Collection and trade of wild-harvested orchids in Nepal
Abishkar Subedi1,2, Bimal Kunwar3, Young Choi4, Yuntao Dai4, Tinde van Andel1, Ram P. Chaudhary3, Hugo J de Boer1,5 and Barbara Gravendeel1*

Abstract
Background: Wild orchids are illegally harvested and traded in Nepal for use in local traditional medicine, horticulture, and international trade. This study aims to: 1) identify the diversity of species of wild orchids in trade in Nepal; 2) study the chain of commercialization from collector to client and/or export; 3) map traditional knowledge and medicinal use of orchids; and 4) integrate the collected data to propose a more sustainable approach to orchid conservation in Nepal.

Methods: Trade, species diversity, and traditional use of wild-harvested orchids were documented during field surveys of markets and through interviews. Trade volumes and approximate income were estimated based on surveys and current market prices. Orchid material samples were identified to species level using a combination of morphology and DNA barcoding.

Results: Orchid trade is a long tradition, and illegal export to China, India and Hong Kong is rife. Estimates show that 9.4 tons of wild orchids were illegally traded from the study sites during 2008/2009. A total of 60 species of wild orchids were reported to be used in traditional medicinal practices to cure at least 38 different ailments, including energizers, aphrodisiacs and treatments of burnt skin, fractured or dislocated bones, headaches, fever and wounds. DNA barcoding successfully identified orchid material to species level that remained sterile after culturing.

Conclusions: Collection of wild orchids was found to be widespread in Nepal, but illegal trade is threatening many species in the wild. Establishment of small-scale sustainable orchid breeding enterprises could be a valuable alternative for the production of medicinal orchids for local communities. Critically endangered species should be placed on CITES Appendix I to provide extra protection to those species. DNA barcoding is an effective method for species identification and monitoring of illegal cross-border trade.

Keywords: Commercialization, DNA barcoding, Orchids, CITES, Traditional medicine

Introduction
Medicinal orchids of Nepal
Orchids are long known for their medicinal value. It is believed that the Chinese were the first to cultivate, describe and use orchids as early as 200 BC [1-3]. In the Indian subcontinent, the Ayurvedic medicinal system uses formulations based on orchid species. Ashtavarga, a group of eight medicinal plants includes four orchid ingredients, Habenaria edgeworthii Hook.f. ex Collet, Habenaria intermedia, Malaxis acuminata D. Don, and M. muscifera* [3-5].

Wild orchids in Nepal are popularly known by the vernacular name Sungava or Sunakhari, which refers to their shiny yellow pseudobulbs. A total of 377 species belonging to 100 genera have been reported from Nepal, including 12 endemic species [6]. Due to inaccessibility of modern health care facilities, about 80% of the population of the country still depends on a wide range of locally available medicinal plants for their basic primary healthcare [7]. By 2004, over 590 studies related to ethnobotany in Nepal had been published [8]. Most of these studies lack detailed knowledge on local therapeutic uses of Nepalese orchids or describe very few
cases only. In contrast to many other plant families, a comprehensive and detailed study of medicinal orchids in Nepal is still lacking [9].

Trade of wild orchids for medicinal and other commercial purposes in Nepal

Trade in wild harvested orchids threatens local biodiversity due to overexploitation and habitat destruction [10]. Vaidya et al. [11] reported that around five tons of tubers of *Orchis latifolia* L. were harvested every year in Nepal to prepare ‘Salep’ for export at an approximate value of USD 900 per ton. The export of valuable medicinal species such as *Dactylorhiza hatagirea* and *Gastrodia elata* from Nepal to China began in the late 1990s, and continues until today despite attempts to ban trade in these endangered species [12]. Bailes [13] reported in 1985 that in eastern Nepal about 100 trucks with 8 tons capacity each, loaded with wild-collected orchids were shipped to India illegally to prepare various Ayurvedic products. These cases all show that wild orchids from Nepal are popular trade items. The unsustainable use of orchid resources and illegal export of commercially important species causes biodiversity erosion and revenue loss to Nepal [12].

At present, it is difficult to come up with alternative, more sustainable methods to exploit wild Nepalese orchids. Efforts are hampered by three main problems. First of all, many illegally traded orchids cannot be identified to species level as they are often collected and traded sterile. Secondly, expertise in Nepal for artificial propagation of wild orchids is still very limited. Thirdly, surprisingly few studies from Nepal have been published on the trade of wild-collected orchids despite growing concerns on overexploitation and illegal national and cross-border trade [14,15].

This study aims to: i) identify the diversity of species in illegal trade in Nepal; ii) map traditional knowledge and medicinal use of wild orchids; iii) study the chain of commercialization from collector to client and/or export; iv) evaluate the efficacy DNA barcoding for orchid identification; and v) integrate the collected data to propose a more sustainable approach to orchid conservation.

Materials and methods

Study areas

Surveys were carried out in three villages in Makwanpur district of central Nepal: Agra, Gogane, and Manahari, and in two markets in the Kathmandu valley: Dakshinkali and Godavari (Figure 1). Surveys were carried out in February-March 2008 and August-November 2009.

Data collection

Primary data were collected from interviews with local villagers involved in orchid collection, middlemen, vendors, local traders and district forest officials. We used a semi-structured questionnaire for the interviews. A detailed inventory of medicinal orchids and their uses in Nepal was prepared by a literature study. Additional information was collected through key informant interviews with local plant healers at the study sites after agreeing to a prior informed consent (PIC) on mutually agreed terms (MAT). A total of 31 people were interviewed.
Plant identification
Wild collected flowering orchids were identified using standard literature [16-18] and cross-referenced with herbarium specimens deposited at Tribhuvan University Central Herbarium (TUCH). Sterile plants were purchased, and small cuttings were cultivated to bloom in an experimental garden in the vicinity of Pokhara for subsequent identification to species level. If no flowering could be initiated, DNA barcoding was applied. Voucher specimens of all orchid species are deposited at TUCH (Table 1).

DNA barcoding of illegally traded orchids
DNA barcoding is a powerful tool used to control trade in species placed on either CITES Appendix I or II [27-29] and to trace cross-border wildlife crime [30].

Purchased orchid samples that were unidentifiable by morphology, and failed to flower in the experimental garden were selected for DNA barcoding identification at the Laboratory of Plant Systematics, Central Department of Botany, Tribhuvan University, Kathmandu. The following methods and materials were applied: Material was ground to powder in a mortar with liquid nitrogen. Total genomic DNA was extracted from 40-100 mg of powder using the DNeasy Plant mini kit (Qiagen Inc.). Parts of the plastid matK gene and nuclear nrITS regions were amplified using the primers-19F; 881R, 731; 2R, and 101F; 102R, respectively [31]. Polymerase chain reactions were carried out on a PXE 0.2 Thermocycler (Applied Biosystems) in a 25 μl volume containing 0.1-50 ng of genomic DNA, 0.1 M of each primer, 10 μM of each dNTP, Qiagen PCR buffer (50 mM KCl, 10 mM Tris–HCl pH 8.7, 1.5 mM MgCl2), and 1.5 units of Taq DNA polymerase (Qiagen, Inc.). The thermal cycling profile started with a 5 min denaturation step of 94°C, then comprised 35 cycles each with 20 s denaturation at 94°C, 20 sec annealing at 48-51°C and 45 s elongation at 72°C, and the program ended with 5 min extension at 72°C. Amplification products were separated on an ABI 3730xl automated sequencer by Macrogen (South Korea) using standard dye-terminator chemistry following the manufacturers protocols (Applied Biosystems). Complementary strands were assembled and edited with Sequencer version 4.01 (Gene Codes Corporation).

NCBI GenBank BLAST searches [32] were used to match DNA sequences generated in this study with those already deposited in the database. Species names were assigned only in cases of a sequence similarity of 100%. DNA sequences generated were submitted to GenBank (accessions JF422074 - JF422082; Table 1).

Results and discussion
Medicinal orchids of Nepal
Sixty species were reported to be used for 38 different ailments (Table 1), representing 15% of the total number of orchids described from Nepal. A recent literature review by Acharya and Rokaya [9] found 82 medicinal orchid species reported from Nepal, 47 of those were also found in this study focusing on a limited area. Hossain [5] in a global literature review on medicinal orchids shows that a total of 129 species are being used for different therapeutic purposes. Eighty-two medicinally used orchids in Nepal imply that the diversity of traditional orchid species in the country is exceptionally high. The high number could be explained by the fact that our study is the first ethnobotanical survey focusing solely on orchids. In addition, the use of DNA barcoding enabled more accurate species identification of sterile material than can be achieved in morphological studies (Table 2).

Acharya and Rokaya [9] recorded 82 medicinal orchid species for Nepal, 34 of which were not recorded in this study, whereas this study recorded 12 additional species. The combined total of both studies comes to 94 medicinally used orchid species. The majority of these are epiphytes, a fourth is terrestrial, and just a few are lithophytes. Coelogynæ, Dendrobiæm, Cymbidium, Bulbophylum, Habenaria, Malaxis and Pholidota are the genera of which most species are being used as traditional medicines. Other reported uses of these medicinal orchids are fodder (25), vegetables (6) and ritual and ceremonial uses (6) [10].

The most common vernacular names for orchids are Sungava and Sunakhari. In addition 23 vernacular names for orchids were recorded to be used by local communities in different parts of Nepal (Table 1). Among these most common are: Thuur or Thurja (moss-like plants growing on tree trunks), Parajivi (parasitic plant), Bankera (pseudobulbs shaped like a wild banana), Banadiwa (ginger-like), Chandigava (silver-coloured flowers), Shaktigumba (pseudobulbs providing energy) and Chadepuhl (flowers inducing vomiting). The vernacular names reflect the vast knowledge of local communities with regard to orchid growing habits, habitats and their potential uses.

Major reported local uses include aphrodisiacs, energizers, and treatments of skin burns, fractured or dislocated bones, headaches, fever, and wounds. Other uses include insect repellent, blood purifier, skin fungi, snake and scorpion bite antidote, inducement of abortions, and recovery from childbirth. Orchids are mainly used as paste, powder or juice, solely or mixed with milk, honey or wheat flour. Orchid extracts are either consumed orally or applied externally. A widespread local use of Coelogynæ is to eat freshly cut slices of the pseudobulb as a thirst quencher.
| Scientific name voucher numbera | Local name | Parts usedb | Traditional use | Reference | Commercial trade |
|--------------------------------|------------|-------------|-----------------|-----------|------------------|
| Acampe praemorsa (Roxb.) Blatt. & McCann (syn. Acampe papillosa (Lindl.) Lindl.) Subedi 170 | Parajivi, Rasna (Sanskrit) | Ra | Powder used in treating rheumatism and for cooling effect. | This study | Medicinal |
| Aerides multiflora Roxb. Kunwar 171 | Parajivi, Thuur | Lf | Powder used in tonic preparation. | This study | Floricultural, medicinal |
| Aerides odorata Lour. Subedi 172 | Parajivi | Lf | Paste of leaves used externally to treat wounds. | This study | Floricultural, medicinal |
| Brachycorythis obcordata (Lindl.) Summerh. Subedi 150 | Gamdol | Tb | Powder mixed with milk and consumed as tonic. | [19] | Medicinal |
| Bulbophyllum careyanum (Hook.) Spreng. Subedi 220 | Banharchul, Thuur, Parajivi | Lf, Pb | Fresh pulp of pseudobulbs externally applied to burns. Powder of leaves used with honey to induce abortions within 3 months of pregnancy and stimulate recovery from childbirth. | [10], This study | Medicinal |
| Bulbophyllum leopardinum (Wall.) Lindl. ex Wall. Subedi 221 | Thuur, Parajivi | Lf, Pb | Fresh pulp or juice externally applied to burns. | This study | Medicinal |
| Bulbophyllum odoratissimum (Sm.) Lindl. ex Hook. f. Subedi 370 | Thurjo | Ep | Powder used in treating tuberculosis, chronic inflammation and fractures. | [20] | Medicinal |
| Calanthe sylvatica (Thouars) Lindl. Subedi 153 | Pakha phul | Fl | Juice applied to stop nosebleeds. | [11] | Floricultural, medicinal |
| Calanthe plantaginea (Thouars) Lindl. Subedi 153 | Ban aduwa | Rz | Dry powder consumed with milk as tonic and as aphrodisiac. | This study | Floricultural, medicinal |
| Calanthe puberula Lindl. Subedi 223 | Ban aduwa | Rz | Dry powder consumed with milk as tonic and as aphrodisiac. | This study | Floricultural, medicinal |
| Coelogyne corymbosa Lindl. Subedi 375 | Chadigava | Pb | Paste applied to the forehead to relieve headaches, fresh juice applied to burns as analgesic. | [7,11,21] | Floricultural, medicinal |
| Coelogyne cristata Lindl. Subedi 224 | Chadigava, Bankera | Pb | Freshly collected paste or juice consumed to relieve headaches, fever and for indigestion. Pulp applied to burnt skin. Juice also applied to skin boils and wounded hooves of cattle. | [1,10,21,22], This study | Floricultural, medicinal |
| Coelogyne fimbrista Lindl. Subedi 225 | Jiivanti (Sanskrit) | Pb | Powder used in tonic preparation. | This study | Floricultural, medicinal |
| Coelogyne flaccida Lindl. Subedi 301 | Chadigava | Pb | Paste applied externally or consumed to relieve frontal headaches. Juice taken for indigestion. | [7,21] | Floricultural, medicinal |
| Coelogyne fuscescens Lindl. Subedi 312 | Bankera | Pb | Paste applied externally or consumed to relieve headaches, fever and stomach ache. Paste applied externally on burns. | This study | Floricultural, medicinal |
| Coelogyne nitida (Wall. ex D.Don) Lindl. Subedi 226 | Banlasun, Thuur | Pb | Paste consumed against headaches and fever. Paste applied externally on burns. | This study | Floricultural, medicinal |
| Coelogyne prolifera Lindl. Subedi 227 | Thuur | Pb | Paste consumed against headaches and fever. Paste applied externally on burns. | [10] | Floricultural, medicinal |
| Coelogyne stricta (D.Don) Schltr. Subedi 314 | Banpyaj | Pb | Paste applied externally against headaches and fever. | This study | Floricultural, medicinal |
| Crepidium acuminatum (D.Don) Szlach. (syn. Malaxis acuminata D. Don) Subedi 321 | Gachno, Gavdamala | Ra, Pb | Powder of roots used against burning sensations, to treat fever and to stop bleeding. | This study | Medicinal |
| Cymbidium aloifolium (L.) Sw. Subedi 228 | Banharchul, Kamaru, Harjor | Ep | Dried powder used as tonic against diarrhea. Fresh paste applied externally over fractured or dislocated bones. | [10,23] | Floricultural, medicinal |
| Cymbidium elegans Lindl. Kunwar 123 | Thuur | Ra, Pb | Fresh juice of pseudobulb consumed to relieve fever. Boiled root juice fed livestock suffering from cold. | [21,22] | Floricultural, medicinal |
| Cymbidium iridioide D. Don Subedi 315 | Thuur | Pb, Lf | Powder of pseudobulb consumed as tonic. Leaf juice applied externally to stimulate blood clotting in deep wounds. | [11], This study | Floricultural, medicinal |
| Orchid Species | Local Name | Part Used | Uses |
|---------------|------------|-----------|------|
| Cypripedium himalaicum Rolfe | Khujukpa | Ep | Powder and juice consumed for urine retention, against kidney stones, heart disease, chest disorders and coughs. |
| Dactylorhiza hatagirea (D.Don) Soo | Paanchaunle, Hatajadi | Tb | Paste consumed against fever. Powder used topically as hemostatic, or to heal fractures. Decoction consumed against intestinal pain. Tuber, eaten raw or as tonic, or mixed with honey or milk used as stimulant. |
| Dendrobium amoenum Wall. ex Lindl. Subedi | Thuur | Pb | Fresh paste applied topically on burnt skin and dislocated bones. |
| Dendrobium densiflorum Lindl. Subedi | Sungava | Pb | Fresh pulp applied to boils and pimples. |
| Dendrobium eriflorum Griff. Kunwar | Thurjo | Pb | Paste mixed with wheat flour and applied on dislocated or fractured bones. Dried powder used as tonic. |
| Dendrobium heterocarpum Wall. ex Lindl. Subedi | Ta | Pb | Paste mixed with wheat flour and applied on fractured or dislocated bones. |
| Dendrobium longicornu Lindl. Subedi | Kause | Ra, Pb | Juice of stems is consumed against fever. Boiled root fed to livestock suffering from coughs. |
| Dendrobium transparens Wall. ex Lindl. Subedi | Parajivi, Thuur | Pb | Paste used on fractured or dislocated bones. |
| Dienia cylindrostachya Lindl. (syn. Malaxis cylindrostachya (Lindl.) Kuntze) Kunwar | Pb | Powder used as tonic. |
| Epipactis helleborine (L.) Crantz | Ra | Juice consumed to cure insanity and gout. |
| Eria spicata (D.Don) Hand-Mazz. Subedi | Parajivi | Pb | Powder consumed during stomach ache, paste applied externally against headaches. |
| Eulophia dabia (D.Don) Hochr. Kunwar | Hatti paila | Rz | Powder consumed against coughs and heart trouble, also used as tonic and appetizer. |
| Eulophia spectabilis (Dennst.) Suresh (syn. Eulophia nuda Lindl.) Kunwar | Amarkand | Tb | Powder used against worm infestation, scrofula, blood disorders, bronchitis and as appetizer. |
| Flickingeria fugax (Rchb.f.) Seidenf. Kunwar | Jiwanti | Ep | Powder used as tonic against general debility and as stimulant. |
| Flickingeria macraei (Lindl.) Seidenf. Subedi | Jiwanti | Ep | Paste used against snake bites, general debility, as stimulant and demulcent. |
| Gastrodia elata Blume | Tb | | Dried powder used as tonic for treating headaches. |
| Gymnadenia orchidis Lindl. Kunwar | Ep | | Powder used to treat gastric, urine and liver disorders. |
| Habenaria intermedia D.Don Subedi | Riddhi | Ra, Lf | Powder used for blood diseases. |
| Habenaria pectinata D.Don Kunwar | Seto musli | Tb, Lf | Leaf juice applied on snake bites. Tuber used against arthritis. |
| Luisia trichorrhiza (Hook.) Blume Subedi | Arjona | Lf | Paste applied externally to treat muscular pain. |
| Luisia tristis (G. Forst.) Hook.f. (syn Luisia zeylanica Lindl.) Subedi | Borjihaar Ep | | Juice used for treating chronic wounds. |
| Malaxis muscifera (Lindl.) Kuntze Kunwar | Jivaka | Pb | Paste applied during diathesis, burning sensation, fever, on sores and as tonic. |
| Otochilus altus Lindl. Subedi | Aankhle laharo Ep | | Powder used as tonic. |
| Otochilus lancilabius Seidenf. Kunwar | Aankhle laharo Ep | | Paste applied to fractured and dislocated bones. |
| Papilionanthe tenes (Roxb.) Schlr. Subedi | Harjor, Thurjo Pb, Lf | | Paste externally applied to treat dislocated bones. |
| Pholidota articulata Lindl. Subedi | Hadjor | Ep | Paste applied on fractured bones and consumed as tonic. |
Wild orchid species in trade

From the total of 60 species of wild orchids recorded as traded from the study sites, 28 species were exported both for medicinal and floricultural purposes, and 32 species for medicinal purposes only. Multiple use-values exacerbate the threat of overexploitation for these species. For medicinal purposes, species belonging to the genera *Acampe*, *Aerides*, *Coelogyne*, *Crepidium*, *Dactylorhiza*, *Dendrobium*, *Gastrodia*, *Eulophia*, *Flickingeria*, *Otochilus*, *Pholidota*, *Satyrium* and *Vanda* are most heavily exploited based on the number of times these were cited by the respondents. *Acampe praemorsa*, *Aerides multiflora*, *Bulbophyllum careyanum*, *Coelogyne cristata*, *C. nitida*, *Crepidium acuminatum*, *Dactylorhiza hatagirea*, *Dendrobium aphyllum*, *D. crepidatum*, *D. eriiflorum*, *D. moschatum*, *Eulophia spectabilis*, *Flickingeria fugax*, *Gastrodia elata*, *Otochilus albus*, *Pholidota pallida*, *Ph. imbricata* and *Vanda cristata* are the most wanted species.

Table 2 DNA barcoding of sterile medicinal orchids

| Medicinal orchid | DNA barcoding |
|------------------|--------------|
| Scientific name  | Voucher number | Marker | Voucher sequence | Reference sequence | Citation for reference sequence |
| *Coelogyne cristata* Lindl. Subedi 224 | nrITS, matK | JF422077, AF302707 | [31] |
| *Coelogyne fimbriata* Lindl. Subedi 225 | nrITS, matK | JF422074, AF302745 | [31] |
| *Coelogyne stricta* (D. Don) Schltr.; Subedi 314 | nrITS, matK | JF422075, AF302757 | [31] |
| *Pleione praecox* D. Don Kunwar 109 | nrITS, matK | JF422076, AF461491 | [33] |

Vouchers are deposited at TUCH; ‡Fl, flowers; Lf, leaves; Pb, pseudobulbs; Ra, roots; Rz, rhizome; Tb, tubers; Ep, entire plant.
for rituals. Coelogyne cristata, Co. flaccida, Co. nitida, Cymbidium iridioides, Dendrobium densiflorum and Vanda cristata are most widely exploited as cut flowers.

Orchid collectors and collecting practices
Collecting wild orchids was predominantly done by local youths, women and children, and a total of 42 collectors were recorded across the study sites. At Dakshinkali, at least 18 local collectors were involved in orchid collection, supplying the 10 local vendors with orchids. The vendors themselves were sometimes also involved in collecting wild orchids. Some local collectors reported to have been involved in orchid collection and selling for more than 25 years.

Medicinal orchids were usually harvested from December up to April with a peak period from January to March. For floriculture, the collection period was found to be throughout the year depending on the availability of flowering individuals. Collectors reported to search far and wide for orchids, frequently traveling more than 10 km on foot through the forest. Epiphytic orchids growing high up in tree canopies were collected by felling the trees if feasible, and preferentially collected in clumps. Terrestrial orchids were collected by unearthing the tubers to take the entire plant.

Collection of wild orchids usually started once a purchase order was received from middlemen. These persons usually stayed nearby orchid collection sites throughout the collection period. Sometimes, the collectors received advance payments. The middlemen usually came from distant districts or even abroad, and provided printed photographs of desired species or small samples of life orchids, and asked collectors to collect similar-looking plants. An example of such a photograph was retrieved from a middleman who received it from international traders based in Hong Kong (Figure 2E). Local people collected all orchids found, also when these did not resemble the species on the photographs provided by the middlemen. None of the orchids collected were discarded at the selling points. Most collectors spent an average of 5-6 h per day in the forest. They carried the orchids in bamboo baskets (Figure 2A-D) or in jute sacks to the nearest selling points. Over the past 15 years, large-scale orchid collection in Nepal has increased year-on-year based on the traded volumes cited by the respondents.

Wild orchids market outlets
Dakshinkali, 22 km from Kathmandu, is the center of wild orchid trade in Nepal, and orchids have been sold for over 25 years. Dakshinkali has at least 10 vendors that are...
specialized in wild orchid trade. Another significant trade hub is Godavari, near Kathmandu, but orchid trade here has gradually declined over the last five years. Dakshinkali is famous for its historic Hindu Kali temple, and every year up to 400,000 pilgrims visit this temple, and purchase wild orchids, which play an important role in ceremonial rituals. Many hotel owners in Kathmandu buy wild orchids at Dakshinkali. These orchids can easily be recognized by their traditional woven bamboo baskets that are specially made for the purpose of selling wild orchids, and not found elsewhere in Nepal.

The east–west highway of the tropical part of central Nepal is another very active site for orchid trade. No fixed orchid selling locations are present here, but every year, the middlemen and/or local traders inform the collectors where the orchids should be brought. At these transitory trade points, the orchids are weighed and traded, with large volumes loaded onto trucks or tractors and transported illegally to India or China.

### Wild orchids trade volume and local income

The peak season for orchid trade at Dakshinkali is from July to October. In this period in the year 2008-2009, each live orchid vendor sold an average of 15-20 pots per day, which averages to 2-2.5 kg of orchids. Extrapolated to yearly trade per vendor this averages to 4.4 tons of orchids per year (2.25 kg × 17.5 pots × 7 days × 16 weeks). The vendors sold both vegetative and flowering orchids, but the latter fetched the highest prices. Popular species such as *Dendrobium densiflorum*, *Coelogyne cristata*, *Cymbidium iridoide* and *Cymbidium erythraeum* traded at the highest values. The price of orchids for floricultural purposes was highly variable and fluctuating, but averaged USD 1.0-1.5 per pot. This average price allows us to make a rough estimate of the annual income per vendor from orchid trade: 17.5 pots × 1.25 USD × 7 days × 16 weeks = 2450 USD.

Local medicinal orchid traders and middlemen reported that orchid trade had declined recently due to the arrest of a number of illegal traders. Collectors were reported to earn an average of USD 2 per kg for medicinal orchids with prices varying between USD 1.5-2.5 depending on the species and quality of the orchids. Based on the interviews we estimate an annual trade of 5 tons for 2008-2009, yielding a combined annual total for Dakshinkali of 9.4 tons of wild orchids for that year.

Detailed export prices of wild orchids collected at the study sites could not be assessed since the traders refused to provide these data. One trader informed us that processed *Dendrobium eriiflorum* sold for 10,000 Hong Kong dollars (~ 1300 USD) per kg. This is in line with the general conception that wild orchids from Nepal fetch higher prices internationally than on the domestic market.

### Legal and illegal trade destinations of Nepalese orchids

Interviews with collectors, middlemen and local traders revealed that most of the wild orchids collected in Nepal are exported to India and China, and occasionally to Hong Kong. None of the actors involved had received permission from local authorities. The local traders mostly exported raw or occasionally semi-processed, dried and cleaned, products. Our findings support previous reports about illegal trade in Nepalese orchids [13,15]. Shakya et al. [14] reported that wild orchids from Nepal were exported to European countries for floricultural purposes, with none of the exported species grown at nurseries. Nepalese newspapers frequently report cases in which orchid smugglers are arrested with huge quantities of wild orchids for export to China.

### Discussion and conclusions

#### Sustainable use of medicinal orchids

Collection and use of wild orchids of Nepal is deeply engrained in the traditional livelihoods of local communities. They form an important part of the traditional health care system and provide a substantial income to subsistence farmers. An increasing number of species are now illegally traded in bulk volumes to some of the most rapidly growing economies in the region, China, India and Hong Kong. This illegal trade creates a severe threat to wild orchid populations in Nepal [34], urging development of alternative strategies for sustainable exploitation. We advocate development of sustainable orchid enterprises focusing on medicinal orchid species grown from cuttings and seed. Cultivated of orchids for raw ingredients of herbal medicine is a niche in the international orchid market that is still relatively undeveloped, and deserves further exploration [2,35,36].

Artificial propagation of orchids has the potential to reduce illegal collecting in the wild through wider availability of stock material, and can also provide large numbers of plants within a short period of time. Artificially propagated plants often have the advantage of being more vigorous than wild collected stock, have a higher survival rate and contain higher contents of compounds with pharmacological effects [37,38]. The establishment of a sustainable national orchid industry based on low cost *in vitro* propagation could be beneficial to the conservation of endangered orchids, and for several species of wild-collected Nepali orchids these techniques have already been developed [39-41]. The potential disadvantage of undercutting local collectors and traders is that as their livelihoods are jeopardized, they are forced to diverge into other sources of supplementary income, such as the collection of other medicinal plant species.
Policies for protection of wild orchids in Nepal
All wild orchids of Nepal are protected under Appendix II of the Convention on International Trade in Endangered species of Wild Fauna and Flora (CITES). The Forest Act 1993, and Forest Regulations 1995, and amendment in 2001 specified all orchids in Nepal as protected. However, contradicting its own policies, the Government of Nepal published a notification on April 14th, 2008 permitting collection of wild orchids for trade. The absence of clear guidelines on sustainable harvesting and weak enforcement of policies could explain the recent increase in illegal trade in orchids [22].

DNA barcoding for identification of sterile orchids
Sterile plant parts sold at local markets can be identified to species level using DNA barcoding. DNA barcoding is increasingly applied for plant species identification [42,43]. This method can both provide the taxonomic identity of samples analyzed, and - if the markers employed are sensitive enough - elucidate the geographical origin of the collected species [28]. For the former scenario, DNA barcoding is increasingly facilitating monitoring trade of CITES-listed species [27]. For the latter, the method is more and more used to trace and substantiate cross-border wildlife crimes [30,44]. We recommend a wider application of DNA barcoding for identification of orchid species in illegal export, as it enables identification of material that is unidentifiable by morphology alone.

Endnote
*Footnote: author names are provided for all species in Table 1. Species not included in Table 1 have author names included in the manuscript body.

Competing interests
The author(s) declare that they have no competing interests.

Authors' contributions
AS, BG, and RC conceived the research. AS and BK were responsible for field research and interviews. AS, BK, BG and RC identified the herbarium vouchers; AS, YC, YD, TA and HB processed the data and performed the quantitative analysis. AS, HB and BG contributed to the manuscript. All authors have read and approved the final manuscript.

Acknowledgements
We thank local plant healers, orchid collectors, vendors, traders and district forest offices of the study sites for providing information. Bijaya Pant, Bhakta Raskoti and Muna Udas are thanked for providing us with relevant literature. Financial support to Abishkar Subedi was provided by the Alberta Minor National Stichting and to Ram Chaudhary by NUFU (Norwegian Council for Higher Education). Raskoti and Muna Udas are thanked for providing us with relevant literature.

Author details
1Naturalis Biodiversity Center, Sylvisuweg 72, P.O. Box 9517, Leiden, The Netherlands. 2Local Initiatives for Biodiversity, Research and Development (L-I-BIRD), P.O. Box 324, Pokhara, Nepal. 3Central Department of Botany, Tribhuvan University, Kirtipur, Nepal. 4Institute Biology Leiden, Einsteinweg 55, 2333 CC Leiden, The Netherlands. 5Department of Organismal Biology, Uppsala University, Norbyvägen 18 D, SE-75236 Uppsala, Sweden.

Received: 26 July 2013 Accepted: 23 August 2013

References
1. Lawler LJ: Ethnobotany of the Orchidaceae. In Orchid Biol Rev Perspect III. Ithaca, NY & London, UK: Comstock Publ. Associates; 1984:27–149.
2. Jalal JS, Kumar P, Pangtey YPS: Ethnomedicinal orchids of Uttarakhand. Western Himalaya. Ethnobot. Leafl 2008; 164.
3. Singh A, Duggal S: Medicinal orchids—an overview. Ethnobot Leafl 2009; 2009:
4. Dhyanis A, Nautiyal BP, Nautiyal MC: Importance of astavarga plants in traditional systems of medicine in Garhwal, Indian Himalaya. Int J Biodivers Sci Ecosyst Serv Manag 2010; 6:1–9.
5. Hossain MM: Therapeutic orchids: traditional uses and recent advances—an overview. Fitoterapia 2011; 82:102–140.
6. Raskoti EB: The orchids of Nepal. Kathmandu, Nepal: Quality printers; 2009.
7. Manandhar NP: Plants and people of Nepal. Portland, OR: Timber Press; 2002.
8. Shrestha KK, Rajbhandary S, Tiwari NN, Poudel RC, Upreti Y: Ethnobotany in Nepal: review and perspectives. WWF Nepal Program Report Series 8.
9. Acharya KP, Rokaya MB: Medicinal orchids of Nepal: are they well protected? Our Nat 2010; 88(2):91.
10. Subedi A: Orchids around Pokhara valley of Nepal. Local Initiatives for Biodiversity, Research and Development: Pokhara, Nepal; 2002.
11. Vaidya BN, Shrestha M, Joshee N: Report on Nepalese orchids species with medicinal properties. In The Himalayan plants, can they save us? Proceeding of Nepal-Japan joint symposium on conservation and utilization of Himalayan medicinal resources. Edited by Watanabe T, Takano A, Bista MS, Salju HK; Japan: Society for the Conservation and Development of Himalayan Medicinal Resources (SCDHMR); 2002:146–152.
12. Bhattarai S, Chaudhary RP, Taylor RSL: Prioritization and trade of ethnomedicinal plants by the people of Manang district, central Nepal. In Veg Soc. their interact: Himalayas. Edited by Chaudhary RP, Subedi BP, Vetaas O: Norway: Tribhuvan University, Nepal and University of Bergen; 2002:151–169.
13. Bailes CP: Orchids in Nepal, the conservation and development of a natural resource, advisory report and recommendations. Richmond, UK: Royal Botanic Gardens Kew; 1985.
14. Shakya LR, Bajracharya BM, Chettri MK: WWF Nepal Program Report Series 8: Conserving the threatened orchids of Kathmandu valley. WWF Nepal Kathmandu, Nepal; 1994.
15. Wildlife Conservation Nepal. Orchids in the Churiya hills and their survival in Nepal. Kathmandu, Nepal: Wildlife Conservation Nepal Conservation and Development of Bhutan, The Charlesworth Group: Huddersfield, UK; 2002.
16. King G, Pantling R: The orchids of the Sikkim-Himalaya. Bengal Secretariat Press: Calcutta; 1986.
17. White KJ, Sharma B: Wild orchids in Nepal: the guide to the Himalayan orchids of the Tribhuvan Rajpath and Chitwan jungle. Bangkok, Thailand: White Lotus Press; 2000.
18. Pearce NR, Cribb PJ: Orchids of Bhutan: flora of Bhutan: vol. 3, part 3. Royal Botanic Garden Edinburgh and Royal Government of Bhutan, The Charlesworth Group: Huddersfield, UK; 2002.
19. Balami NP: Ethnomedicinal uses of plants among the Newar community of Pharping village of Kathmandu district. Nepal Tribhuwan Univer J 2004; 24:13–19.
20. Chen Y, Xu J, Yu H, Qing C, Zhang Y, Liu Y, Wang J: 3,7-Dihydroxy-2,4,6-trimethoxyphenanthrene, a new phenanthrene from Bulbophyllum odoratissimum. J-Korean Chem Soc 2010; 51:352.
21. Pyakurel D, Gurung K: Enumeration of orchids and estimation of current stock of traded orchids in Rolpa district: final report. Rolpa, Nepal: District forest office Rolpa; 2008.
22. Thakur RB, Yadav RP, Thakur NP: Enumerating the status of orchid species of Makawanpur district. Hamlo Kalpabricha 2010, 20:1–18.
23. Gewali MB: Aspects of traditional medicine in Nepal. Toyama, Japan: Inst. Nat. Med. Univ. Toyama; 2008.
24. Lama YC, Ghimire SK, Aumeeruddy-Thomas Y: Medicinal plants of Dolpo: Amchis’ knowledge and conservation. Kathmandu, Nepal: WWF; 2001.
25. Nepal IUCN: National register of medicinal & aromatic plants. Kathmandu, Nepal: IUCN Nepal Country Office for His Majesty’s Government of Nepal, Ministry of Forests and Soil Conservation; 2004.
26. Tenzu Y, Ji L, Hirano H, Ueda M, Nagashima K, Kikuchi T: Studies on the constituents of orchidaceous plants IX constituents of Spiranthes sinensis (Pers.) Ames var. amoena (M. Bieberstein) Hará(2).
structures of spiranthesol, spiranthequinone, spiranthol-C and spiransineol-B, new isopentenyldihydrophenanthrenes. Chem Pharm Bull (Tokyo) 1990, 38:629–635.

27. Eurlings MCM, Gravendeel B: TmL-trnF sequence data imply paraphyly of Aquilaria and Gyrinops (Thymelaeaceae) and provide new perspectives for agarwood identification. Plant Syst Evol 2005, 254:1–12.

28. Eurlings MCM, Heuveling van Beek H, Gravendeel B: Polymorphic microsatellites for forensic identification of agarwood (Aquilaria crassna). Forensic Sci Int 2010, 197:30–34.

29. Ogden R, Dawnay N, McEwing R: Wildlife DNA forensics—bridging the gap between conservation genetics and law enforcement. Endanger Species Res 2009, 9:179–195.

30. Dawney N, Ogden R, McEwing R, Carvalho GR, Thorpe RS: Validation of the barcoding gene COI for use in forensic genetic species identification. Forensic Sci Int 2010, 197:30–34.

31. Gravendeel B, Chase MW, De Vogel EF, Roos MC, Mes TH, Bachmann K: Molecular phylogeny of Coelogyne (Epidendroideae; Orchidaceae) based on plastid RFLPs, matK, and nuclear ribosomal ITS sequences: evidence for polyphyly. Am J Bot 2001, 88:1915–1927.

32. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ: Basic local alignment search tool. J Mol Biol 1990, 215:403–410.

33. Gravendeel B, Eurlings M, van den Berg C, Cribb PJ: Phylogeny of Pleione (Orchidaceae) and parentage analysis of its wild hybrids based on plastid and nuclear ribosomal ITS sequences and morphological data. Syst Bot 2004, 29:50–63.

34. Subedi A: Orchids and sustainable livelihoods: initiatives in Nepal to manage globally threatened biodiversity. In Proc. 18th world orchid conf. Edited by Raynal-Roques A, Roguenant A, Prat D. Turriers, France: Naturalia Publications; 2005:470–474.

35. Kong JM, Goh NK, Chia LS, Chia TF: Recent advances in traditional plant drugs and orchids. Acta Pharmacol Sin 2003, 24:7–21.

36. Maikhuri RK, Rao KS, Chauhan K, Kandari LS, Prasad P, Rajasekaran C: Development of marketing of medicinal plants and other forest products: can it be a path way for effective management and conservation? Indian For 2003, 129:169–178.

37. Kalimuthu K, Senthilkumar R, Vijayakumar S: In vitro micropropagation of orchid, Oncidium sp. (dancing doll). Afr J Biotechnol 2007, 6:1171–1174.

38. Chugh S, Guha S, Rao IU: Micropropagation of orchids: a review on the potential of different explants. Sci Hort 2009, 122:507–520.

39. Swar S: Micropropagation of Cymbidium triflorus D. Don and Coelogyne cristata Lindl. (Orchidaceae). MSc thesis. Tribhuvan University: Central Department of Botany, 2003.

40. Gurung R: In vitro propagation of Aerides odorata Lour. by shoot tip culture. MSc thesis. Tribhuvan University: Central Department of Botany, 2005.

41. Shrestha A: Ex situ conservation of Coelogyne ovalis Lindl. (Orchidaceae) through micropropagation. MSc thesis. Tribhuvan University: Central Department of Botany, 2005.

42. Coghlan M, Haile J, Houston J, Murray D, White N, Moolhuijzen P, Bellgard M, Bunce M: Deep sequencing of plant and animal DNA contained within traditional Chinese medicines reveals legality issues and health safety concerns. Plos Genet 2012, 8:e1002657.

43. Wasser SK, Mailand C, Booth R, Mutayoba B, Kisamo E, Clark B, Stephens M: Using DNA to track the origin of the largest ivory seizure since the 1989 trade ban. Proc Natl Acad Sci 2007, 104:4228.