

Material and Methods

3.1. Planning of the Survey

Prior to visiting the different areas, a thorough survey of the existing literatures were made to assess and estimate the species richness in the study areas. The firsthand information has been gathered from literature of (Hooker, 1888–1890, King & Pantling, 1898, Pradhan, 1976, 1979, Pearce & Cribb, 2002, Pradhan & Pradhan, 1997, Bruhl, 1926, Hara, 1966, 1971, Ohashi, 1975, Mathew, 1966, Hedge, 1990, Das & Chanda, 1988, Kumar *et al.* 2013 and other standard Orchid literature Chowdhery, 1998 and Lucksom, 2007) and prepared checklists with their habitat, altitudinal range, phenology and availability.

Map procured from the Forest Department was thoroughly studied and altitudes of the various places of the Darjeeling Himalaya were recorded. In the process, trekking routes or paths and camp sites were identified; porters were employed to carry the luggage, specially for the survey of high hills region. Different forest areas were selected with the help of past experiences and Forest Department personnel. Availability of water, health centres, villages and local markets were identified with the help of local village motor drivers, villagers, teachers, senior citizens and students. Sometime helps of traffic police were also taken to select the appropriate route for transportation during survey. The different areas of the Darjeeling district as low as ± 120 m from Siliguri-Sukuna to as high as 3660 m Sandakphu-Phalut were visited repeatedly in different seasons so as to collect and record Orchid species from the region. The Orchid species samples were randomly collected from all the accessible parts of the region during the field surveys. Initially many short field trips were undertaken in order to acclimatize the research team in the entirely different set of climatic conditions of the region, and the subsequent field

trips were arranged for longer duration upto three weeks. The floristic survey for Orchid Flora special efforts were made to identify and note their detailed information like diversity, altitudinal range, present availability status, habit, habitat, phenology, and distribution data from the herbaria as well as literature. Herbarium scrutinies were done at Herbarium of Lloyd Botanical Garden, Darjeeling; NBU, Siliguri; BSI, Gangtok and CAL, Howrah.

The planning of the surveys and field trips took into consideration the following points:

- (i) All the Sub-Divisions for the Darjeeling district were covered.
- (ii) Collection was done from different altitudinal (vertical) and lateral (horizontal) gradients.
- (iii) It represented different ecological niches showing differences with respect to exposure to light, precipitation, aspect and wind
- (iv) Included all types of habitat such as forests, deforested areas, cultivated lands, tea gardens, river sides, wastelands, villages and nurseries.
- (v) Spread over different seasons throughout the year with specified programmes during three different seasons in case of the regions in high altitudes.

3.2. Collection of Specimens and Processing of Materials

Both extensive and intensive surveys were carried out for a period of eight years between 2007 to January 2015 and recorded the distribution and habitat status of different taxa. In fact, quite a large number of places were visited during over 60 field trips. During the field trips representative specimens of Orchid Flora were collected either in flowering and fruiting condition for better identification. Repeated field trips were done covering entire altitudinal range extending from as low as ± 120 m amsl at Bidhan Nagar Siliguri to 3660 m amsl at Sandakphu throughout the year to record the exact period of flowering and fruiting. Because Orchid species were bloom almost round the year except many terrestrial species which were bloom only

in monsoon and post monsoon season. Care was taken to cause minimal damage to the wild population as far as possible. Normally, only 2-3 specimens of each species in flowering and fruiting stage were collected keeping the plant intact in the nature. Relevant field notes, freehand drawing and dissection of fresh floral parts and their measurement were made on the spot and description and data compilation task done in the laboratory. The collected specimens were then properly tagged and recorded in the Field Note Book. The tagged specimens were temporarily kept in polythene bag, the mouth of which, were closed to prevent desiccation. The specimens were brought to the field camp or the laboratory for further processing. In the field camp, the specimens after cleansing of unwanted material were poisoned by treating them with formalin. Herbarium sheets were prepared using the standard technique (Jain & Rao, 1977). The specimens were then transferred to portable plant presses and brought to the laboratory. In the laboratory they were transferred to large presses with regular changes of blotting paper to dry them under pressure. On completion of drying the specimens were treated with 6% Mercuric Chloride (HgCl_2) solution prepared in rectified spirit (Ethanol), dried again under pressure using blotting paper.

Photographs of the specimens including their natural habitats along with close up view were taken prior to collection. Photographs of some specimens were also taken from floral nurseries, botanical garden and research institution. Digital display with measurement of floral parts of some collected specimens were also prepared with the help of Adobe Photoshop CS 8.0 version and save as JPEG format.

Dried specimens were then mounted on standard herbarium sheets (41.5×28 cm) using adhesive glue and stitched with threads. A herbarium label (15.5×10 cm) was pasted at the bottom right hand corner of the sheet. Field notes such as the collection number, date of collection, name of the plant, family, local names (wherever available), habitat, habit, locality, altitude, flower colour, local availability status, information on medicinal uses etc were written from the field notes to the herbarium labels. Dried smaller parts such as floral parts, fruits, etc. that could not be settled on the herbarium sheet were placed in paper packed or envelopes and affixed to the left hand top corner of the herbarium sheet. The specimens were then stored temporarily in wooden cabinets in the laboratory for further study.

3.3. The Field Note Book

All the relevant and necessary information related to the representative specimen such as the collection number, location, altitude, date of collection, availability status, habitat, habit, flower colour, flowering and fruiting periods, aroma, peculiarities, local name, uses etc. were recorded in the Field Note Book then transferred to the labels of the herbarium sheets. All information regarding the available Orchid species including those in floral nurseries and research institutes were made during the visit of these sites. After the conclusion of the dissertation, the Field Note Book will be deposited at the Herbarium in Department of Botany, St. Joseph's College, North Point, Darjeeling.

3.4. Identification

Preliminary identification of specimens were mainly done at Plant Taxonomy Laboratory, NBU, Department of Botany of St. Joseph's College and Cluny Women's College, using all available literature and matching them with available predetermined specimens in the Lloyd Botanical Garden Herbarium; National Research Centre for Orchids, Indian Council of Agriculture Research, Darjeeling Campus and Pakyong, Sikkim. For this wide range of literature (floras, monographs, revisions etc.) were consulted including Hooker (1849-1851, 1872-1897), Hara (1966, 1971), Hara *et al.* (1982), Ohashi (1975), Pradhan (1976, 1979), Pradhan & Pradhan (1997), Pearce & Cribb (2002), Lucksom (2007), Misra (2014). These were then confirmed by matching them at Central National Herbarium, Howrah (CAL). The nomenclature of the Orchid species were checked and updated by consulting recent works.

3.5. Deposition and Storing of the Specimen

After completion of the work, the first and second sets of specimens except the type specimens of the new variety that was discovered during the present study have been deposited and stored in the herbarium of the Department of Botany, St. Joseph's College, North Point, Darjeeling and the third set in the herbarium of Taxonomy and Ethnobiology Research Laboratory, Cluny Womens' College, Kalimpong. The type specimens of the new variety *Geodorum densiflorum* (Lamk.)

Schltr. var. *kalimpongense* Rajendra Yonzone, D. Lama, R. B. Bhujel & Samuel Rai. Holotype No.1213A (CAL) has been deposited in the Central National Herbarium, Botanical Survey of India, Indian Botanical Garden, Howrah and its Isotype 1213B (NBU) at herbarium of Department of Botany, North Bengal University and another Isotype 1213C (CWC) in the herbarium of Taxonomy and Ethnobiology Research Laboratory, Cluny Womens' College, Kalimpong now is shifted to herbarium of St. Joseph's College, SJC. Digital images of type specimens were taken and conserved for future course of action.

3.6. Utilization Studies

A review of the Orchid species use for medicinal and other ethnobotanical purposes served as the secondary source of data. Data on Orchids having ornamental importance were collected through visits made to all the different Orchid nurseries in the study area. Data on the hybrid varieties along with the wild species used for commerce were obtained during such visits

3.7. Citation of references

For each taxon, reference to the protologues have been provided after the author citation. After this, major taxonomic references related to the taxon have been cited in this work. Following abbreviations have been used in the citation for the well-known books, journals, periodicals, etc. so as to make the presentation shorter.

Important Books

- FBI : *Flora of British India* by J.D. Hooker (ed.)
FEH : *Flora of Eastern Himalaya* by H. Hara & H. Ohashi (ed.)
FB : *Flora of Bhutan, Orchids of Bhutan* by Pearce & Cribb.

Important Journals

- Bull. Bot. Surv. India : *Bulletin of Botanical Survey of India*
J. Bomb. Nat. Hist. Soc. : *Journal of Bombay Natural History Society*
J. Econ. Tax. Bot. : *Journal of Economic and Taxonomic Botany*
J. Jap. Bot. : *Journal of Japanese Botany*
Kew Bull. : *Kew Bulletin*

Am. J. Bot.	: <i>American Journal of Botany</i>
Ind. J. Forestry	: <i>Indian Journal of Forestry</i>
Amer. Orch. Soc. Bull.	: <i>American Orchid Soc. Bulletin</i>
Bot. Journal	: <i>Botany Journal</i>
J. Phytol. Res.	: <i>Journal of Phytol Resesearch</i>
Environ. Boil. Conserve.	: <i>Environment Biology Conservation</i>
Linn. Soc. Bot. J.	: <i>Linnius Society Botany Journal</i>
Ind. For. Res. Ser.	: <i>Indian Forest Research Survey</i>
J. Orchid Soc. India	: <i>Journal of Orchid Society of India</i>
Acta Phytotax. Sin.	: <i>Acta Phytotaxonomy Sin</i>
J. Assam Sci. Soc.	: <i>Journal of Assam Science Society</i>
Annals Missouri Bot. Gard.	: <i>Annals of Missouri Botanical Garden</i>
Bot. Mus. Leafl.	: <i>Botanical Museum Leaflets</i>
Nordic J. Bot.	: <i>Nordic Journal of Botany</i>
Bull. Medico-Ethnobot. Res.	: <i>Bulletin of Medical Ethnobotany Research</i>
Orchid Rev.	: <i>Orchid Review</i>
Ind. J. Forestry	: <i>Indian Journal of Forestry</i>
Ann. Tsukuba Bot. Gard.	: <i>Annals of Tsukuba Bot. Gard.</i>
Ind. Forester	: <i>Indian Forester</i>
Proc. Ind. Acad. Sci.	: <i>Proceeding of Indian Academy of Sciences</i>
J. Indian Bot. Soc.	: <i>Journal of Indian Botanical Society</i>
Plant Conservation Bull.	: <i>Plant Conservation Bulletin</i>
J. Asiat. Soc. Bengal	: <i>Journal of Asiatic Society of Bengal</i>
Ann. Roy. Bot. Gard.	: <i>Annals of Botanical Garden</i>
Bot. Notiser.	: <i>Botanical Notiser</i>
Bot. Reg.	: <i>Botanical Register</i>
J. Proc. Linn. Soc. Bot.	: <i>Journal of Proceeding Linneus Society Botany</i>
Plant Syst. Evol.	: <i>Plant System Evolution</i>
Blumea Suppl.	: <i>Blumea Supplements</i>
Indian. J. Tradn. Knowl.	: <i>Indian Journal of Traditional Knowledge</i>
J. Orissa Bot. Soc.	: <i>Journal of Orissa Botanical Society</i>
Norw. J. Bot.	: <i>Norway Journal of Botany</i>
Austral. Orchid Rev.	: <i>Australian Orchid Review</i>

J. Non. Timb. Forest Prod.	: <i>Journal of Non Timber Forest Products</i>
Proc. Him. Geol. Sem.	: <i>Proceeding of Himalayan Geological Seminar</i>
Edinb. J. Bot.	: <i>Edinburg Journal of Botany</i>
Bull. Arunachal For. Res.	: <i>Bulletin of Arunachal Forest Research</i>
Econ. Bot.	: <i>Economic Botany</i>
Bot. Notiser	: <i>Botany Notiser</i>
J. Linn. Soc.	: <i>Journal of Linneus Society</i>
Dansk Bot. Ark.	: <i>Dansk Botanisk Arkiv</i>
Opera Bot.	: <i>Opera Botany</i>
Malayan Nat. J.	: <i>Malayan Natural Journal</i>
Bull. Jard. Bot. Buitenz.	: <i>Bulletin of Jarden Botany Buitenz</i>
Bot. Mag.	: <i>Botany Magazine</i>
Curr. Sci.	: <i>Current Science</i> :
Acta Bot. Neerl.	: <i>Acta Botanica Neerlandica</i>
Res. Bull. (Sci.) Punj. Univ.	: <i>Research Bulletin (Science) Punjab University</i>
McAllen Int. Orchid Soc. J.	: <i>The McAllen International Orchid Society Journal</i>
Int. J. Pharm. Lif. Sci.	: <i>International Journal of Pharm. and Life Sciences</i>
J. Env. Eco.	: <i>Journal of Environment and Ecology,</i>
Biosci. Discov.	: <i>Bioscience Discovery</i>
Int. J. Pharm. Biosc.	: <i>International Journal of Pharm. and Biosciences</i>
Asian J. Pharm. Lif. Sci.	: <i>Asian Journal of Pharm. and Life Sciences</i>
J. Golden Res. Thoug.	: <i>Journal of Golden Research Thought</i>
Ind. Hort. J.	: <i>Indian Horticulture Journal</i>
J. Interacad.	: <i>Interacademia Journal of Interacademia</i>
Nat. J. Lif. Sci.	: <i>National Journal of Life Science.</i>

Other abbreviations used in the enumeration

<i>c.</i>	: about (<i>circa</i>)
<i>nom. Illeg.</i>	: illegitimate name (<i>nomen illegitimum</i>)
<i>nom. nud.</i>	: <i>nomen nudum</i>
<i>sensu</i>	: in the sense of author indicated and not as originally intended
<i>ssp./subsp.</i>	: sub-species
<i>var.</i>	: variety
<i>var. nov.</i>	: new variety
<i>fig.</i>	: figure.

3.8. Methods of Enumeration

The detail morphology studies of each specimen were undertaken at both the Department of Botany, St. Joseph's College, North Point, Darjeeling and Taxonomy and Ethnobiology Research Laboratory, Cluny Womens' College, Kalimpong. Artificial key for the recorded genera have been provided. Similar keys have been provided for each genus recorded with more than one species. Keys for varieties were included within the species key.

Correct and updated nomenclature have been used for all the taxa after consulting relevant literatures, that included flora, monographs and latest nomenclature from IPNI web sites. Protologues have been consulted to decipher nomenclatural problems and identity of doubtful specimens. Taxonomic descriptions highlighting the flowering and fruiting, specimen cited, date of collection, present availability status, local distribution and general distribution (from available literature or study of deposited specimen in different Herbaria) of all taxa have been provided. Synonyms along with basionym from important literatures have been provided for each specimen.

The metric unit system (cm and mm) has been used with the first figure in the dimension indicating the length and the second the breadth *e.g.*, dimension of leaves is given as 9 × 5 cm; where only a single dimension is given it indicates the length or the height *e.g.* anther 2 mm.

Photographs on habitat, different interesting and/or rare species of Orchids and herbarium species have been provided along with the line drawing and digital photo display of many of the species. Floral morphological and colour variation of species were provided along with colour photo plates.

The basis of framing up of present Orchid Flora of Darjeeling Himalaya is the classification presented by Pearce & Cribb (2002). However, with the availability of recent literature including Lucksom (2007), Chowdhery & Agrawala (2013), Misra (2014) facilitated the work. As far as possible up-to-date nomenclature of plants has been used in terms of the provisions of ICBN. Proper artificial dichotomous keys were provided for the identification of genera, species and varietal categories. The legitimate correct name of the species is printed in italic-bold and that is followed by basionym and synonym(s), if any, in italics only. The present availability status of the species in its natural habitat, flowering and fruiting, specimen cited, date of collection, collection number, altitudinal range and the local and general distribution for each taxa have also been clearly indicated.