. tribuloides* Delile (1813: 70), *A. vogelii* subsp. *fatimensis*], Haryana (*A. tribuloides*), Rajasthan (*A. tribuloides*) and Gujarat (*A. vogelii* subsp. *fatimensis*). *Astragalus vogelii* subsp. *fatimensis*, first reported from Punjab Province based on Edgeworth's collections made during 1836 (Bunge1868, Baker 1876, Ali 1961), is considered a rare taxon in India (Cooke 1902). Subsequently, some collections were also made during 1883/85 by Drummond from Punjab (Ali 1961). After that, it has not been recollected from Punjab in recent years, as is evident from the ongoing work on the genus in India (Chaudhary & Srivastava 2007, Chaudhary 2018). A second collection

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of this taxon was reported from Gujarat as *A. prolixus* Sieb. ex Bunge (1968: 9) from two districts (Kutch-Mundra and Broach-Bhadbhut) (Jain & Kanodia 1960, Shah 1978, Raghavan *et al.* 1981). However, Chaudhary (2018) was not able to record it from Gujarat as he could not locate or examine any of specimens mentioned by Shah (1978). Podlech & Zarre (2013) have also recorded it only from Punjab in India.

Recently, during botanical exploration in North Gujarat region, the authors (SP & PD) collected an interesting species of *Astragalus* from a new locality near Jaloya Village in Suigam Taluka of Banaskantha District in Gujarat State of India in February 2020. After a critical study of collected specimens and relevant literature (Baker 1876, Cook 1902, Ali 1961&1977, Shah 1978, Podlech 1984, Podlech & Zarre 2013, Chaudhary 2018), these were identified as *A. vogelii* subsp. *fatimensis*. The present collections represent an extended distribution of the taxon in Gujarat in India. A detailed description along with taxonomic information, field photographs and line drawings are provided for easy identification of this taxon.

Astragalus vogelii subsp. ***fatimensis*** Maire, Mém. Soc. Hist. Nat. Afr. Nord 3: 126. 1933. (Figs. 1 & 2)

Annual herbs, prostrate, caespitose, stems slender, up to 12 cm long, pilose with appressed, medifixed, white hairs. *Stipules* 1.5–2.0 × ca. 1 mm, free from petiole, erect or half spreading, triangular-lanceolate, pilose with white medifixed hairs on both surfaces, ciliate along margins. *Leaves* 4–5 cm long, imparipinnately compound; petiole ca. 6 mm long; hairy as on stem; rachis 9–12 mm long; leaflets 3–7 pairs, opposite to subopposite, subsessile, ovate or oblong, 3–5 × 1–2 mm, cuneate at base, obtuse or minutely retuse at apex, entire along margins, densely pilose on both surfaces with appressed, medifixed, white hairs. *Inflorescence* axillary, peduncled umbel or capitate raceme, 2–13-flowered; peduncle 2–5 (10–40.5) mm long, generally distinctly shorter than subtending leaf or sometimes equal to subtending leaf, hairy as on stem. *Bracts* ca. 1 mm long, longer than pedicel, linear, pilose with white hairs. *Flowers* 3–5 mm long, pinkish to violet, subsessile, erect; pedicel less than 1 mm long, pubescent. *Calyx* ca. 3 mm long, campanulate, pilose generally with white hairs outside, sometimes with black hairs especially on teeth, glabrous inside, tube ca. 1.5 mm long, teeth linear, equal to tube. *Petals* slightly longer than calyx; standard ca. 3 mm long, oblong-elliptic with emarginate tip; wing petals ca. 2.5 mm long, shorter than standard and keel petals, lamina ca. 1.5 mm long, narrowly ovate with subobtuse apex, claw ca. 1 mm long; keel petals ca. 3 mm long, equal to standard, lamina ca. 1 mm long, oblong with subobtuse apex, almost straight, claw ca. 2 mm long. *Staminal sheath* ca. 2 mm long, obliquely cut at mouth (obtuse at mouth in open condition), free filaments minute; vexillary filament ca. 2 mm long, free from staminal sheath. *Ovary* ca. 2 mm long, sessile, densely pilose with white hairs; style minute, incurved; stigma capitate, glabrous. *Pods* 5–10 × 2.5–3.0 mm, sessile, straight, turgid, spreading in all directions, ovoid-oblong, deeply grooved dorsally, shortly acuminate, covered with appressed or spreading, white, subbasifixed hairs, unilocular, 4–6-seeded. *Seeds* 1.5–2.0 × ca. 1 mm, oblong-reniform, yellowish-brown, turgid, irregularly depressed, smooth, glabrous.

Flowering & Fruiting:—January to March.

Habitat:—In India, the taxon is restricted to saline habitats only along road margins or sides in very thin and fragmented populations. Only about 26 individuals were noticed in a 2–3 km long stretch. The Banaskantha District borders with the Sabarkantha District in the east, Kachchh District in the west, Patan and Mehsana Districts in the south and Rajasthan State in the north, hence there is a probability that this taxon occurs in adjacent areas and attempts will be made to collect more specimens to assess its conservation status in India.

Distribution in India:—Gujarat, Punjab. No specimens have been observed from Rajasthan as reported in Sanjappa (1992). Kumar and Sane (2003) have wrongly reported it from Maharashtra.

Distribution in world:—Algeria, Egypt, Eritrea, Iran, Iraq, Oman, Pakistan, Saudi Arabia, Sudan, Yemen (Podlech & Zarre 2013).

Notes:—*Astragalus vogelii* subsp. *fatimensis* differs from subsp. *vogelii* by its dense inflorescence head (vs lax inflorescence) and spreading pods (vs erect pods), but sometimes in the specimen 'Fatma valley, *W. Schimper* 843 (P photo !)' the inflorescence is more elongated with lax flowers. Generally the peduncles are distinctly shorter than the leaves, however, in our specimens they are shorter to almost equal to the leaves. The hairs on the calyx are generally white or sometimes intermixed with black hairs. Podlech & Zarre (2013) and Maire (1933) also observed this feature of the calyx. The pods are unilocular in this taxon, however, Baker (1876) has mistakenly mentioned bilocular pods.

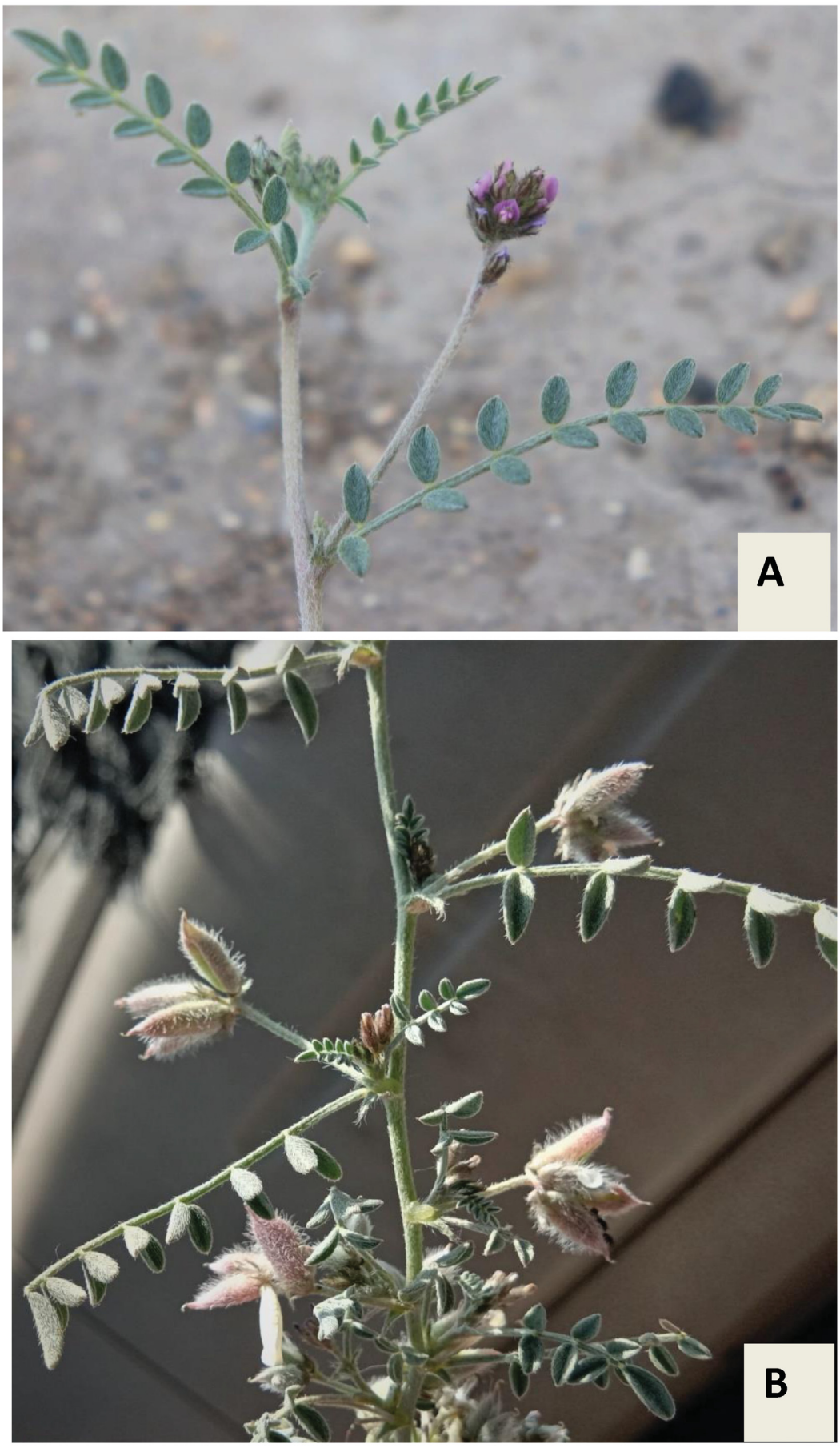

FIGURE 1. ***Astragalus vogelii*** subsp. ***fatimensis*** Maire: A, A twig with flowers; B, A. twig with pods.

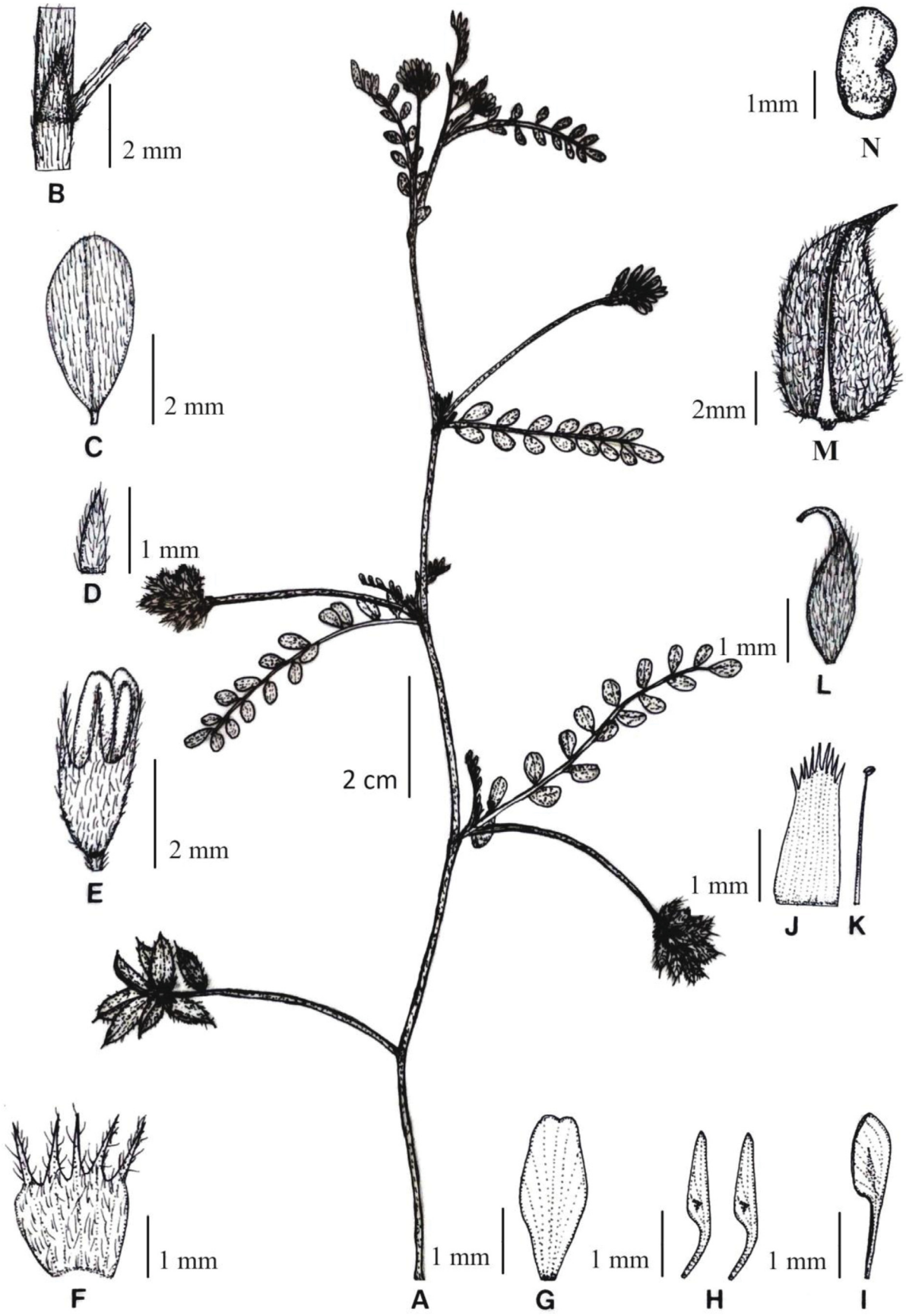

FIGURE 2. ***Astragalus vogelii*** subsp. ***fatimensis*** Maire: A, Habit; B, Stipules with a portion of stem and petiole; C, Leaflet; D, Bract; E, Flower; F, Calyx (opened, outer surface); G, Standard; H, Keel petals; I, Wing petals; J, Staminal sheath (splitted); K, Vexillary filament; L, Carpel; M, Pod; N, Seed (Drawn from *Patel & Desai* SKP-85 by L. B. Chaudhary).

Specimens examined

INDIA. Gujarat State: Banaskantha District, Suigam Taluka, Jaloya Village, 24⁰11'8530" N & 71⁰14' 6920" E, 14 m, 16. 2. 2020, *S.K. Patel & P.R. Desai* SKP-85 (BSI, LWG, Gujarat Arts and Science College, Ahmedabad, Gujarat); Kachchh District, Mandvi Taluka, Bhuj, Gujarat Institute of Desert Ecology, 20. 1. 2016, *R. Patel* 1276 (GUIDE Kachch).

Nomenclature of the taxon

Astragalus vogelii subsp. ***fatimensis*** Maire, Mém. Soc. Hist. Nat. Afr. Nord 3: 126. 1933; Kumar & Sane, Legum. South Asia: Checkl. 244. 2003; L. B. Chaudhary, Rev. gen. *Astragalus* L. (Legum.-Papilio.) India 75. 2018. Type: (Saudi Arabia), Prope Pag. Uasert in Valle Wadi Fatme Pr. Mecca, 3 Feb. 1836, *W. Schimper* 843 (P!; K-000895560!, 000895564! in Herb. J.Ball).

= *A. arabicus* Ehrenb. ex Bunge, Mém. Acad. Imp. Sci. Saint Pétersbourg 11 (16): 9. 1868, in clave & 15 (1): 6. 1869, descriptio, nom. illegit., non Kotschy, 1866. Type: Arabia, El Gidon, Jan. 1825, *Ehrenherg s. n.* (lecto: P!; iso: K!).

= *A. prolixus auct. non* Sieb. ex Bunge, 1868; Baker in Hook. f., Fl. Brit. India 2: 121. 1876; Cooke, Fl. Bombay 1: 329. 1902; Shah, Fl. Gujarat State 1: 183. 1978.

= *A. fatmensis* Chiov., Ann. Ist. Bot. Roma 8 (Pirotta, Fl. Eritrea, i. Fasc. 1): 95. 1903. Type: based on type of *A. arabicus* Ehrenb. ex Bunge.

= *A. fatimensis* Hochst. ex Blatt., Rec. Bot. Surv. India 8 (1): 156. 1921; Ali, Biologia 7: 79. 1961 & in Nasir & Ali, Fl. W. Pakistan 100: 209. 1977 (as *A. fatmensis*); Sanjappa, Legum. India 87. 1992. Type: India, Punjab, Ludhiana ('Lodiana'), *Edgeworth s.n*. (lecto: K-001090897!), lectotype designated by Ali (1961).

= *Podlechiella vogelii* subsp. *fatimensis* (Chiov.) Maassoumi & Kazempour Osaloo, Plant Syst. Evol. 242: 22. 2003; L.B.Chaudhary & Srivastava, Taiwania 52 (1): 32. 2007; Podlech & Zarre, Tax. Rev. gen. *Astragalus* L. (Legum.) Old World 1: 169. 2013 (as *Podlechiella vogelii* subsp. *fatimensis* (Maire) Maassoumi & Kazempour Osaloo).

This taxon (*A. vogelii* subsp. *fatimensis*) was first described as *A. arabicus* Ehrenb. ex Bunge (1868: 9), which is a later homonym of *A. arabicus* Kotschy (1866: 264). Ali (1961) has discussed this issue in his work and also mentioned that *A. prolixus* Sieb. ex Bunge is different from *A. arabicus* Ehrenb. ex Bunge. It has been observed that there are two epithets '*fatimensis'* and '*fatmensis'* that available for *A. arabicus* Ehrenb. ex Bunge. These epithets have been variously and inconsistently used in different publications and online databases (Table 1) for the same taxon based on the same type. Chiovenda (1903) was the first to provide a new name *A. fatmensis* Chiov. (1903: 95), while Blatter (1921) published *A. fatimensis* Hochst. ex Blatt. (1921: 156) for *Astragalus arabicus* Ehrenb. ex Bunge at species rank. However, Maire in 1933 treated this taxon as a subspecies of *A. vogelii* based on a specimen 'Fatma valley, *W. Schimper* 843' (that was named as *A. fatmensis* by Hochst.) but used the epithet '*fatimensis'* without citing Chiovenda (1903) and Blatter (1921). Probably, the epithet *fatimensis* was adopted from the geographical area Wadi Fatima (on Type specimen it is 'Fatme'), Province Macca of Saudi Arabia. Subsequently, this treatment of Maire (1933) was accepted by Podlech (1984), Podlech & Zarre (2013) and Chaudhary (2018) and *A. fatmensis* of Chiovenda (1903) and *A. fatimensis* of Blatter (1921) were treated as synonyms. On the other hand, Kazempor Osaloo *et al*. (2003) and Chaudhary & Srivastava (2007) used *A. fatmensis* Chiov. as basionym while transferring *A. vogelii* to a new genus *Podlechiella* [*P. vogelii* (Webb) Maassoumi & Kazempour Osaloo subsp. *vogelii* (2003: 22) and *P. vogelii* subsp. *fatimensis* (Chiov.) Maassoumi & Kazempour Osaloo (2003: 22)], however, they applied *fatimensis* epithet in place of *fatmensis* as used by Chiovenda (1903).

Maire (1933) made his new combination (*Astragalus vogelii* subsp. *fatimensis* Maire) based on an unpublished name on Schimper's specimen No 843. In our opinion Maire's *fatimensis* should be considered as new subspecies despite the fact that he refers to Hochst.'s manuscript/unpublished name and treats it as a new combination. This name is separate from *A. fatmensis* Chiov. (1903) and *A. fatimensis* Hochst. ex Blatt. (1921) that are all based on the same type (Arabia, El Gidon, Jan. 1825, *Ehrenberg s.n.* (P; iso: K), because as per ICN Article 11.2 (Turland *et al.*, 2018), a name has no priority outside the rank at which it is published. Therefore, the correct name should be ***Astragalus vogelii*** subsp. ***fatimensis*** Maire, Mém. Soc. Hist. Nat. Afr. Nord 3: 126. 1933.

TABLE 1. Epithet variants of *fatimensis* vs *fatmensis* used in different publications for the same taxon.

A. fatimensis	*A. fatmensis*
Blatter (1921)	Chiovenda (1903)
Maire (1933)	Pirotta (1903–1908)
Podlech (1984)	Ali (1961, 1977)
Sanjappa (1992)	Monod (1977)
Kumar & Sane (2003)	IPINI
Kazempor Osaloo *et al*. (2003)	Tropicos
Chaudhary & Srivastava (2007)	POWO
Podlech & Zarre (2013)	
Chaudhary (2018)	
The Plant List	

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Ephedra karumanchiana (Ephedraceae) *sp. nov.* from Gujarat State, India

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A new species, *Ephedra karumanchiana* is described from the scrubland area (arid zone) of North Gujarat, India. Identity of *E. karumanchiana* was further confirmed by sequences from the nuclear ribosomal internal transcribed spacer region (ITS2-S2F-ITS4) and chloroplast DNA regions employing three different primers, *viz. rbcL, psbA-trnH* and *trnL-trnF* intergenic spacer regions. A detailed description, phenology, and conservation status are given.

Key Words: Bracts; synangia; DNA sequencing; conservation status; gymnosperm; Kachchh.

Introduction

Ephedraceae Dumort. is a monotypic family containing the genus *Ephedra* Linnaeus (1753). Members of this genus are commonly known as joint firs, which are adapted to varied to extreme conditions and having highly diversified distinct morphology from rest of other gymnosperms. It is reported and noticed abundantly in dry and open habitats, such as deserts, rocky slopes, grasslands and maritime areas (Stapf 1889, Price 1996; Ickert-Bond and Renner, 2016). Approximately, 55-65 species have been reported worldwide, of which 13 species and a variety are found to be distributed and noticed in different biogeographical zones of India (Sharma & Singh 2016). Most of the species occurring in India are reported from the cold deserts while, *E. foliata* Boiss. ex C.A.Mey. (1846) and *E. przewalskii* Stapf. (1889) are reported from hot deserts of Rajasthan as well as semiarid regions of Gujarat State respectively (Sharma *et al.* 2013). Formerly, *E. foliata* Boiss. ex C.A.Mey. (1846) was the only species reported wildly from Gujarat state (Sharma *et al.* 2013). As a result, earlier researchers always neglected this genus in Gujarat under the notion that an entire population occurring in the state is *E. foliata.*

During our recent field visits, a unique population of *Ephedra* was observed growing naturally at Ramsan (North Gujarat, arid region) in 2014 and in subsequent years it is also noticed and collected from several localities of North Gujarat to Rajasthan. The present collection differs from *E. foliata* reported from Kachchh region of the Gujarat state. After critical study of morphological, vegetative and reproductive structures, it was concluded that the population of Northern Gujarat might be a new variant and an undescribed species. Therefore, the main aim of the present communiqué is to elucidate the variations within *E. foliata.* For further confirmation of the identity of the species, molecular analysis of ITS and chloroplast DNA regions were carried out and a phylogenetic tree was generated with *Ephedra* species *viz E. intermedia* Schrenk & C.A. Mey. (1846), *E. Foeminea Forssk., E. Przewalskii Stapf* and *E. foliata Boiss. ex C.A.Mey.,* that are reported from hot deserts of India and adjacent countries.

Materials and methods

Collection of plant specimens: Extensive field surveys were carried out from June 2012 to January 2020 from different locations of Gujarat (Kachchh–Navinal, Kala Dongar and Dhinodhar hills; Banaskatha - Dhroba, Dhakha, Vasan) and Rajasthan (Jalor -Panseri, Pooran, up to Sunadha Mata temple), India (Map–1). Specimens were collected in sterile polyethylene bags and brought to the laboratory for morphological and molecular studies. Also, some samples were processed for herbarium preparation.

Preparation of voucher specimens: Twigs (5-8 twigs from different individuals per locality) with male and female strobili each were collected from Navinal, Kala Dungar and Dhinodhar hills of Kachchh region and Banaskatha, Dhroba, Dhakha,

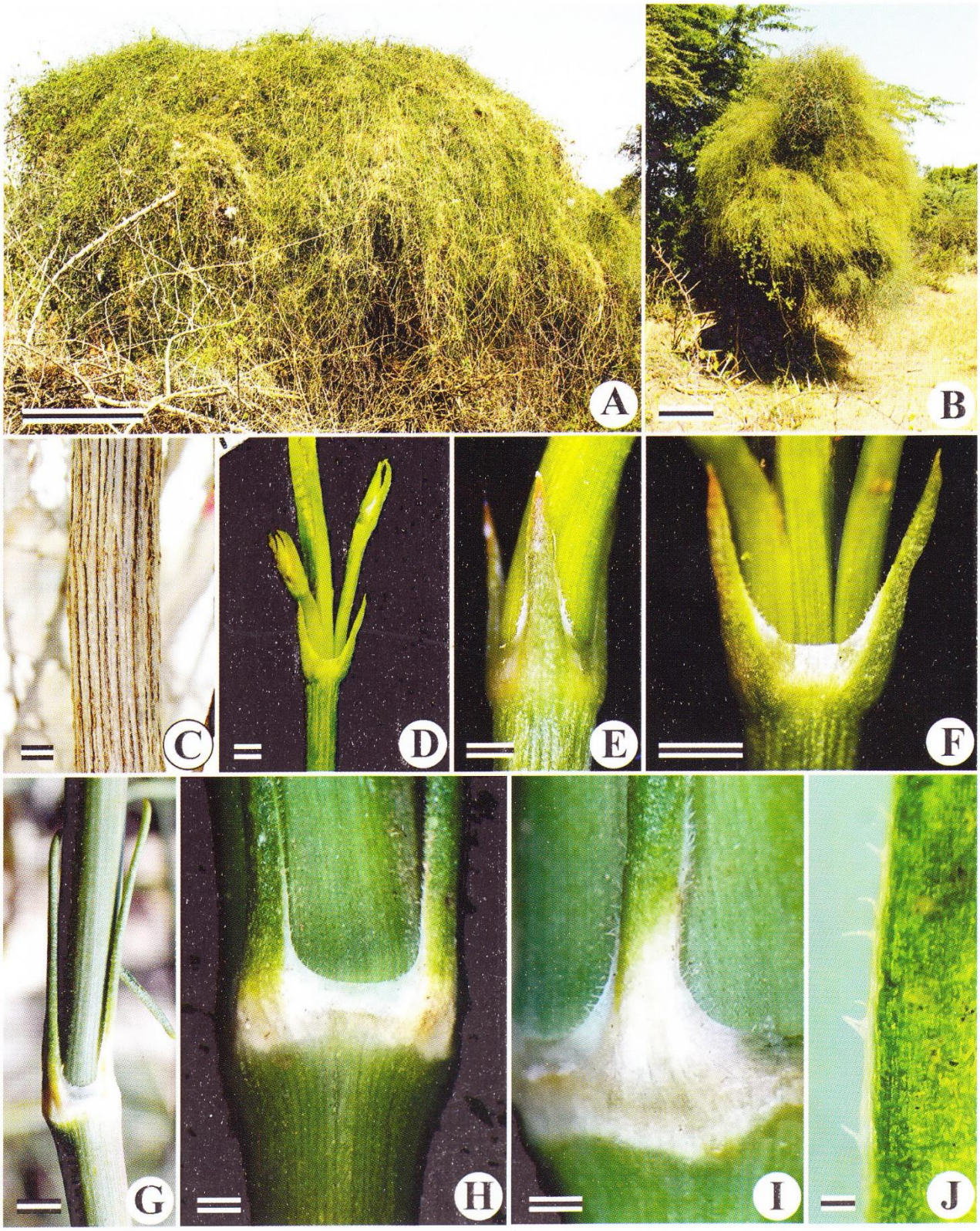

Plate 1. *Ephedra karumanchiana sp.nov.* A-B, Habit, C, Stem. D, Young branches emerging from the nodal portion. E, Young leaf. F, Sheathing of young leaf. G, Mature leaf. H, Enlarged portion of leaf sheath. I, Enlarged portion of leaf base showing hairy in the marginal areas. J, Enlarged portion of hairs on leaf margin. *Scale bars*: A-B, 1 m. C, 1 cm. D-F, H, I, 1 mm. G, 5 mm. J, 200 µm.

Vasan from north Gujarat. Dry specimens were treated with 0.5% mercuric chloride dissolved in ethanol, air dried under fan and deposited in BARO herbarium, Department of Botany, Faculty of Science, the Maharaja Sayajirao University of Baroda, Vadodara, Gujarat (India).

Photography and microscopy: Photographs of naturally growing plants were taken in the field with a digital camera (Cannon SLR 1200D). Micro-morphological characters were studied under binocular stereo-zoom microscope (Leica MSZE6).

Literature survey and identification: The morphological analysis and description are based on observations made in the field and examination of live specimens. Identification of specimens was carried out by referring to relevant literature (Blatter, 1916; Sahni 1990; Sharma and Uniyal 2008; Sharma *et al.* 2010; Sharma and Singh 2015, 2016).

Identity of specimens was ascertained by consulting protologues, type specimens and specimens of related species. Types and voucher specimens in the virtual herbaria (Global Plants on JSTOR and websites of K) were reviewed. Acronyms of herbaria are updated from Index Herbariorum (Thiers, 2016).

Molecular identification and sequencing of DNA: Molecular identification and sequencing of DNA was carried out by using the method given in Doyle & Doyle (1990), White *et al.* (1990), Taberlet *et al.* (1991), Levin et al. (2003), Kress and Erickson (2007) and Chen *et al.* (2010). Details of the methods followed for DNA isolation, its purification, amplification, sequencing and construction of phylogentic tree is described elsewhere (Patil *et al.*, 2018).

Phylogenetic analysis: The incongruence test was done to evaluate the potential incongruence and combine ability of the cpDNA and nr ITS data. For generating the phylogenetic tree, sequences of other morphologically close species of *Ephedra* were obtained from the GenBank (Table 1). The nucleotide sequences were aligned with ClustalW (Thompson et al. 2002) embedded in MEGA X (Kumar *et al.* 2018). Separate and combined molecular phylogenetic analyses were performed using the Maximum likelihood (ML) method. The concatenated dataset was analyzed in Partition Finder (Lanfear *et al.* 2012) to select the best partitioning scheme. The same partition scheme was selected for ML analyses. ML analyses were employed to infer the phylogenetic relationships in RaxML (Silvestro and Michalak 2012). ML analysis inference was drawn using the sequences of *Gnetum gnemon* L. and *Welwitschia mirabilis Hook.f.* as outgroup. ML analysis was run for 1000 bootstrap replicates under the GTR + I model to assess clade support.

Results

Taxonomic Description

Ephedra karumanchiana S.K.Patel, S.M.Patil, Raole & K.S.Rajput *sp. nov.*, (Figs 1, 2).

Type: INDIA. Gujarat, Banaskantha district, Ramsan, 135–160 m, 8 March 2015, *Patel et al.*160 (**Holotype** CAL!, **Isotypes** BARO!, SUK!, K!).

Description: Plant dioecious (strictly), terrestrial, scandent shrub, 4–8 m in height; stems 3–7 m (rarely 6) height, 5–15 cm (rarely up to 22 cm) thick, green–brown when young, dark-brown at maturity, woody, branched, having distinct nodes and internodes; branchlets 1–2 cm thick (rarely 4 cm), 2–5, arranged in whorls, scabrous, green, having ridges and furrows; leaves 2–4 cm long, 2-5 mm broad, 2–4 per node (rarely 5), scaly, hairy, whorled, green–pale green, sheath white–yellow, linear-lanceolate or connate, apex narrow, acute, base broad; male strobili compound, like biparous-multiparous cyme, pedunculate; single male strobilus 0.5–1.5 cm x 2–3 mm, green when young, pink at maturity, strobili 6–12 per node in 2–4 groups, whorled, ovate–lanceolate, bracteate, bract 10–24 per strobilus, binate, bi-colours, centre dark green, margin hyaline, ciliate; male flowers 3–8mm, 6–12 pairs per strobilus (mature); sporangiophores 1–3 mm long, pinkish-brown, with synangia, synangia usually 3–6; pollen grains golden yellow, dimorphic, ellipsoidal or widely ellipsoidal; female strobili 0.5–1.2 x 0.2–0.5 cm, 4–14, stalked, terminal, sometimes on nodal branches, bracteate, bract 3–6 pairs, opposite, pink, non-mucronate, obtuse, median bracts sub-acute, base connate; ovules 2–7 x 2–3 mm, 1–2 (rarely 3), ovate, green-black; seeds 1–2 (rarely 3), covered with fleshy pink colour bract, ovate, black with persistent micropyle.

Phenology: Flowering in January-February;

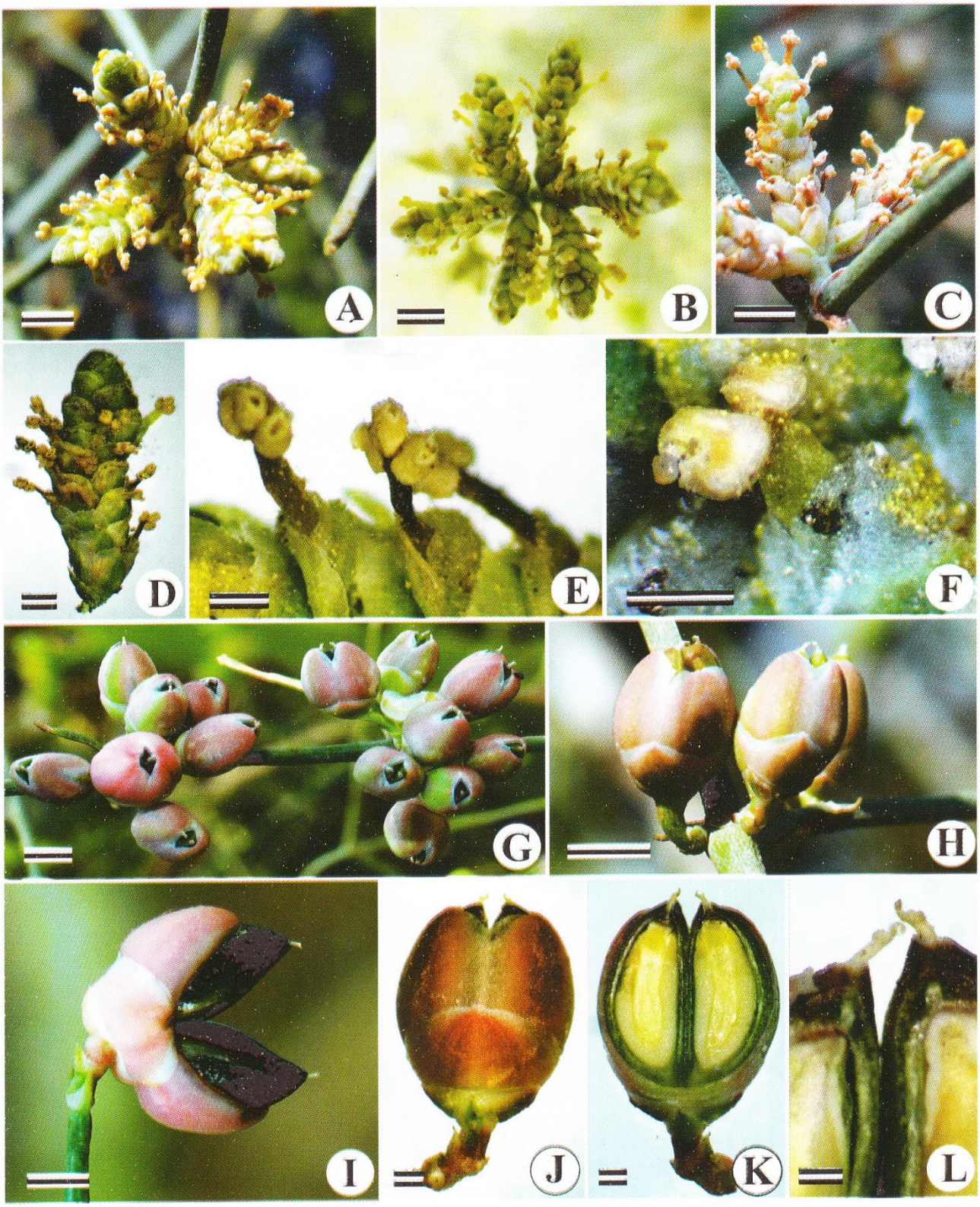

Plate 2. *Ephedra karumanchiana*. A-C, Arrangement of male cones. D, Single male cone showing arrangement of anther. E Anthers emerging from bracts. F, Enlarged portion of anther. G-H, Arrangement of female cones. I, Single female cone. J, A portion of female cone showing the arrangement of bracts. K, Longitudinal section showing female gametophyte. L, Enlarged portion of the stigma. *Scale bars*: A, 2 mm. B, 5 mm. C, G, H, 1 cm. D, 3 mm. E, 1 mm. F, 0.5 mm. I, 5 mm. J, K, 2 mm. L, 1 mm.

fruiting in March-May.

Etymology: The species is named after Prof. (Dr.) Karumanchi Sambhasiva Rao, Former Head, Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar (Gujarat) for his valuable contribution in the field of plant anatomy.

Distribution: INDIA, Gujarat state: Banaskantha Dist., Ramsan, Dhroba, Dhakha, Vasan; Rajasthan state: Jalor Dist., Panseri, Pooran (Plate 3).

Ecology: *E. karumanchiana* grows in semiarid-arid regions of Gujarat and Rajasthan at an altitude of < 300 m asl. Mature individuals of *E. karumanchiana* start producing strobili at the end of February with increasing temperature (beginning of summer), and reaches full bloom in March–April when the average day temperature ranges between 38–42° C. Maturation of cones and seed dispersal takes place in the month of May when the day temperature is the highest for the year (exceeding 45–48°C per day).

IUCN category: DATA DEFICIENT

Conservation status: *E. karumanchiana* is an endemic to western India, constituted by a population of about 100–130 individuals per km^2 on an area of occupancy (AOO) of 50–70 km^2. Therefore, according to IUCN Red List categories and criteria version 3.0 (IUCN 2016), it is assessed as data deficient. Therefore, further survey and exploration are required in adjacent areas of North Gujarat and Southern part of Rajasthan sharing similar geomorphological and climatic conditions.

Specimens Examined: INDIA. Gujarat, Banaskatha Dist., Ramsan, <300 m, 4 August 2014 *K.S. Rajput* (BARO); Ramsan, 13thFebruary, 2016;26 April, 2017 *Patel and Rajput* (BARO); Dhroba, 5 April, 2018, *Patel et al.* (BARO); Dhakha, 20 March, 2019, *Patil, Rajput and Patel* (BARO); Rajasthan, Jalor Dist., Panseri, 18 November, 2018, *Kachhiyapatel and Rajput* (BARO).

Molecular identification: Sequences of *E. karumanchiana* using nuclear ribosomal internal transcribed spacer region (ITS2-S2F-ITS4) and chloroplast DNA primers (*rbcL-F-rbcL-R*, *psbA-trnH* and *trnL-trnF*) were compared with the available molecular data base in the NCBI. Sequences of the *rbcL* marker shows that the new species is closely related to *E. frustillata* Miers (1863) (GQ248600) submitted by CBOL Plant Working Group (2009). Similarly, *trnH-psbA (*MF096972; Liu *et al.* 2017) *and trn*L-*trn*F sequences (AP010819; Wu *et al.* 2009) showed a close relationship between the new species and *E. equisetina* Bunge (1851).

Molecular Phylogeny

Majority of Indian species of Ephedra are found growing in temperate climate except *E. foliata*. Molecular analysis and phylogenetic studies on Indian species of *Ephedra* are rarely reported. No molecular sequences of Indian species are available in NCBI database except *E. foliata*. Therefore, to carryout phylogenetic analysis, sequences available in the NCBI database from the world were used (Figure 4, Appendix I). Ickert-Bond & Wojciechowski (2004) studied phylogenetic analysis of *Ephedra* and suggested that use of molecular sequence data would play an important role and can serve as an essential tool to resolve relationship problem for taxonomic studies. In the present study, phylogenetic tree was generated using *rbc*L, ITS and chloroplast DNA regions employing three different primers, *viz. rbcL*, *psbA-trnH* and *trnL-trnF* intergenic spacer regions.

Discussion

Taxonomy

The present population of *Ephedra* species is under observation in the field since 2014. Meanwhile, similar population is also reported from several other locations (i.e., Dhroba, Dhakha, Vasan; Rajasthan state: Jalor Dist., Panseri, Pooran, up to Sunadha Mata temple). Authors are regularly visiting these cites since 2014 but no such unknown parasites were observed in these areas. Recently, Meena *et al.* (2019) analysed genetic diversity of *Ephedra foliata* occurring in Gujarat and Rajasthan and concluded that the population of *Ephedra* from Gujarat is genetically distinct than Rajasthan. Dendrogram provided by Meena *et al.* (2019) also depicting that samples from Gujarat formed separate clade which is an indirect evidence that samples collected from northern part of Gujarat state are supporting to raise status as a new species.

The newly reported population of *Ephedra* is reaching height up to 5m (rarely 6m) and 2-4 cm

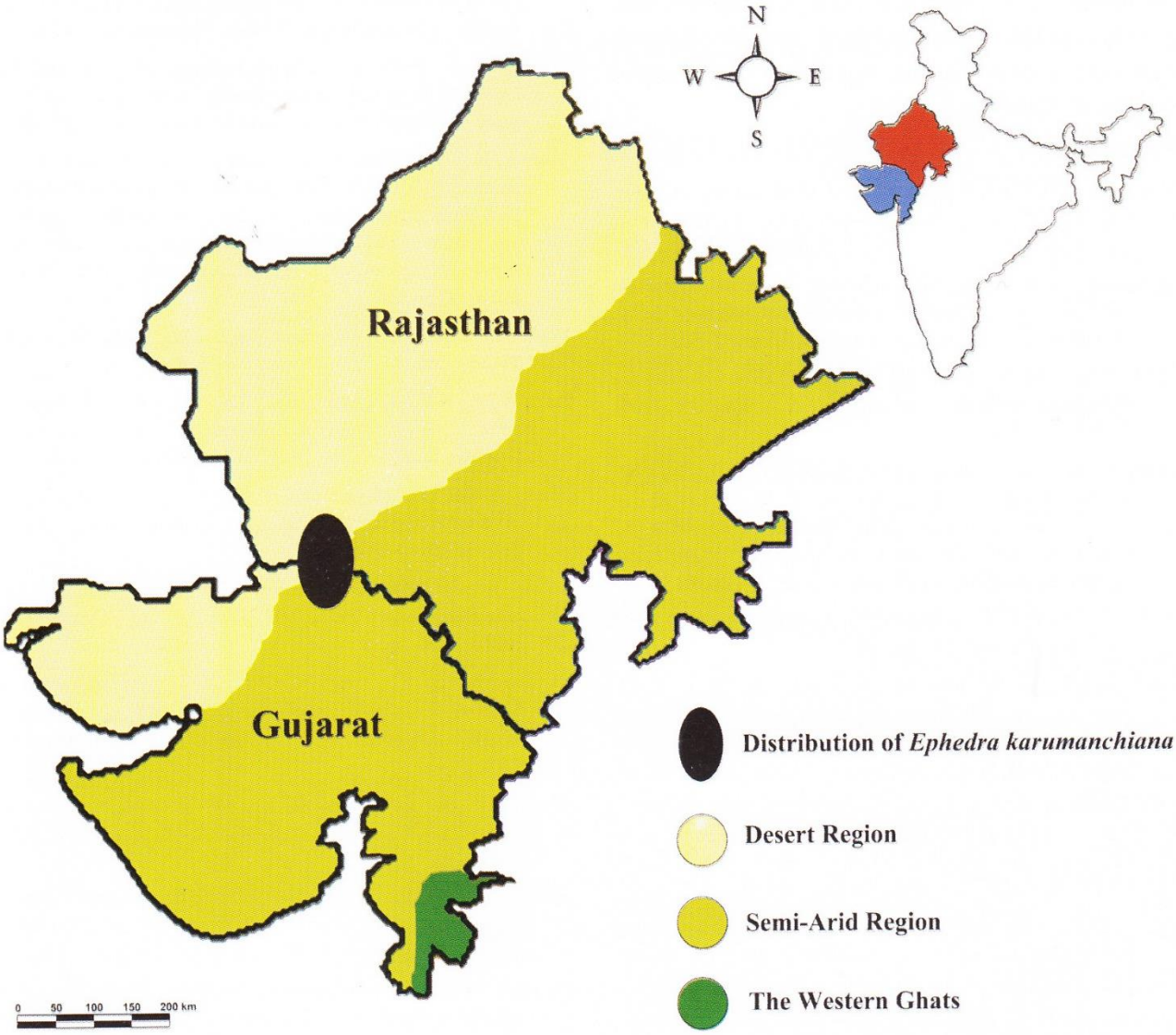

Plate 3. Distribution map of *Ephedra karumanchiana* in the western part (Gujarat and Rajasthan states) of India.

thick stems; climbing habit, scaly leaves and jointed stem which are quite distinct in young branches; linear-lanceolate leaves, 10–15 male strobili in biparous cyme and 5–10 female strobili, ovoid, pink with non-mucronate bract. In addition to above, present species differs from its closely related hot desert species and can be easily differentiated in the characters are listed in Table 1.

Phylogenetic analysis: Phylogenetic tree showed that *E. karumanchiana* is placed in a separate clade from its morphologically as well as habitat wise closely related species like *E. foliata* Boiss. ex C.A.Mey. and *E. przewalskiiStapf* with 100% bootstrap support (Plate 4).

Conclusion

All species of *Ephedra* growing in India occur in temperate regions except *E. foliata* and *E. przewalskii*. Further, molecular phylogeny study reveals that *E. karumanchiana* stands as a distinct species.

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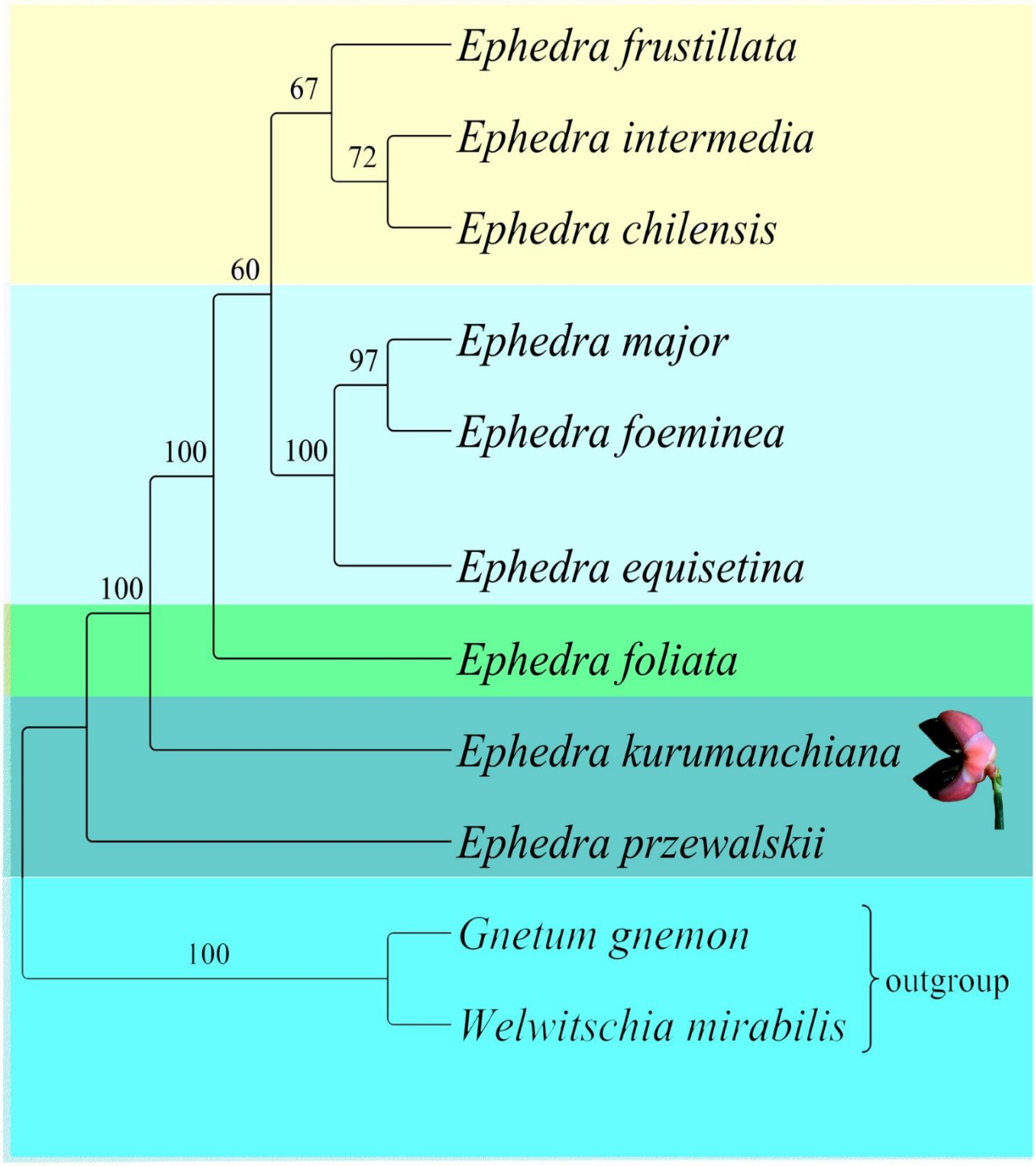

Plate 4. Phylogenetic analyses of combined nuclear ITS-1 and chloroplast *rbcL* + *psbA-trnH* + *trnLF* sequences of *Ephedra*. The tree shows the 50% bootstrap consensus from Maximum likelihood analysis. Numbers above the branch show bootstrap values

Multiple sequence alignment using ClustalW and ClustalX. *Current protocols in Bioinformatics* **1**:2–3.

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Table 1: Comparative morphology of *Ephedra karumanchiana* anditsclosely resembling species, *viz.* *E. foliata, E. frustillata, E. przewalskii* and *E. patchyclada*

Morphology/ Species	*E. karumanchiana*	*E. foliata*	*E. przewalskii*	*E. patchyclada*
Habit	Large, scandent-trailing, shrub	Small, scandent-trailing shrub	Medium, erect, ascending shrub	Small, erecto-patent undershrub
Stem	3–5 m (rarely 6 m or more), woody, scabrous, green	0.5–1 m, woody, climbing, slender, yellow-green, grooved	2–4 m tall, young branchlets green to pale brownish	0.5–1.5 m tall, woody, green or bluish grayish green, virgate
Male strobilus	03–13 per node, sometime bifurcate, whorled, stalked	Solitary or 2–3 per node, sessile	3–4, whorled, sessile	solitary or in clusters of 3–4 per node, sessile or shortly stalked
Bract	10–24 bracts per strobilus, bract bicolorous, center dark green, margin hyaline, ciliate	3–7 bracts per strobilus, connate, obtuse, sessile, dark green, ciliate	3–4, connate at base	5–8 bracts per strobilus, bract connate
Anthers	10–15 per strobilus, short stalked	3–4 per strobilus, sessile	5–8, short stalked	6–8 per strobilus, sessile
Female strobilus	4–14, whorled, pedunculate, bracteate, pink, non-mucronate	4–8, whorled, pedunculate, white, non-mucronate	4–5, whorled, sub-globose, bracteate, bracts completely free, connate at base, light brown	2–4, opposite, shortly pedunculate, bracteate, red, fleshy
Ovules	1–2, ovate, green-black	2, globose, white	2–3, elongate-ovoid	1 or 2, elongate-ovoid
Seeds	1–2 (rarely 3), ovate, black, persistent micropyle	2, brownish-black, micropyle non-persistent	(2 or) 3, elongate-ovoid, concealed by scarious bracts	1, elongate-ovoid

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Comparison Between Agriculture Soil and Common Land Soil in Relation to Soil Edaphic Factors and Nematode Community

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Abstract: ***Plant-parasitic nematodes may cause mechanical damage to the roots, stems, leaves, and flower structures of many plants. The host plant is more important in the nematode population. The control of these nematodes is more difficult than that of other pests because they mostly inhabit the inner part of the crops. Some Edaphic factors and secondary metabolites of plants play an important role in nematode control. The present study aims to analyze the comparision between agriculture soil and common land soil in relation to soil edaphic factors and nematode community. For testing nematode infection in crops, different agricultural fields were selected from different areas in and around Junagadh District. We have selected some agriculture sites in which nematode population were widely found. On the other hand, common land soil were no nematode population found. Edaphic factors like soil pH, Temperature, Moisture, Organic Carbon, Electrical Conductivity, Phosphorus, and Potassium were effective in the nematode growth. Results indicate that Soil temperature and Moisture were more affected in the nematode community. Further studies for the control of these nematodes are underway.***

Keywords: Nematode, Edaphic factors, Temperature, Moisture, Organic Carbon

I. INTRODUCTION

A natural population of the plant-parasitic nematodes is usually polyspecific. Species have different niche dimensions. The same species may be present in different proportions in different environmental conditions at different times. The host plant affects populations of plant-parasitic nematodes more than do soil factors. Even though the host-parasite associations are strong and the living part of a community cannot be separated from the physical part. If the species composition of populations is casual, then density-dependent factors are weak. (Norton, 1989)

Biological control of these plant-parasitic nematodes would be incomplete without some consideration of the soil environment. Plant-parasitic nematodes spend most of their life cycle stages in soil. They also develop their relationships with roots during the feeding process, it is considered them as occupying the soil–root interface rather than the bulk soil mass. Eggs of some plant-parasitic nematodes diffuse from roots and feeding takes place of the root system near root tips, root hairs, and in regions where lateral roots emerge. The bodies of ectoparasitic nematodes attach with the surface area of root; adult females of some sedentary endoparasites protrude into this zone and the eggs of many species are aggregated on the root surface. (Stirling, 2018)

Edaphic factors such as soil pH, Temperature, Moisture, Organic Carbon, Electrical Conductivity, Phosphorus, and Potassium were effective in the nematode growth (Norton, 1989). Some Edaphic factors and secondary metabolites of plants play an important role in nematode control.

The present study aims to analyze the comparision between agriculture soil and common land soil in relation to soil edaphic factors and the nematode Community.

II. MATERIAL AND METHODS

A. Study Area and Soil Sampling

This research was conducted on the different areas in and around Junagadh district which is situated in the Southwestern part of the Gujarat state. Soil samples were collected by the Random sampling method. The soil was collected from the 15 cm depth where nematode population was mostly found. Soil samples were collected in polythene bags from different sites. Each bag marked with name of the site and the date. Then soil samples were estimated in a laboratory for different edaphic factors. Based on edaphic factors we found an effect of nematode population in different soil.

B. Soil Edaphic Factors

Soil Temperature [by thermometer at a depth of 15 -20 cm below the ground]

Soil Humidity [in terms of percentage on a dry weight basis. A known amount of soil sample was taken from the different locality and it was dried in an oven at 80ºC for 48 h and weighed again. The percentage of moisture content was calculated by using the following equation:

$$\text{Moisture} = \frac{\text{weight of moist soil - the weight of dry soil} \times 100}{\text{weight of dry soil}}$$

Soil pH [by pH meter]

Soil Electrical conductivity [by ELICO CM 183EC-TDS Analyser]

Organic carbon [by volumetric method (Walkley and Black, 1934)]

Available Phosphorus [by Bray's and Olsen's method (Bray and Kurtz, 1945 and Olsen, 1954)]

Potassium [by flame photometry (Toth and Prince, 1949)]

C. Nematode Extraction and Identification

Nematodes were found from 15-20 cm depth of soil and root materials. For that, we have used the Baerman funnel technique. The extracted nematodes were observed under a stereo microscope and Binocular light microscopes. Identification of nematodes was based on their morphological characteristics. For that, we used 2 different types of identification keys which are the interactive diagnostic key to plant-parasitic, free-living and predaceous nematodes (Tarjan et al., 1977) and the identification key for agriculturally important plant-parasitic nematodes (Mekete et al., 2012).

III. RESULTS AND DISCUSSION

Results show the statistical data for the soil analysis in and around Junagadh district and the status of the nematode population. Table-1 shows the statistical data, where the average has been calculated. In which common land soil have no nematode population found. On the other hand, agriculture soil were found to be rich in nematode populations. In which average temperature and pH were low in agricultural soils as compared to common land soils. Where as average Humidity, EC, Organic Carbon, Phosphorus and Potash were high in agricultural soils as compared to common land soils.

Soil Edaphic factors	Common Land soil	Agriculture soil
Temperature (˚C)	29	25
Humidity (%)	22	26
Soil pH	8.1	7.7
Electrical Conductivity (EC) (dS/m)	0.25	1.11
Organic Carbon	0.87	1.95
Phosphorus (kg/ha)	41.56	52.02
Potash (kg/ha)	276	459

Table I : Stastical data of soil edaphic factors in agriculture soil and common land soil

A. Classification of Nematode

In our study, We have identified 4 nematode species viz., *Monhystera sp., Mesorhabditis sp.*, *Ditylenchus sp.* and *Meloidogyne sp.* from agriculture soil rich in amount. The classification and general characters for each nematode species are as follows:

B. Monhystera sp. Identification

Body mostly tapering considerably posteriorly. Caudal sucker small, somewhat pointed. Esophagus uniform, cylindrical. Vulva about a posterior third of the body. Uterus unsymmetrical. Viviparous or oviparous. Spicules long. (Bastian, 1865). With the above characteristics, the collected specimen was identified by an Interactive diagnostic key to nematodes (Tarjan et al., 1977).

Classification :

Kingdom	:	Animalia
Phylum	:	Nematoda
Class	:	Adenophorea
Order	:	Monhysterida
Family	:	Monhysteridae
Genus	:	*Monhystera*
Species	:	*Monhystera sp.*
Host Plant	:	Ground nut

(*Arachis hypogaea* L.)

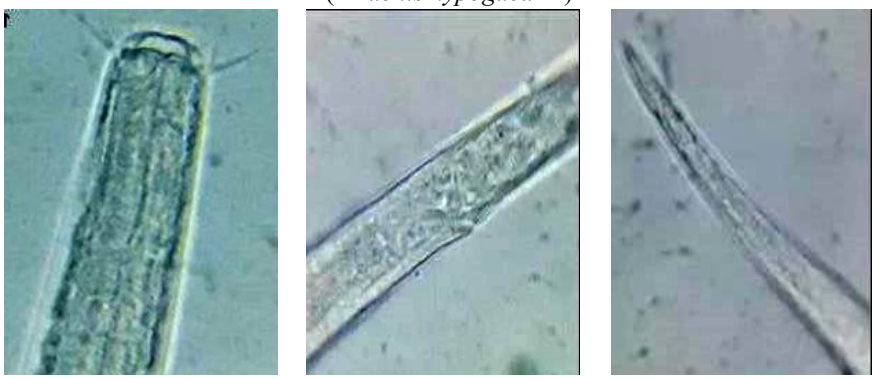

Fig. 1 *Monhystera sp.* A) Mouth part with cephalic setae, B) Vulva opening at the posterior part of the body and
C) Tail region

C. Mesorhabditis sp. identification :

Relatively small nematodes. Lips lobate, strongly cuticularized and in some species, deeply incised. Stoma long and narrow, never prismatic. The anterior part of esophagus is marked with transverse ridging; a median bulb present. No definite oesophageal collar. Vulva posterior and ovary single; female tail conical shaped. Bursa open and not radially arranged. Spicules long and slender, proximally knobbed, distally fused. 2 pairs of preanal papillae (Osche, 1952). With the above characteristics, the collected specimen was identified by Interactive diagnostic key to nematodes (Tarjan et al., 1977).

Classification :

Kingdom	:	Animalia
Phylum	:	Nematoda
Class	:	Chromadorea
Order	:	Rhabditida
Family	:	Rhabditidae
Genus	:	*Mesorhabditis*
Species	:	*Mesorhabditis sp.*
Host Plant	:	Saptaparni

(*Alstonia scholaris*)

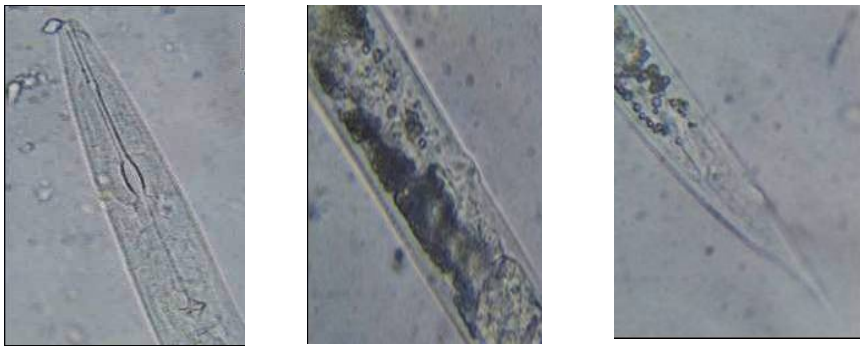

Fig. 2 *Mesorhabditis sp.* A) Mouth part with a stoma, Mid esophagus bulb and Posterior esophagus bulb well developed, B) vulva opening and C) Tail region

D. Ditylenchus sp. Identification

Median bulb with or without valve; isthmus not separated from glandular bulb by a constriction; glandular bulb short or long, when long may overlap the intestine for a short or long distance. Ovary short or long, sometimes reaching esophageal region and/or flexed; oocytes in one/two rows; columned uterus with four rows of four cells; post-uterine sac (PUS) present or absent. Testes usually without flexures; caudal alae leptoderan, short adanal or long, but never reaching tail end. Mature female not or slightly swollen. Mycetophagous or parasites of higher plants, found in soil or above ground. (Thorne, 1945) With the above characteristics, the collected specimen was identified by an Interactive diagnostic key to nematodes (Tarjan et al., 1977).

Classification :

Kingdom	:	Animalia
Phylum	:	Nematoda
Class	:	Secernentea
Order	:	Tylenchida
Famil	:	Anguinidae
Genus	:	*Ditylenchus*
Species	:	*Ditylenchus sp.*
Host Plant	:	Chickpea

(*Cicer arietinum*)

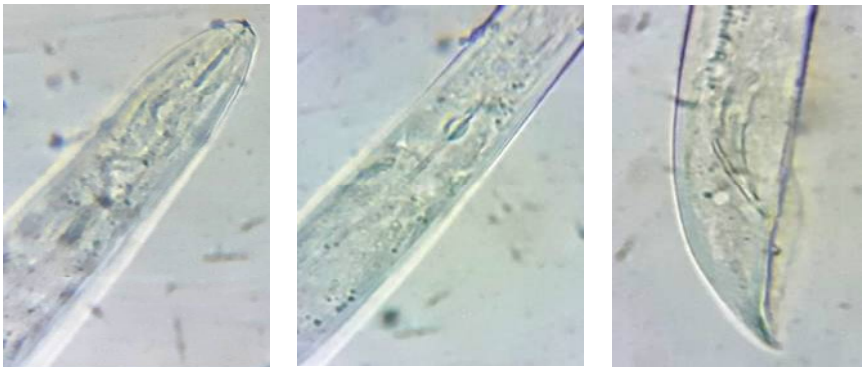

Fig. 3 *Ditylenchus sp.* A) Mouth part with stylet, B) Midian bulb and C) Tail region with spicule and bursa

E. Root-knot Nematode Identification

Cuticle not abnormally thick, annulated in all stages of the male and female. The cephalic framework of medium sclerotization; lateral sectors equal to wider than submedian sectors.

1) *Female:* Sedentary, globose with projecting neck. No preadult vermiform female stage. Cuticle moderately thick; annulationforming finger-print like pattern around vulva and anus. Labial disc dumb-bell shaped, not detached from labial sectors. Cephalic framework and spear delicate. The excretory pore is anterior to the median oesophageal bulb, often only slightly posterior to the stylet base. Vulva and anus terminal; perineal region flush or slightly raised. No cyst stage. Eggs are not retained in the body but deposited in a gelatinous matrix.
2) *Male:* Labial area low, not set-off, irregularly annulated. Lateral field with four lines.
3) *Juveniles:* Second stage juveniles migratory, vermiform. Cephalic framework and spear delicate. Labial area not set-off. Late second-stage sedentary, swollen (spike-tailed). The third and fourth stages occurring within the second stage cuticle, devoid of stylet.

With the above characteristics, the collected specimen was identified by an Interactive diagnostic key to nematodes (Tarjan et al., 1977).

Classification :

Kingdom: Animalia
Phylum : Nematoda
Class : Secernentea
Order : Tylenchida
Family : Heteroderidae
Genus : *Meloidogyne*
Species : *Meloidogyne sp.*
Host Plant : Ground nut

(*Arachis hypogaea* L.) & Chickpea
(*Cicer arietinum*)

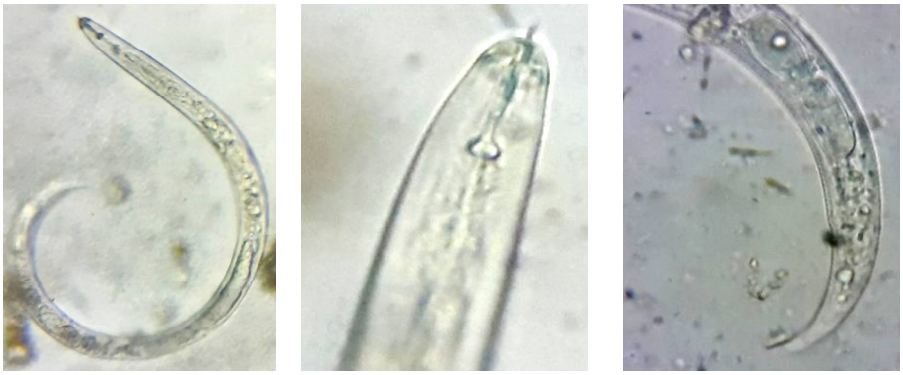

Fig. 4 *Meloidogyne sp.* A) Full Body of *Meloidogyne sp*., B) Mouth part with knob stylet C) Tail region

IV. CONCLUSIONS

From our study, we concluded that these edaphic factors are play important role in the different life cycle stages of the nematode population. We found that agricultural soils are rich in nematode population as compared to common land soil. Average Temperature and pH were low in agricultural soils as compared to common land soil. Where as average Humidity, EC, Organic Carbon, Phosphorus and Potash were high in agricultural soils as compared to common land soil due to the use of fertilizers. So these Agriculture land soil were most effective in the growth of the nematode population. The nematode diversity increased while moving from acidic to alkaline pH and in abundant to phosphorus, Potash, Organic carbon.

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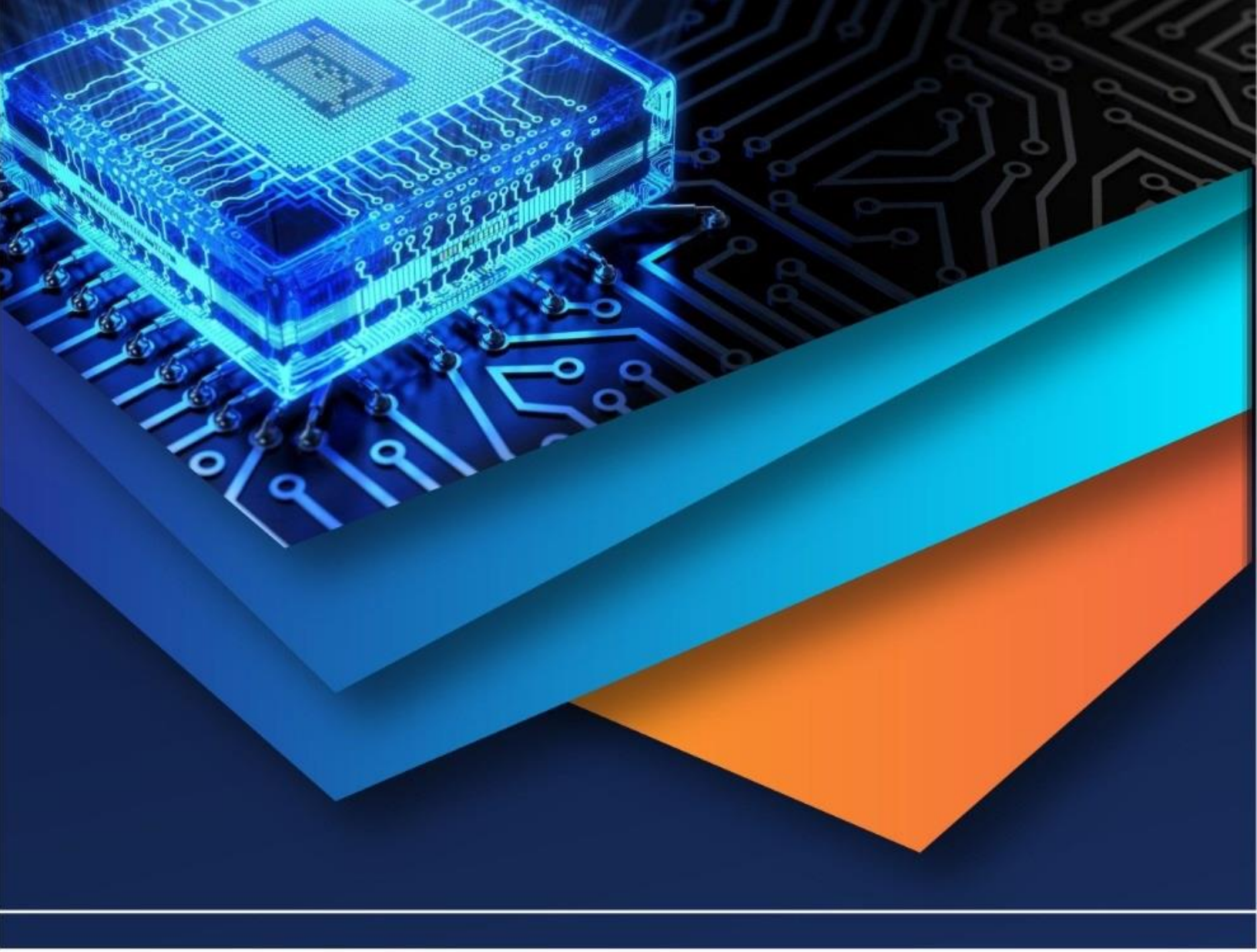

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Effect of Size and Shape on Thermo-Elastic Properties of Nano-Germanium

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Abstract

Germanium is a semiconductor with varied applications in the field of nanoscience and other fields of science. With known information about the bulk properties of germanium, an effort has been made to investigate the characteristics of germanium when it is in nanoscale size. The effective use of germanium and its compound in nanotechnology and other fields requires an intensive study of the thermo-elastic properties in nano scale. Effect of size and shape on the melting temperature, thermal expansivity, and bulk modulus has been studied for pure nano germanium. An attempt has been made to compute Young's modulus using two different formalisms. A comparative study of both the formalisms with experimental values is presented in this work. The comparative study for Young's modulus brings out the most suitable formalism for germanium nano crystal to calculate this modulus.

Keywords: *Melting point, Thermal expansivity, Bulk Modulus, Young Modulus, Nanosolid.*

1. INRODUCTION

Germanium is a very important semi-conductor material found on the earth [1, 2]. Although not readily available, like its silicon counterpart, it still has various uses in transistors, integrated circuits, etc. Though its bulk properties have been known to many for ages, investigation to understand the properties of nano solid had been started recently [3]. The use of nanomaterial is vast and has got many real-life applications [4]. The effect of size and shape plays a very important role to understand the thermoelastic properties of the nanomaterial.

The unavailability of experimental data for the size and shape dependence on the thermodynamic properties of semiconducting nanomaterials led us to investigate the effect of size and shape on the thermodynamic as well as thermoelastic properties of nanosolids. In the present communication, size dependency of melting point, bulk modulus, and coefficient of volume thermal expansion and Young modulus of nano-germanium has been reported. Two different theoretical formalisms [5, 6] have been used to compute the ratio of Young modulus of nano germanium to bulk germanium. Our predicted results are compared with the available results [7,8]. A comparative study of both formalisms is presented in the present work.

2. METHODOLOGY

The variation in the melting point against the size of nano-solids can be understood using the W.H.Qi model [9]. This model has predicted the size dependent melting temperature of nanoparticles, nanowires and nanofilms. Melting temperature of nanosolid based on the Qi model reads as follows

$$T_{mp} = T_{mb}\left(1 - \frac{N}{2n}\right) \qquad (1)$$

where *n* is the total number of atoms in a nanosolid and the total number of atoms on the surface of the nanosolid is N. Here T_{mp} and T_{mb} are the melting temperatures of the nanosolid and the corresponding bulk material respectively. The value of ratio N/n depends upon the shape and size of the nanosolid and the expressions of N/n for spherical nanosolids, nanowires and nanofilms have been tabulated in Table 1 [9].

Table 1. *N/n for three different types of nanosolids. Here d and D are the diameter of the atom and nanoparticles respectively. For the disk-like nanosolid l and h are the diameter of nanowire and width of nanofilm respectively.*

Nanosolid	N/n
Nanosphere	$4d/D$
Nanowire (h>>1)	$(8/3)(d/l)$
Nanofilm (l>>h)	$(4/3)(d/h)$

The coefficient of volume thermal expansion of nanosolids based on R. Kumar et al. model [10] and also given in [11] reads as follows.

$$\alpha_{nm} = \alpha_b \left(1 - \frac{N}{2n}\right)^{(-1)} \qquad (2)$$

where α_{nm} and α_b are the coefficients of volume thermal expansion of nanosolid and corresponding bulk material respectively.

Equation of isothermal bulk modulus developed by Pandya et al. [3] reads as follows

$$B = B_0\left(\frac{V}{V_0}\right)\left(1 + (B_0' + 1)\left(1 - \frac{V}{V_0}\right)\right) + B_0\left(\frac{V}{V_0}\right)\left(\frac{(B_0'+1)}{2}\left(1 - \frac{V}{V_0}\right)^2\right) \qquad (3)$$

where B_0 is the isothermal bulk modulus at zero pressure, B_0' is the pressure derivative of bulk modulus. V_0 is the volume at zero pressure and V is the volume at pressure P.

To compute the ratio of Young modulus, of nano-crystal to bulk crystal, two different formalisms [5,6] have been used. Following Qi model [9] S. Patil et al. [5] proposed the expression for Young modulus of nanosolids which reads as follows:

$$\frac{Y_{nm}}{Y_{bm}} = exp\left(\pm\frac{S_{vib}-1}{\frac{r}{r_0}-1}\right) \qquad (4)$$

Here Y_{nm} and Y_{bm} are the young modulus of the nanosolid and corresponding bulk value respectively and r_0 is the critical radius at which all the atoms of the nano-crystal are located on the surface [5]. The value of `r_0' is given by [5,12]

$$r_0 = (3 - d)h \qquad (5)$$

where *h* is the atomic diameter and d=0 for spherical nanosolids, d=1 for nanowires and d=2 for nanofilms [12]. Ratio [5] of mean square displacement of atoms on the surface and that in the interior of the nanosolid can be derived from the vibrational entropy expression and is given by

$$S_{vib} = \left(\frac{2S_{nm}}{3R}\right) + 1 \qquad (6)$$

Here *R* is the ideal gas constant and S_{nm} is nano melting entropy given by [13, 16]

$$S_{nm} = S_{mb} + \left(\frac{3R}{2} ln\left(1 - \frac{N}{2n}\right)\right) \qquad (7)$$

Here S_{mb} is the bulk melting entropy given as $S_{mb}=H_{mb}/T_{mb}$ [13, 16]; H_{mb} is melting enthalpy for bulk materials and T_{mb} is the bulk melting temperature. The value of the ratio N/n can be obtained from Table 1. Using the approach adopted by G. Patel et al. [6] the ratio of Young modulus can be computed as follows

$$\frac{Y_{nm}}{Y_{bm}} = 1 + \left(1 - \left(1 - \frac{(\beta)(S)A}{6}\right)\right) \qquad (8)$$

where β is material constant [6,14] and S is the shape factor [6,15] of the material. `A' is the surface to volume ratio [6,14].

3. RESULT AND DISCUSSION

Using eq. (1) the melting temperatures for nano-germanium have been evaluated and the predicted results for spherical nanosolids, nanowires and nanofilms are

reported in Figure 1. Figure 1 provides a comparative study of variation of melting temperatures against the size of the nanosolid. In Figure 1 we have included our predicted results for the nanosolids having size less than 13nm because for the nanosolids having higher size our results are analogous to their corresponding bulk counterpart.

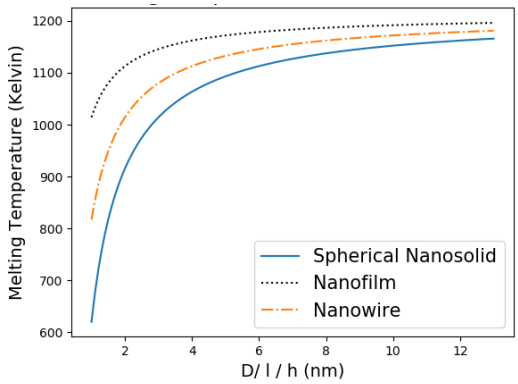

Figure 1. *Variation of melting temperatures of nanogermanium for spherical nanosolid (D nm), nanowire (l nm) and nanofilm (h nm) shapes against the size.*

It is found that the melting temperature decreases as the size of nanosolid decreases and the trend of variation in melting temperature is almost similar for different shapes. It is found that effect of shape on the melting temperature is significant in the case of spherical nanosolid. Variation in melting temperature is noteworthy for smaller particles. At nano-level the surface to volume ratio increases drastically, resulting in alteration of the thermodynamic and thermal properties. At the nanoscale range as the size of the particle decreases melting temperature depresses. This is due to the enhanced surface to volume ratio at the nanoscale size. At the size below 20nm surface to volume ratio increases considerably so the number of atoms on the surface increases. On the surface 50 percent of the bonds are dangling bonds that causes in the reduction of melting temperature considerably below 20nm size.

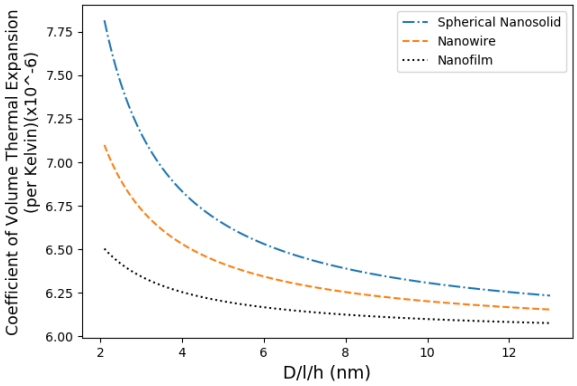

Figure 2. *Variation of coefficient of volume thermal expansion of nanogermanium for spherical nanosolid (D nm), nanowire (l nm) and nanofilm (h nm) shapes against the size.*

Using eq. (2) the coefficient of volume thermal expansion for nanogermanium has been computed. The predicted results for spherical nanoparticles, nanowires and nanofilms are shown in Figure 2. The variation in the coefficient of volume thermal expansion (α) for the nanosolids is significant for the size less than 10nm for all the shapes. In this range, it is found that (α) increases as the size of the nanosolids decreases. Results of the coefficient of volume thermal expansion (α) for nanosolids having a size greater than 10nm are similar to the results of their corresponding bulk counterparts for all the cases.

The variation of isothermal bulk modulus against compression, calculated using eq. (3), is shown in Figure 3. The input parameters used for the computation of isothermal bulk modulus are tabulated in Table 2.

Table 2. *Input parameters used for the computation of bulk modulus.*

B_0'	B_0' (GPa)	Particle Size
4	112	13 nm
4	92	49 nm
3	74.9	Bulk Germanium

Our results on the study of compressibility of nano germanium

demonstrate that nano size samples are less compressible than bulk materials which are in agreement with the Hall-Patch effect [3]. Our results indicate that bulk modulus increases with decrease in particle size, which may be the effect due to the larger surface between grains in nanosized particles that provides energy leading to the increase in hardness. Hence it is found that nanogermanium gradually hardens with an increment of pressure.

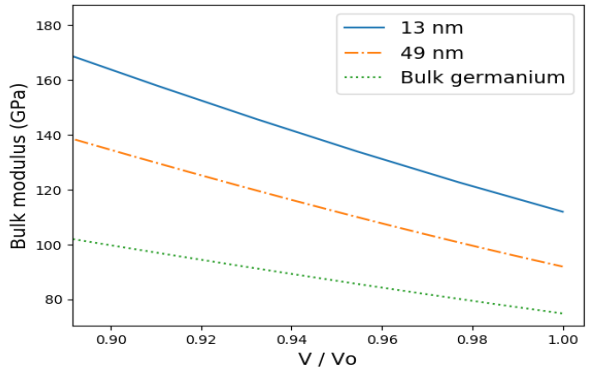

Figure 3. *Bulk modulus versus volume compression for germanium and nanogermanium having spherical shape.*

The predicted results for the ratio of Young modulus of nanosolids to the bulk solid computed using eq. (4) and eq. (8) are compared in Figure 4. As the size decreased of nanowire surface to volume ratio increases and number of atoms on the surface increases so on the surface interatomic distance decreases which enhances tangential force resulting into the increment of young modulus.

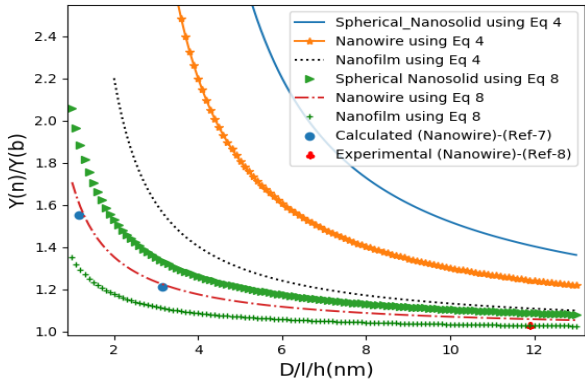

Figure 4. *Ratio of size-dependent Young modulus of nanosolid to bulk Ge computed using (4) and (8)*

For the case of nanowire, it is found that the predicted results of the ratio of Young modulus using eq. (8) are in good agreement with the available experimental finding [13] and other available computed results [7]. It is found that results obtained by Patil et al [5] formulation deviate largely as compared with the work of G. Patel et al. [6]. Patil's formulation is based on Lindemann's criterion of melting. Patel and co-workers have used a liquid drop [14] model to derive the empirical relation between Young modulus and the size of nano solids. With reduction in size the binding energy increases and because of this increase in the binding energy the Young modulus increases with reduced size. This large difference between the results of these two formalisms is observed because each of the formalism is based on different assumptions.

4. CONCLUSION

It is concluded that the melting temperature decreases as the size of nanosolid decreases. We have found that nano size samples are less compressible than bulk materials. Nanogermanium gradually hardens with an increment of pressure and the coefficient of volume thermal expansion of nanosolids increases with decreasing size for all different shapes.

In the present work, two different formalisms are critically analysed by studying the thermoelastic properties of nano germanium. It is found that the Young modulus of nanosolids decreases as the size of nanosolid increases, the reason being the binding energy of nanogermanium. These predictions may be of current interest to the researchers engaged in the experimental studies and this model may be applicable to binary semiconductors.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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