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Photographic Key for the Microhistological Identification of Some Plants of Indian Trans-Himalaya

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Abstract

Microhistology techniques have been used in many studies regarding food habits of herbivores. The absence of detailed reference materials and time consumed in creating reference plant materials for a particular study species and area hampers an understanding and extensive use of the technique. On the other hand, the use of direct sighting procedure of animals to study the dietary spectrum of herbivores is interrupted by tough terrain and harsh climatic condition in the Trans-Himalaya. The current study provides a photographic key for identification of 38 plants species belonging to 35 genera and 21 families. Structures such as types of stomata, trichomes and epidermal cells are discussed for different species of plants collected from Kargil, Ladakh. The given information is expected to help researchers working on feeding ecology of mammals in the Indian Trans-Himalaya.

Keywords: dietary spectrum, feeding ecology, herbivore, microhistology, plant species

Introduction

Feeding habits of mammals are in the centre of interest of population biology (Lodé, 1996) and ecology (Mátrai *et al.,* 1998). A number of methods have already been evolved and used investigating dietary composition (Holechek *et al.,* 1982; Smith and Shandruk, 1979; Shrestha and Wegge, 2006). One of the indirect techniques for determining diet composition of herbivores is identification of food items through epidermis fragments in the stomach contents or faecal pellets as proposed by Baumgartner and Martin (1939). Later the technique was further advanced by Dusi (1949) and Holechek and Gross (1982) and since then it is being frequently used to study dietary composition of a range of wild and domestic herbivores (Uresk, 1986; Green, 1987; Alipayo, *et al.,* 1992; Ilyas and Khan, 2004; Shrestha *et al.,* 2005; Wegge *et al.*, 2006; Wingard *et al.,* 2011).

Microhistological analysis of plant remains in the faeces has several unique advantages which accounts for its popularity as a research tool (Holechek *et al.*, 1982a; Smith and Shandruk, 1979). There are few shortcomings as well, such as low accuracy depending on herbivore and plant species studied (Caron *et al.,* 1985), sample preparation technique used (Holechek *et al.,* 1982b; Johnson *et al.*, 1983a), observer training (Holechek and Gross, 1982) digestion and fragmentation (Johnson *et al.,* 1983b), number of slides prepared and frequency of observation per slide (Holechek and Vavra, 1981). Furthermore, the absence of detailed reference material or excessive costs in time and effort to create reference material for a particular study species and area mostly hamper a better understanding and extensive use of microhistological techniques (Carrière, 2002). Probably considering this, Satakopan (1972) described a key to the identification of plants remains in animal droppings. Similar efforts were made elsewhere; Hurst and Beck (1988) developed a reference key based on microhistological characteristics of aquatic plants of Florida, Lindstöm *et al.* (1998) did the same for perennial grass in central Argentina, while Carrière (2002) provided photographic key for the microhistological identification of some Arctic vascular plants. Such reference keys can be of great help to the researchers working on feeding habits of herbivores by reducing time and efforts to develop reference. This paper is also aimed to develop a key for identification of some plant species of Indian Trans-Himalaya by illustrating characters of stomata, trichomes and epidermal cells.

Materials and methods

Study area

The study was conducted in Rangdum valley (34° 06' 55" to 33° 56' 32" N and 75° 57' 04" E to 76° 19' 02" E) situated in the south-western part of Kargil district, Ladakh. The villages within the valley are Panikhar, Achambur, Tangole, Parkachik, Zuildo and Tashi-tonge (Fig. 1). The valley covers an area of 200 km^2 between an elevation of 3,245 and 4,550 m. The valley is bounded by Penzilla in the east, the Nun Kun peak in the South, Parkachik in the West and Wakkha Nallah in the North. A combination of arctic and desert type condition prevails in the region, where precipitation is generally in the form of snowfall and rainfall remains less than 100 mm per year.

The study area support populations of carnivores like snow leopard (*Panthera uncia)*, Himalayan brown bear (*Ursus arctos isabellinus*), Tibetan wolf (*Canis lupus*), red fox (*Vulpes vulpes*), mountain weasel (*Mustela altaica*) and stoat (*Mustela erminea*). Herbivore species of the valley include large livestock population along with wild species like Asiatic ibex (*Capra ibex*), Royle's pika (*Ochotona roylei*) and plateau

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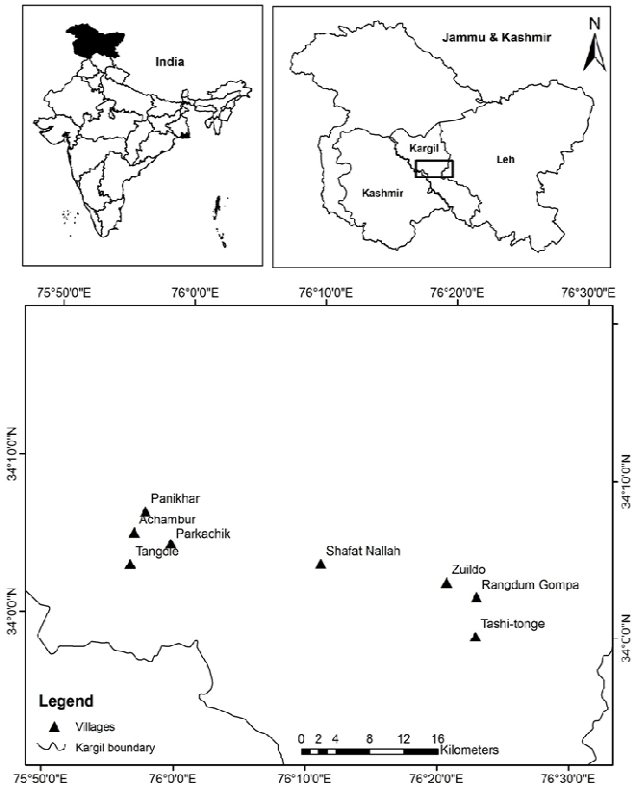

Fig. 1. Outline map of the Kargil

pika (*Ochotona curzoniae*). Long-tailed marmot (*Marmota caudata*), Silvery mountain vole (*Alticola argentatus*) and Stoliczka's mountain vole (*Alticola stoliczkanus*) represent the rodent community of the valley (Shoeb, 2011).

Methodology

All potential food plants that were considered to form the diet of herbivores in the study area were collected between May and July 2013 to prepare reference material. Plants species were identified using specialised materials on plants of Ladakh (Polunin and Stainton, 1997; Chaurasia and Singh, 1998; Chaurasia and Singh, 2001; Chaurasia *et al.,* 2008). Two different principles are available for preparing reference material to study microhistological character of plant epidermal fragments. The reference material could either (1) simulate the small and torn appearance of fragments found in animal droppings after ingestion and digestion (e.g. Ellis *et al.*, 1998), or (2) show as many identification features as possible (Johnson *et al.*, 1983b). For diet analysis, reference material must simulate the small fragment found in the sample following mastication and digestion. Hence, first principle was preferred to prepare reference materials of plants following Satakopan (1972).

A few bits of leaves were taken from each food plant sample as stem and vascular tissue are much harder to identify (Green, 1987). They were shredded coarsely and placed in a test tube. Nitric acid along with distilled water (1:3) was added to the material in the test tube. The tube containing the sample plant material was heated and agitated on a low flame for about five to ten minutes. The material was then removed from the flame and was allowed to cool, then again put above the flame for further heating. The heating was continued till the material became transparent. The duration of the warming/heating of material depended on the hardness of the plant species; hard plants required more time to become transparent, while the soft and young plants took less time. The tube was allowed to cool, the liquid drained off and washed repeatedly in distilled water. All the transparent material was poured in a petri dish. Then a few pieces of transparent plant material were taken and dehydrated by passing through the grades of absolute alcohol (AA) and distilled water (DW) mixture (AA:DW; 1:3, 1:1, 3:1) and finally through the absolute alcohol. After dehydrating, the transparent plant material was treated with safranine stain solution and then washed with absolute alcohol to remove excess colour. After this plant specimen was put on the slide and was left for few seconds till it was dried properly. Finally the specimen was mounted using Canada balsam. After mounting, the diagnostic features were recorded by photomicrography using a digital microscope. Broad anatomical features of leaf e.g. stomata, trichomes, epidermal cells and cell wall were focused and their characters were noted. Stomata were classified following Prabhakar (2004). Trichomes were categorised as glandular and non glandular (Johnson *et al.,* 1983a). Epidermal cells were categorised on the basis of their shape e.g. rectangular, angular, lobed and angular to lobed.

Results and discussions

Histological characters of 38 plants species were investigated and the data showed they are belonging to 35 genera and 21 families (Table 1). Only broad anatomical features e.g. epidermal cell, stomata and trichomes were observed. These broad anatomical characters proved to help in recognition of species (Yagueddú *et al*., 2009) and have also been used earlier to identify food plant species (Ilyas and Khan, 2004).

Four types of stomata e.g. anomocytic, isotricytic, paracytic and diacytic were observed. Anomocytic stomata were found in 23 species belonging to families Berberidaceae, Boraginaceae, Brassicaceae, Gentianaceae, Geraniaceae, Lamiaceae, Leguminosae, Onagraceae, Orobanchaceae, Papaveraceae, Polygonaceae, Ranunculaceae, Rosaceae, Scrophulariaceae. Paracytic stomata were observed in 11 species belonging to families Convolvulaceae, Colchicaceae, Amaryllidaceae, Brassicaceae, Boraginaceae, Leguminosae, Apiaceae, Ranunculaceae, Leguminosae and Asteraceae. Isotricytic stomata were observed in two species of Polygonaceae and Gentianaceae. Only one family, Caryophyllaceae, contained diacytic stomata (Table 1).

All member of family Ranunculaceae (5 species) contained anomocytic stomata, except one species *Delphinium brunonianum* which had paracytic stomata. Javed *et al.* (2012) also reported the presence of anomocytic stomata in this family. Species belonging to Leguminosae contained two types of stomata, anomocytic (3 species) and paracytic (2 species). Similar finding were also advocated in various members of this family (Saheed and Illoh, 2010). Among the members of Polygonaceae, anomocytic and isotricytic stomata were observed. Metcalfe and Chalk (1950) described that stomata in Polygonaceae were nearly always of anomocytic type in Polygonaceae, while Yasmin *et al.* (2010)

Table 1. Identification key of some plants of Kargil, Indian Trans-Himalaya

	Group A stomata present, cells present, trichomes absent			Fig. No.
A1	Anomocytic stomata			
	Angular to lobed epidermal cells	*Trifolium repens*	Leguminosae	2.a
		Pedicularis bicornuta	Orobanchaceae	2.b
	Angular with linear cell wall	*Corydalis crassifolia*	Papaveraceae	2.c
		Epilobium latifolium	Onagraceae	2.d, 2.e
		Oxyria digyna	Polygonaceae	2.f
	Lobed with sinous anticlinal	*Draba amoena*	Brassicaceae	2.g
		Thymus serpyllum	Lamiaceae	2.h
		Anemone spp.	Ranunculaceae	2.i
		Gentianella paludosa	Gentianaceae	2.j
	Lobed with wavy cell wall	*Meconopsis aculeate*	Papaveraceae	2.k, 2.l
		Podophyllum hexandrum	Berberidaceae	3.a, 3.b
A2	Isotricytic stomata			
	Angular with linear cell wall	*Rheum spiciforme*	Polygonaceae	3.c
	Lobed with weakly sinous anticlinal	*Exacum tetragonum*	Gentianaceae	3.d
A3	Paracytic stomata			
	Angular to lobed with linear cell wall	*Dactylorhiza hatagirea*	Orchidaceae	3.e
		Convolvulus arvensis	Convolvulaceae	3.f
	Rectangular with linear cell wall	*Colchicum luteum*	Colchicaceae	3.g
		Allium carolinianum	Amaryllidaceae	3.h
	Lobed with linear cell wall	*Draba gracillima*	Brassicaceae	3.i
	Group B: Stomata present, cells visible, trichomes present			
B2	Anomocytic stomata			
	Angular with linear cell wall			
	Non glandular, long, ligulate trichomes with one celled base and blunt tip	*Oxytropis lapponica*	Leguminosae	3.j
	Non glandular, long, erect, ligulate trichomes	*Geranium pratense*	Geraniaceae	3.k
	Non glandular, long, smooth ribbon like trichomes	*Anemone replica*	Ranunculaceae	3.l, 4.a
	Non glandular, medium sized trichomes with blunt tip	*Cynoglossum glochidiatum*	Boraginaceae	4.b
	Non glandular, medium sized trichomes with pointed tip and one celled base	*Ranunculus brothersii*	Ranunculaceae	4.c
	Non glandular small, nail shaped trichomes with pointed tip and broad base	*Aconogonum molle*	Polygonaceae	4.d, 4.e
	Non glandular, unicellular to segmented trichomes with pointed tip	*Marrubium vulgare*	Lamiaceae	4.f, 4.g
	Lobed with smooth linear cell wall			
	Non glandular, medium multicellular tube like trichomes	*Aquilegia fragrans*	Ranunculaceae	4.h
	Lobed with sinous anticlinal cell wall			
	Non glandular, long Jumble unicellular tube like trichomes	*Potentilla eriocarpa*	Rosaceae	4.i,
	Lobed with wavy cell wall			
	Non glandular, compound trichomes (Branched)	*Verbascum thapsus*	Scrophulariaceae	4.j
	Medium, unicellular sword like ligulate trichomes	*Delphinium cashmerianum*	Ranunculaceae	4.k, 4.l
	Glandular, multicellular trichomes with one celled base and bulbous tip	*Cicer microphyllum*	Leguminosae	5.a, 5.b
B2	Diacytic stomata			
	Angular with linear cell wall			
	Non glandular, medium, unicellular, ligulate and smooth trichomes	*Silene edgeworthii*	Caryophyllaceae	5.c, 5.d
B3	Paracytic stomata			
	Angular to lobed with linear cell wall			
	Non glandular, small to long ligulate trichomes with pointed tip and base	*Arnebia euchroma*	Boraginaceae	5.e, 5.f
	Angular with linear cell wall			
	Non glandular, medium sized wavy trichomes with pointed tip	*Melilotus officinalis*	Leguminosae	5.g, 5.h
	Lobed with wavy cell wall			
	Non glandular, ligulate trichome, sword like with pointed tip	*Selinum tenuifolium*	Apiaceae	5.i
	Lobed with sinous anticlinal			
	Glandular, Long, straight, ligulate trichomes with pointed tip and long trichomes with a pointed tip and bulbous or pot like base	*Delphinium brunonianum*	Ranunculaceae	5.j, 5.k
	Unicellular, medium sized trichomes	*Lindelofia stylosa*	Boraginaceae	5.l
	Epidermal cells not clearly visible			
	Non glandular, thin ribbon like trichomes	*Taraxacum officinale*	Asteraceae.	6.a, 6.b
B4	Stomata not clearly visible			
	Not clearly visible			
	Non glandular multicellular trichomes with pointed tip and one celled base	*Astragalus rhizanthus*	Leguminosae	6.c

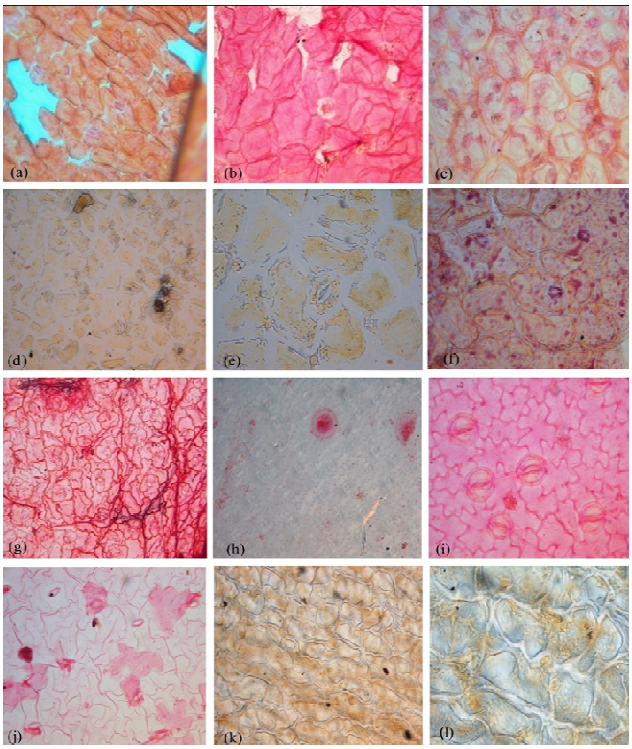

Fig. 2. a. *Trifolium repens*; b. *Pedicularis bicornuta*; c. *Corydalis crassifolia*; d. *Epilobium latifolium* (Cells); e. *Epilobium latifolium* (Stomata); f. *Oxyria digyna*; g *Draba arnoena*; h. *Thymus serpyllum*; i. *Anemone* spp.; j. *Gentianella paludosa*; k. *Meconopsis aculeate* (Cells); l. *Meconopsis aculeate* (Stomata)

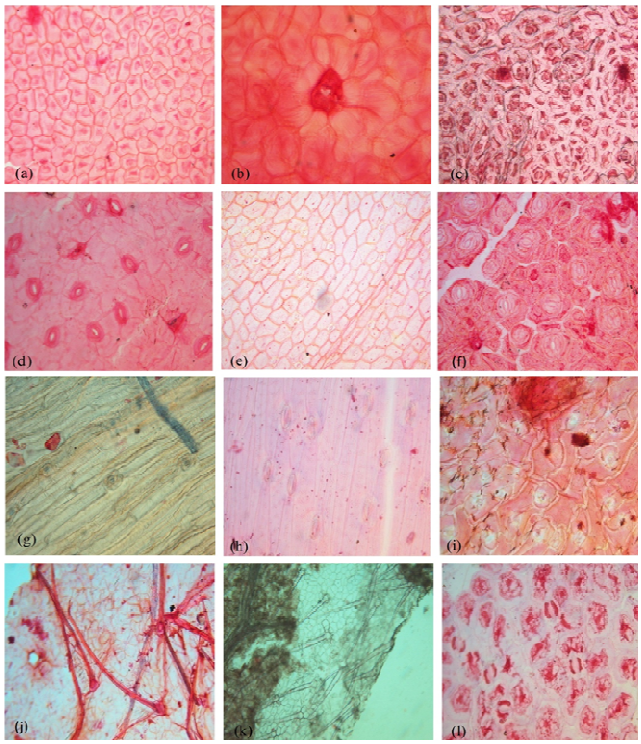

Fig. 3. a. *Podophyllum hexandrum* (Cells); b. *Podophyllum hexandrum* (Stomata); c. *Rheum spiciforme*; d. *Exacum tetragonum*; e. *Dactylorhiza hatagirea*; f. *Convolvulus arvensis*; g. *Colchicum luteum*; h. *Allium caroliniarum*; i. *Draba gracillima*; j. *Oxytropis lapponica*; k. *Geranium pratense*; l. *Anemone replica* (Cells)

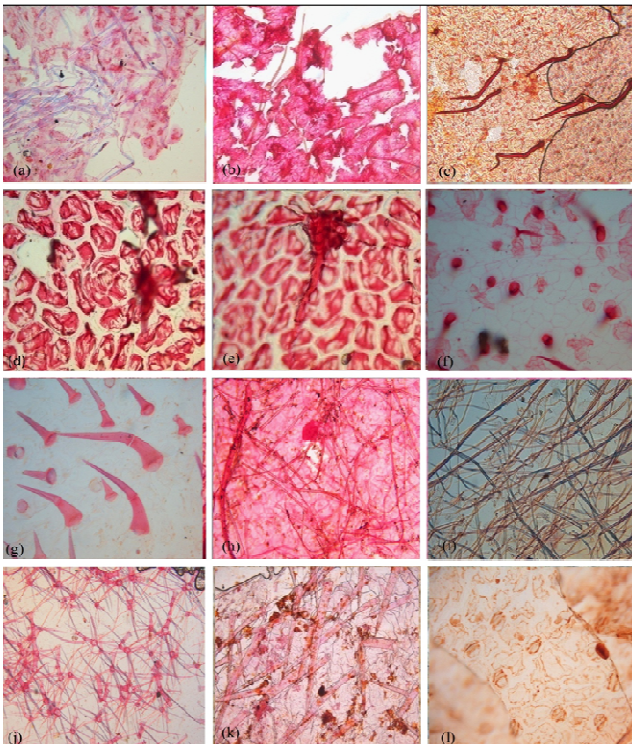

Fig. 4. a. *Anemone replica* (Trichomes); b. *Cynoglossum glochidiatum*; c. *Ranunculus brotherusii*; d. *Aconogonum molle* (Cells); e. *Aconogonum molle* (Trichome); f. *Marrubium vulgare* (Cells); g. *Marrubium vulgare* (Trichomes); h. *Aquilagia fragrans*; i. *Potentilla eriocarpa* (Trichomes); j. *Verbascum thapsus*; k. *Delphinium cashmerianum* (Trichomes); l. *Delphinium cashmerianum* (Cells and Stomata)

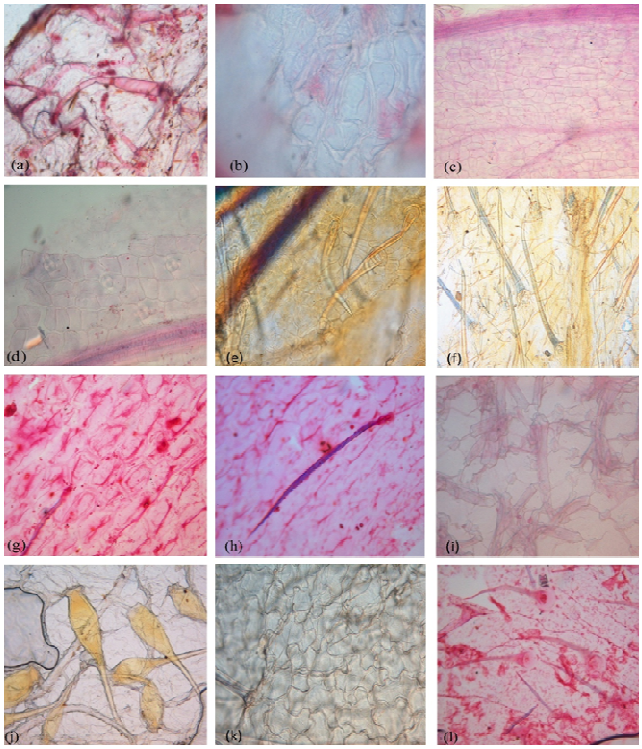

Fig. 5. a. *Cicer microphyllum* (Trichomes); b. *Cicer microphyllum* (Cells and Stomata); c. *Silene edgeworthii* (Cells); d. *Silene edgeworthii* (Stomata); e. *Arnebia euchroma* (Trichomes); f. *Arnebia euchroma* (Cell and Trichomes); g. *Melilotus officinalis* (Cells); h. *Melilotus officinalis* (Trichome); i. *Selinum tenuifolium*; j. *Delphinium brunonianum* (Trichomes); k. *Delphinium brunonianum* (Cells); l. *Lindelofia stylosa* (Trichome)

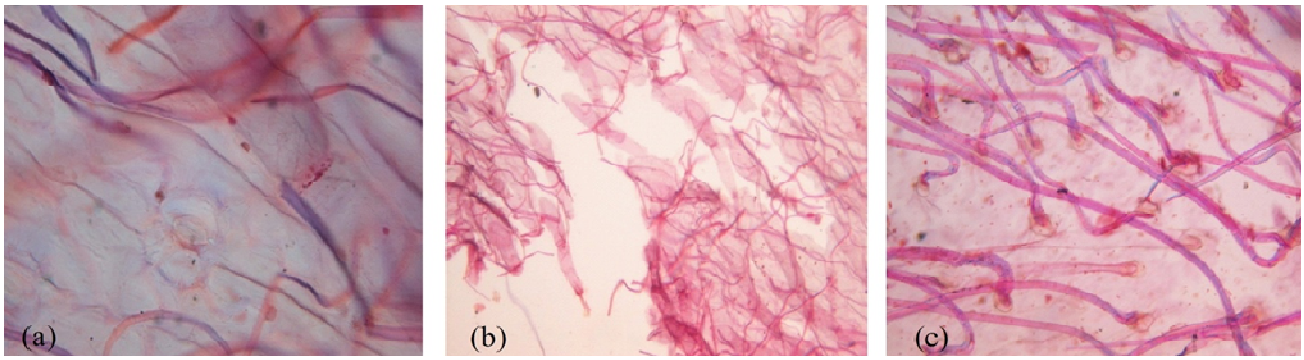

Fig. 6. a. *Taraxacum officinale* (Stomata); b. *Taraxacum officinale* (Trichomes); c. *Astragallus rhizanthus*

observed variability among stomata types within this family. Members of family Lamiaceae (2 species) and Papavaraceae had anomocytic stomata only. Brassicaceae contained anomocytic and paracytic stomata, while Gentianaceae contain anomocytic and isocytic stomata.

Four types of epidermal cells were recorded in the examined species. These were angular, angular to lobed, lobed and rectangular. The lobed epidermal cells were observed in 16 species (Figs. 1g-1l, 2a-2b, 2d, 2i, 3h-3l, 4a, 4b and 4i-4l), angular in 13 species (Figs. 1c-1f, 2c, 2j-2k, 3a-3g, 4c-4d and 4g-4h), angular to lobed in five species (Figs. 1a, 1b, 2e, 2f, 4e and 4f) and rectangular in two species (Figs. 2g and 2h). The epidermal cells were not clearly visible in two species e.g. *Taraxacum officinale* and *Astragalus rhizanthus* (Figs. 5b and 5c). Lobed epidermal cells were observed in Berberidaceae, Apiaceae, Boraginaceae, Brassicaceae, Gentianaceae, Lamiaceae, Leguminosae, Papaveraceae, Ranunculaceae, Rosaceae and Scrophulariaceae. Angular cells were present in Boraginaceae, Geraniaceae, Lamiaceae, Leguminosae, Polygonaceae, Ranunculaceae, Caryophyllaceae and Onagraceae. Angular to lobed epidermal cell were present in Leguminosae, Orobanchaceae, Boraginaceae, Convolvulaceae and Orchidaceae. Amaryllidaceae and Colchicaceae contained rectangular cells. However, in two members of Asteraceae and Leguminosae epidermal cell were not visible.

The shape and number of cells making up a trichome is the best starting point for identifying a dicot species (Johnson *et al*., 1983a). Characters such as size of the base, shape of the apex, texture of the surface and degree of tapering are useful in identification. Two types of trichomes, glandular and non glandular, were observed having various shapes, like ligulate, ribbon like, nail shaped, tube like, compound, branched and sword like (Table 1). *Cicer microphyllum* and *Delphinium brunonianum* belonging to families Leguminosae and Ranunculaceae respectively contained glandular trichomes, while the rest 36 species had non glandular trichomes. Saheed and Illoh (2010) also confirmed the occurrence of glandular trichomes in family Leguminosae. The presence of glandular trichomes in the genus *Delphinium* had earlier been reported (Torres *et al.,* 2000).

Conclusions

The purpose of this study is to provide a set of illustrations to identify plant species in the faecal remains of mammals inhabiting Kargil and Ladakh regions of Trans-Himalaya at an altitude of 3,500 m ASL and above, which is also a prime habitat of snow leopard. The Asiatic ibex, Ladakh urial, long tailed marmot, pica and livestock form the prey base of snow leopard. There are evidences of competition for food between ever increasing livestock population and naturally occurring prey species and therefore monitoring population status of prey species and their interaction with habitat is central to the management of natural prey base of snow leopard. However, the harsh climatic condition and rough terrain of the study area hamper food habits studies through direct observation in the region. Microhistology is a widely used procedure to study the food habits of mammals around the world through indirect observations. The researchers are making efforts to carry out studies on dietary spectrum of different prey species and in doing so they have to spend a great deal of time and labour to collect plant material and subsequently develop reference catalogue to identify species in the remains of animal faeces. Using this key, food habits of snow leopard's prey base can be investigated with relative ease and it is expected to save researchers' time that can be devoted in setting conservation targets.

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