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continued on the back inside cover

Caption: Malabar Slender Loris *Loris lydekkerianus malabaricus* © Dileep Anthikkad.



A cytomorphological investigation of three species of the genus *Sonchus* L. (Asterales: Asteraceae) from Punjab, India

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Abstract: Three species of the genus *Sonchus* L. (*Sonchus asper*, *S. oleraceus* and *S. wightianus*) were collected from the Malwa region of Punjab during 2019 to 2020. These species were studied for cytomorphological variations. The species under investigation were identified based on their morphological descriptions. *Sonchus asper* (L.) Hill and *Sonchus wightianus* DC. possess the same number of chromosomes ($2n=2x=18$) whereas *Sonchus oleraceus* (L.) L. is tetraploid with $2n=4x=32$ chromosomes. Chromosome number of *S. wightianus* ($2n=2x=18$) was worked out for the first time from the state of Punjab. *Sonchus oleraceus* has larger pollens than *S. asper* and *S. wightianus*. This study will be useful for researchers, taxonomists and cytologists for accurate identification of these three species.

Keywords: Chromosome number, involucre bract, meiosis, palynology, *Sonchus*, taxonomy.

Sonchus L. is a member of the family Asteraceae with 95 species distributed throughout the world including western Morocco, Ethiopia, southern Sudan, South Africa, Canary Island, Europe, Iran, Iraq, Egypt, Afghanistan, and Turkistan (Boulos 1960; Cho et al. 2019). *Sonchus* species are annual to perennial herbs with a milky latex. The stem is clasping, toothed or pinnatifid, segmented leaves; terminal, umbellate, yellow, ligulate-homogamous heads; ovoid, ellipsoid, compressed, ribbed achenes with white hairy pappus which are the important features of the genus *Sonchus* L. (Quireshi

et al. 2002; Rahman et al. 2008). Earlier four species of *Sonchus* (*S. asper* Vill., *S. arvensis* L., *S. oleraceus* L., & *S. maritimus* L.) were reported from British India (Hooker 1882) and undivided Punjab (Bamber 1916). Sharma (1990) enlisted *S. asper*, *S. oleraceus*, and *S. wightianus* from Punjab. Later on, Sidhu (1991) recorded *S. asper*, *S. arvensis*, and *S. oleraceus* from the state of Punjab. *Sonchus asper* and *S. oleraceus* were common in the previous studies whereas *S. wightianus* or *S. arvensis* were frequently misplaced under confusing species.

Morphological parameters have been used for the identifications of plant species for a long time. It is one of the basic, simple and cost effective tools. Morphological features such as leaf shape and color; flower color and type; number, position and nature of androecium and gynoecium; shape and type of fruit and seeds are used for identification of species (Singh & Dey 2005). Chromosome number is also important in the identification of species because species, genera and families have their own unique chromosome numbers in general and basic chromosome number in particular. Variations in chromosome numbers are useful in taxonomic studies (Raven 1975; Jones 1979).

The present study is an attempt to differentiate between previously reported two (*S. arvensis* and

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S. wightianus) species (Sharma 1990; Sidhu 1991). Therefore, it is important to look into the detailed morphology of the three species under investigation. Keeping this in view, the present study has been planned to characterize three species of *Sonchus* from the state of Punjab based on morphological and cytological observations.

MATERIALS AND METHODS

Collection of study materials

The present study has been undertaken in the Malwa region of the state of Punjab, India. The study material of three species of *Sonchus* was collected during 2019 to 2020. The collected plant specimens were cleaned thoroughly, pressed, and dried at room temperature. After this, the plant specimens were pasted on herbarium sheets. Herbarium specimens were deposited in the Herbarium, Department of Botany, Punjab University Chandigarh (PAN-21994, 21996 and 21997).

Morphological study

Morphological features of a leaf (arrangement, shape, type, color), stem (glabrous, hairy), flower (colour, type, shape), androecium (number, shape, nature), gynoecium (shape, number, nature) were examined to establish the identity of each of the three *Sonchus* species. The available literature (Hooker 1882; Bamber 1916; Turner et al. 1961; Walter & Kutta 1971; Boulos 1972; Hsieh et al. 1972; Nair 1978; Mejias & Andres 2004; Cho et al. 2019) have been looked into to describe the *Sonchus* species in question. The Herbarium, Department of Botany, Panjab University Chandigarh and online Herbaria have also been consulted for identification.

Meiotic and pollen study

Meiotic analysis has been carried out in three *Sonchus* species to examine their chromosome numbers. Young flower buds were collected and fixed in the fixative (ethanol 3: glacial acetic acid 1) for 24 hours then shifted to 70% ethanol till further use. Anthers were excised from young flower bud on the glass slide having a drop of acetocarmine and crushed with the help of a glass rod. The material was covered with a micro cover-slip and pressed in two folds of filter paper after gentle heating. Slides were observed under the microscope. Photographs of the pollen mother cells containing countable chromosomes have been taken. For pollen study, mature anthers were taken on the slide and squashed in glycerol acetocarmine (1:1), covered with a cover-slip and observed under the microscope after 24 hours. Uniformly stained pollens (S.P.) were considered

fertile whereas, poorly stained or unstained pollens as sterile. The percentage of pollen fertility was calculated using (Pollen fertility = S.P. / Total Pollens x 100) formula. Pollen size has been measured with the help of camera-lucida technique.

RESULTS AND DISCUSSION

Three species of the genus *Sonchus*, i.e., *Sonchus asper*, *S. oleraceus*, and *S. wightianus* were collected from the Malwa region of Punjab during 2019 to 2020. All the three species are annual with erect habit. Leaves of *S. oleraceus* are smooth, glabrous, and light green whereas they are dark green in the case of *S. wightianus*. In *S. asper*, leaves are spined and bluish-green. Leaves are elliptic-oblong, half amplexicaul with round auricles in *S. asper* and *S. wightianus* but auricles are spreading in the case of *S. oleraceus* (Image 1,2). Similarly, leaf auricles were found to be round in *S. asper* and pointed to acute in *S. oleraceus* (Barber 1941; Quireshi et al. 2002; Cho et al. 2019). *S. asper* and *S. oleraceus* are very similar to each other in flower colour, i.e., pale yellow to dark yellow whereas the flower colour in *S. wightianus* is orange yellow. Involucral bracts are smooth in *S. oleraceus*, glandular hairy in *S. wightianus* and spiny-



Image 1. Habitat of *Sonchus* L. species (a-c): a—*Sonchus asper* | b—*Sonchus oleraceus* | c—*Sonchus wightianus*. © Rai Singh



Image 2. Morphological details of *Sonchus* species (a–d): a—leaf | b—leaf auricles | c—capitulum | d—involucral bracts. © Rai Singh

hairy in *S. asper* (Image 2). Rahman et al. (2008) also observed glandular and hairy involucral bracts in *S. wightianus* which supports the present study. This feature is important and useful for establishing the identity of *S. wightianus*. Achenes are wrinkled with ribs in *S. asper*, compressed in *S. oleraceus* and finely compressed in *S. wightianus* (Image 3).

Identification key (morphology)

- 1 (a) Leaf auricles acute..... *S. oleraceus*
- 1 (b) Leaf auricles round..... 2
- 2 (a) Involucral bracts with glandular hairs
..... *S. wightianus*
- 2 (b) Involucral bracts with spiny hairs *S. asper*

Both *Sonchus asper* and *S. wightianus* are diploid and contain $2n=2x=18$ chromosomes. Nine bivalents were observed at diakinesis and metaphase-I in *S. asper* and equal segregation of chromosomes (9-9) at anaphase-I in *S. wightianus* (Image 4.a,b,d). Razaq et al. (1994) also

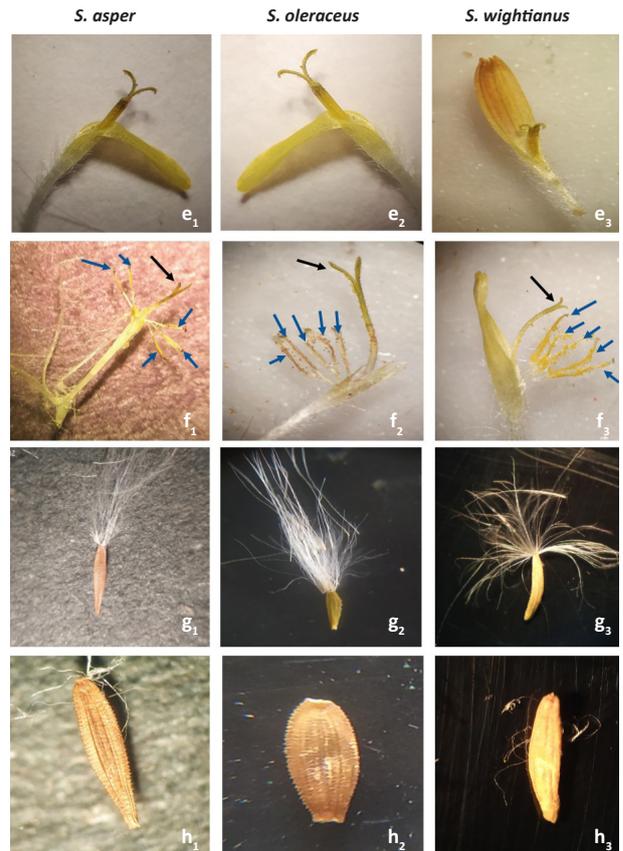


Image 3. Morphological details of *Sonchus* species (e–h): e—flower | f—flower (black arrow showing stigma and blue arrows showing stamens) | g—achene with pappus | h—achene. © Rai Singh

reported chromosome numbers $2n=18$ in both *Sonchus asper* and *S. wightianus* and $2n=32$ in *S. oleraceus* from Pakistan.

Sonchus oleraceus is a tetraploid and has shown 16 bivalents at diakinesis stage (Image 4c). Present chromosome findings of *S. oleraceus* is in consonance with Ishikawa (1911) who also reported $2n=4x=32$ chromosome in this species. It has suggested the genetic stability of species even after more than 100 years. But a diploid form of *S. oleraceus* ($2n=16$) and tetraploid ($2n=32$) were previously reported by Marchal (1920) and Cooper & Mahony (1935), respectively. More studies had described *S. asper* as diploid ($2n=18$) and *S. oleraceus* as tetraploid ($2n=32$) (Turner et al. 1961; Walter & Kutta 1971; Boulos 1972; Hsieh et al. 1972; Gupta & Gill 1983; Sidhu et al. 2011; Kaur & Singhal 2015). The variation of chromosome number in *Sonchus* species points towards the incidence of aneuploidy that has happened over time in the genus *Sonchus*.

Pollen size of *S. oleraceus* is $36.25 \times 32.5 \mu\text{m}$ – $40 \times 33.75 \mu\text{m}$ followed by *S. wightianus* ($33.75 \times 32.5 \mu\text{m}$ – $36.25 \times 33.75 \mu\text{m}$) and *S. asper* ($31.25 \times 28.75 \mu\text{m}$ – 35

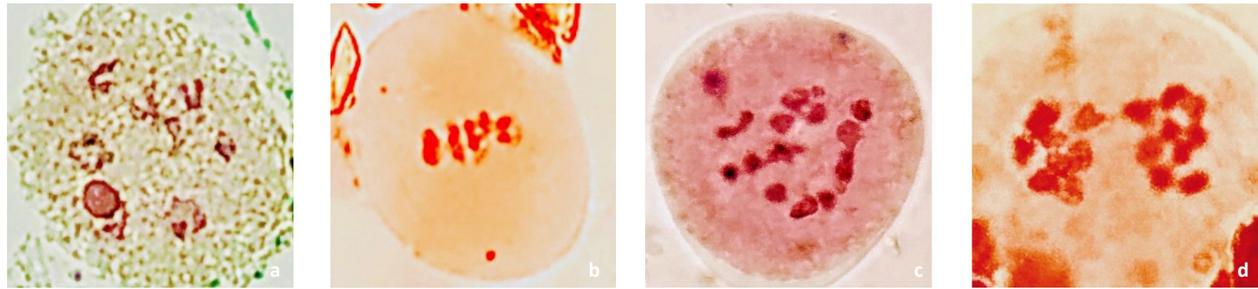


Image 4. Chromosome details of *Sonchus* L. species (a–d): a–b—*S. asper* (n= 9) | c—*S. oleraceus* (n= 16) | d—*S. wightianus* (n= 9). © Rai Singh



Image 5. Pollen grains of three *Sonchus* L. species (a–c): a—*S. asper* | b—*S. oleraceus* | c—*S. wightianus*. © Rai Singh

x 32.5 μm) (Image 5 a–c). Pollen size of *S. asper* and *S. wightianus* is almost similar which may be due to the same number of chromosomes ($2n=2x=18$). Pollens of *S. oleraceus* are larger than the other two species which may be because of its tetraploid ($2n=4x=32$) nature. Pollen fertility was maximum in *S. oleraceus* (94.33%), followed by *S. wightianus* (92.13%) and *S. asper* (88.88%). High pollen fertility in *S. oleraceus* suggested that it is an allotetraploid. These observations are in consonance with Poole (1932) who found that amphidiploids possess a greater degree of pollen fertility.

Earlier three species of *Sonchus* such *S. asper*, *S. oleraceus*, & *S. wightianus* (Sharma 1990) and *S. asper*, *S. oleraceus*, & *S. arvensis* (Sidhu 1991) were documented from the state of Punjab, India. But according to available literature (Shumovich & Montgomery 1955; Mamgain 1998) *S. arvensis* grows exclusively in Europe and is likely confused with *S. wightianus* in India. In literature, from the state of Punjab third species of *Sonchus* was considered as *S. arvensis* but it is actually a *S. wightianus*.

Cytological details of *Sonchus* species are also incomplete from the state of Punjab, India. Previously, Gupta & Gill (1983) had worked out chromosome numbers of three *Sonchus* species (*S. asper* (L.) Hill, *S. brachyotus* DC and *S. oleraceus* L.) from the state of Punjab. However, they have not worked out the

chromosome of *S. wightianus*. Consequently, information about the chromosome number of *S. wightianus* is not known. Therefore, the present study has been carried out for cytomorphological characterization of *Sonchus* species from the state of Punjab India. The findings of the present study will be useful for researchers, cytologists, and taxonomists for correct identification of *Sonchus* species based on morphological, cytological, and palynological details.

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Articles

Understanding human-flying fox interactions in the Agusan Marsh Wildlife Sanctuary as basis for conservation policy interventions

– Sherryl L. Paz & Juan Carlos T. Gonzalez, Pp. 19431–19447

Argentinian odonates (dragonflies and damselflies): current and future distribution and discussion of their conservation

– A. Nava-Bolaños, D.E. Vrech, A.V. Peretti & A. Córdoba-Aguilar, Pp. 19448–19465

Communications

The diel activity pattern of small carnivores of Western Ghats, India: a case study at Nelliampathies in Kerala, India

– Devika Sanghamithra & P.O. Nameer, Pp. 19466–19474

Distribution and threats to Smooth-Coated Otters *Lutrogale perspicillata* (Mammalia: Carnivora: Mustelidae) in Shuklaphanta National Park, Nepal

– Gopi Krishna Joshi, Rajeev Joshi & Bishow Poudel, Pp. 19475–19483

Wildlife hunting practices of the Santal and Oraon communities in Rajshahi, Bangladesh

– Azizul Islam Barkat, Fahmida Tasnim Liza, Sumaiya Akter, Ashikur Rahman Shome & M. Fazle Rabbe, Pp. 19484–19491

Ethnozoological use of primates in northeastern India

– Deborah Daolagupu, Nazimur Rahman Talukdar & Parthankar Choudhury, Pp. 19492–19499

Factors influencing the flush response and flight initiation distance of three owl species in the Andaman Islands

– Shanmugavel Sureshmarimuthu, Santhanakrishnan Babu, Honnavalli Nagaraj Kumara & Nagaraj Rajeshkumar, Pp. 19500–19508

Birds of Barandabhar Corridor Forest, Chitwan, Nepal

– Saneer Lamichhane, Babu Ram Lamichhane, Kapil Pokharel, Pramod Raj Regmi, Tulasi Prasad Dahal, Santosh Bhattarai, Chiranjibi Prasad Pokharel, Pabitra Gotame, Trishna Rayamajhi, Ram Chandra Kandel & Aashish Gurung, Pp. 19509–19526

On some additions to the amphibians of Gunung Inas Forest Reserve, Kedah, Peninsular Malaysia

– Shahriza Shahrudin, Pp. 19527–19539

Reviews

A review of research on the distribution, ecology, behaviour, and conservation of the Slender Lorises *Loris lydekkerianus* (Mammalia: Primates: Lorisidae) in India

– Mewa Singh, Mridula Singh, Honnavalli N. Kumara, Shanthala Kumar, Smitha D. Gnanaolivu & Ramamoorthy Sasi, Pp. 19540–19552

Bivalves (Mollusca: Bivalvia) in Malaysian Borneo: status and threats

– Abdulla-Al-Asif, Hadi Hamli, Abu Hena Mustafa Kamal, Mohd Hanafi Idris, Geoffery James Gerusu, Johan Ismail & Muyassar H. Abualreesh, Pp. 19553–19565

Disentangling earthworm taxonomic stumbling blocks using molecular markers

– Azhar Rashid Lone, Samrendra Singh Thakur, Nalini Tiwari, Olusola B. Sokefun & Shweta Yadav, Pp. 19566–19579

A reference of identification keys to plant-parasitic nematodes (Nematoda: Tylenchida\ Tylenchomorpha)

– Reza Ghaderi, Manouchehr Hosseinvand & Ali Eskandari, Pp. 19580–19602

Short Communications

Catalogue of herpetological specimens from Meghalaya, India at the Salim Ali Centre for Ornithology and Natural History

– S.R. Chandramouli, R.S. Naveen, S. Sureshmarimuthu, S. Babu, P.V. Karunakaran & Honnavalli N. Kumara, Pp. 19603–19610

A preliminary assessment of odonate diversity along the river Tirthan, Great Himalayan National Park Conservation Area, India with reference to the impact of climate change

– Amar Paul Singh, Kritish De, Virendra Prasad Uniyal & Sambandam Sathyakumar, Pp. 19611–19615

A checklist of orthopteran fauna (Insecta: Orthoptera) with some new records in the cold arid region of Ladakh, India

– M. Ali, M. Kamil Usmani, Hira Naz, Tajamul Hassan Baba & Mohsin Ali, Pp. 19616–19625

New distribution records of two *Begonias* to the flora of Bhutan

– Phub Gyeltshen & Sherab Jamtsho, Pp. 19626–19631

Rediscovery of *Aponogeton lakhonensis* A. Camus (Aponogetonaceae): a long-lost aquatic plant of India

– Debolina Dey, Shrirang Ramchandra Yadav & Nilakshee Devi, Pp. 19632–19635

***Glyphochloa acuminata* (Hack.) Clayton var. *laevis* (Poaceae): a new variety from central Western Ghats of Karnataka, India**

– H.U. Abhijit & Y.L. Krishnamurthy, Pp. 19636–19639

A cytomorphological investigation of three species of the genus *Sonchus* L. (Asterales: Asteraceae) from Punjab, India

– M.C. Sidhu & Rai Singh, Pp. 19640–19644

***Dryopteris lunanensis* (Dryopteridaceae) - an addition to the pteridophytic diversity of India**

– Chhandam Chanda, Christopher Roy Fraser-Jenkins & Vineet Kumar Rawat, Pp. 19645–19648

Notes

First record of Spotted Linsang *Prionodon pardicolor* (Mammalia: Carnivora: Prionodontidae) with photographic evidence in Meghalaya, India

– Papori Khatonier & Adrian Wansaindor Lyngdoh, Pp. 19649–19651

First record of the Eastern Cat Snake *Boiga gocool* (Gray, 1835) (Squamata: Colubridae) from Tripura, India

– Sumit Nath, Biswajit Singh, Chiranjib Debnath & Joydeb Majumder, Pp. 19652–19656

First record of the genus *Tibetanja* (Lepidoptera: Eupterotidae: Janinae) from India

– Alka Vaidya & H. Sankararaman, Pp. 19657–19659

***Austroborus cordillerae* (Mollusca: Gastropoda) from central Argentina: a rare, little-known land snail**

– Sandra Gordillo, Pp. 19660–19662

Intestinal coccidiosis (Apicomplexa: Eimeriidae) in a Himalayan Griffon Vulture *Gyps himalayensis*

– Vimalraj Padayatchiar Govindan, Parag Madhukar Dhakate & Ayush Uniyal, Pp. 19663–19664

Two new additions to the orchid flora of Assam, India

– Sanswarg Basumatary, Sanjib Baruah & Lal Ji Singh, Pp. 19665–19670

Wildlife art and illustration – combining black and white ink drawings with colour: some experiments in Auroville, India

– M. Eric Ramanujam & Joss Brooks, Pp. 19671–19674

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