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ARTÍCULO DE INVESTIGACIÓN

COLOMBIAN FROZEN BIODIVERSITY: 16 YEARS OF THE TISSUE COLLECTION OF THE HUMBOLDT INSTITUTE

La biodiversidad congelada de Colombia: 16 años de la colección de tejidos del Instituto Humboldt

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ABSTRACT

Collections of frozen tissue samples stand as keystone sources of molecular information to construct biodiversity knowledge, and are particularly challenged if they focus on megadiverse countries. In 1998 the Humboldt Institute (*Instituto de Investigación de Recursos Biológicos Alexander von Humboldt*) began a tissue collection of Colombian biodiversity (IAvH-CT) and the aim of this work is to present a diagnostic and an historical perspective for that collection, constructed by compiling information and experiences on its management as well as by organizing and curating the information of each catalogued sample. After 16 years, the IAvH-CT harbors 16,469 samples, which represent around 2530 species from 1289 genera, and 323 families of the Colombian biodiversity. Samples are biased toward plants (44 %) and birds (40 %), but also include other animal taxa. Geographically, IAvH-CT includes samples from all Colombian departments, but there is broad variation in their coverage. When compared with other international collections, IAvH-CT fulfills several standards of sample storage and data management, but its major weakness is that several tissues seem to lack a vouchered specimen. Tissues housed at IAvH-CT have been included in at least 48 studies published in several scientific journals. IAvH-CT is implementing strategies to improve curatorial standards, fill-in taxonomic gaps, and to explore the potential of its samples to understand the outstanding Colombian biota in a cooperative research framework among institutions.

Keywords: biological collections, biorepository, birds, cryoconservation, Neotropics, plants.

RESUMEN

Las colecciones de tejidos son fuentes fundamentales de información molecular para el conocimiento de la biodiversidad, y son particularmente desafiantes si están enfocadas en países megadiversos. En 1998 el Instituto Humboldt (Instituto de Investigación de Recursos Biológicos Alexander von Humboldt) inició una colección de tejidos de la biodiversidad colombiana (IAvH-CT). El objetivo de este trabajo es presentar un diagnóstico y una perspectiva histórica de esta colección, mediante la compilación de información y de experiencias sobre su manejo y organizando y curando la información de cada muestra catalogada. Después de 16 años IAvH-CT resguarda 16,469 muestras, que representan alrededor de 2530 especies de 1289 géneros y 323 familias de la biodiversidad colombiana. El número de muestras está sesgado hacia plantas (44 %) y aves (40 %), pero también incluyen otros taxones animales. Geográficamente, IAvH-CT incluye muestras de todos los departamentos colombianos, pero hay una gran variación en su cobertura.

Al ser comparada con otras colecciones internacionales se encuentra que IAvH-CT cumple varios estándares de almacenamiento de muestras y de manejo de datos, pero su gran debilidad es que varios tejidos aparentemente carecen de un ejemplar de referencia. Varios tejidos almacenados en IAvH-CT han sido incluidos en al menos 48 estudios publicados en varias revistas científicas. IAvH-CT está implementando estrategias para mejorar la curaduría, llenar vacíos taxonómicos y explorar el potencial de sus muestras para entender la impresionante biota colombiana en un marco de investigación en cooperación con otras instituciones.
Palabras clave: aves, biorepositorio, colecciones biológicas, crioconservación, Neotrópico, plantas.

INTRODUCTION

The role of biological collections from megadiverse countries as a tool to conduct scientific research about biodiversity, evolution, or conservation is relevant and challenged because these countries not only harbor a large proportion of the life forms, but also several species and ecosystems of conservation concern (Mittermeier *et al.*, 1998; Myers *et al.*, 2000; Hillebrand, 2004; Orme *et al.*, 2005; Kier *et al.*, 2009). Such is the case of Colombia, a medium-size country located in northern South America that probably hosts more than 10 % of the species in the world (Rangel-Ch, 1995; Samper, 1997; Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, 1998; Myers *et al.*, 2000; Rangel-Ch., 2006; Sistema de Información sobre Biodiversidad de Colombia [SiB-Colombia], 2013). Although Colombia has faced a series of complex social problems during the last century, Colombian researchers and institutions have managed to maintain scientific exploration and increased the production of scientific knowledge about biodiversity, supporting the growth of biodiversity representation in biological collections and the open access to the data (Arbeláez-Cortés, 2013b; Sistema de Información sobre Biodiversidad de Colombia [SiB-Colombia], 2013).

The Humboldt Institute (*Instituto de Investigación de Recursos Biológicos Alexander von Humboldt*, hereafter IAvH) is part of the Colombian National Environmental System (*Sistema Nacional Ambiental*, SINA), which aims to store and share data to generate knowledge about Colombian environment and ecosystems. One of the main goals of IAvH is to carry out scientific research on biodiversity in the continental territory of Colombia, including not only terrestrial ecosystems and species, but also aquatic and genetic resources. The biological collections of IAvH, most of which were inherited from the former *Instituto Nacional de Recursos Naturales* (INDERENA), currently host around 450,000 cataloged objects, of more than 14,000 species in different specimen type-oriented collections (*i.e.*, vertebrate, eggshell, herbarium, entomology and other-invertebrates), natural sound collection, and tissue collection. The biological collections of IAvH are among the largest and more representative in Colombia and have proved to be crucial for understanding Colombian biodiversity through the support of several research programs (C. A. Medina, unpublished data). Particularly, the tissue collection of IAvH (IAvH-CT) holds more than 16,000 samples of over 2500 species of plants and animals ready to be included in studies gathering molecular information with non-commercial purposes.

Cryogenic tissue collections store biological material usually frozen at temperatures below -80 °C, but other methods could be used in other kinds of tissue collections, allowing long-term availability of tissues as useful and optimal sources to obtain molecular information (Corthals and Desalle, 2005; Hanner and Gregory, 2007; Zimkus and Ford, 2014). Moreover, tissue collections are crucial in tropical countries where access through all their territory in order to obtain fresh tissues for molecular studies is sometimes excessively expensive or even dangerous for researchers (Anderson and Maldonado-Ocampo, 2013; Regalado, 2013). Besides, a tissue collection maintained over several years allows the access to reliable molecular information across different periods, which could be useful to detect changes in species of short generation time, and if maintained longer also for other species. The scientific value of tissue collections is enormous, especially if their samples are associated to voucher material in specimen type-oriented collections (*e.g.*, Ruedas *et al.*, 2000; Suarez and Tsutsui, 2004; Cuervo *et al.*, 2006; Pyke and Ehrlich, 2009; Astrin *et al.*, 2013; Gaudeul and Rouhan, 2013; Rocha *et al.*, 2014). Although the development in the fields of ancient and environmental DNA is opening a broader spectrum to access molecular information (*e.g.*, Rasmussen *et al.*, 2011; Meyer *et al.*, 2012; Shokralla *et al.*, 2012; Orlando *et al.*, 2013), tissue collections remain keystone sources of material to obtain molecular data for studies on biodiversity.

Our aim is to present a diagnostic and an historical perspective for IAvH-CT. To do this we compiled information and experiences from the first 16 years of IAvH-CT and basic data for other similar collections around the world. In particular, we depict the taxonomic and geographical coverage of the collection as well as the knowledge obtained by using their tissue samples, highlighting the potential of IAvH-CT to produce new knowledge about Colombian biodiversity.

MATERIAL AND METHODS

Data curation and diagnosis of the geographic coverage, taxonomy, and vouchered material

We compiled the information of each catalogued sample at IAvH-CT from August 1998 to August 2014, to construct a database using Access (Microsoft, 2010). The taxonomy

and geography at the departmental level (*i.e.*, major administrative and geopolitical division of Colombia) were curated to homogenize the information of all sample records. We used Catalogue of Life 2013 (Roskov *et al.*, 2013) to homogenize the taxonomic information. The list matching service, available on the webpage of Catalogue of Life, was used to compare the taxa names in our database, allowing to identify synonyms and misspellings, which were corrected in an additional field of our database. It is necessary to note that the species name of every sample as it was originally written was kept in another field of the database ("Determination Remarks"). In a few cases, some names did not appear in Catalogue of Life; when these names corresponded to plants we curated them using the Tropicos database (Missouri Botanical Garden, 2013), which is a more comprehensive source of taxonomic information for Neotropical plants than Catalogue of Life. A few other names that we did not find were defined as "non existent" and excluded from the analyses. The curation of the geographic information consisted on a revision for misspelled department names and filling in the database field for department adscription for records that only included information about locality or municipality.

To depict and quantify the taxonomic coverage we conducted queries in different taxonomic ranks. IAvH-CT is also organized by "biological groups" (*e.g.*, plants, birds, fishes, invertebrates), which do not correspond to the same taxonomic rank, or sometimes do not represent a valid taxon, but are useful to ascribe samples to general groups. Therefore, the samples of this field were also quantified. Additionally, we ranked the families with the larger number of species and samples. In order to obtain a picture of the taxonomic representation of Colombian biodiversity at IAvH-CT we compared the number of species represented in the collection with the number of species reported for Colombia for taxa with available information (Mendoza *et al.*, 2004; Mendoza and Ramírez, 2006; Rangel-Ch., 2006; Maldonado-Ocampo *et al.*, 2008; Salaman *et al.*, 2009; Galeano and Bernal, 2010; Donegan *et al.*, 2013; Sistema de Información sobre Biodiversidad de Colombia [SiB-Colombia], 2013; Solari *et al.*, 2013; Acosta, 2014). To examine the geographical pattern of the samples of IAvH-CT we mapped their geographic provenience at department level using five ranks and the method of natural breaks in ArcGis 9.1 (ESRI, 2009).

We also searched the database to know if there is information for a voucher specimen supporting the samples. The IAvH-CT's database includes two fields where this information can be queried. One field ("museum number") indicates the catalog number assigned to the specimen voucher in a collection specialized in the taxon, while another field ("specimen location") indicates the biological collection where the specimen voucher was sent. Hence, we made a query for the samples with no information in both fields. After this query, we explored the taxa included in order to know if they were collected in the context of population genetics projects, which usually do not include specimen vouchers.

An historical perspective for IAvH-CT and its place among other tissue collections

We compiled information on the management of IAvH-CT since its foundation to construct an historical perspective. Basic information on the collection was recovered from printed texts, electronic files, and personal records of the authors who have been involved as curators, or associated researchers, during different periods. Some issues of this history had been already addressed by Palacio-Mejía (2006).

We also ranked the IAvH-CT in a global context by comparing it with other tissue collections around the world. To do this, we conducted *ad libitum* searches in institutional webpages of several tissue collections and extracted information from the abstracts published for the Global Genome Biodiversity Network (GGBN) meeting of 2014 (Global Genome Biodiversity Network, 2014) and from the list of collections presented in Zimkus and Ford (2014). For information available we searched the following data of each collection: geographic location, year of foundation, preservation method, biological groups, sample numbers, and geographic coverage. However, such information was incomplete for every collection. Therefore our comparison of each item of information for IAvH-CT must be limited to the information available for subsets of other collections.

Publication survey

Finally, to quantify the role of IAvH-CT in generating knowledge about Colombian genetic diversity we compiled the publications that have used its samples. To do this we selected the published works included in a list of research projects conducted using the collection between 1998 and 2010, which is part of the archive at IAvH-CT. Additionally, other studies were recovered, and added to this list, by searching the works of researchers that have received tissue grants from IAvH-CT and by searching the acronym IAvH in GenBank. We restricted the analysis to papers in scientific journals. The complete text of each paper was revised to identify whether there was an explicit mention of samples from IAvH-CT. In certain cases it was found that some papers did not mention IAvH-CT (or IAvH-BT, acronym of the former name), but the use of IAvH-CT samples in these works was tacit (see Results), and therefore these papers were also included. The bibliographic records were compiled in another database and were organized and analyzed using basic bibliometric methods, in a similar manner as in Arbeláez-Cortés (2013b). The 2013 impact factor (Thomson Reuters, 2014) of each journal was annotated for each publication.

RESULTS

Taxonomic coverage

At least 2530 species from 1289 genera, 323 families, 124 orders, 16 classes, and eight phyla of Colombian biodiversity are represented in the 16,469 samples housed at IAvH-CT. By sample, we refer to the tissue or DNA of a single individual that in some cases is stored in several cryovials (one to ten), of which IAvH-CT includes around 21,000. The precise number of species remains unknown because several samples are identified only at the genus or family ranks and could correspond to species already represented in IAvH-CT. The samples are biased toward plants (44 %) and birds (40 %). Regarding species numbers, birds are well represented (around 905 species), including almost half of the Colombian species (Table 1), but plants are underrepresented in spite of the high number of species included (1139 species, 4 % of Colombian richness). Nonetheless, freshwater fishes (182 species), reptiles (72 species), and mammals (77 species) represent between 12 and 16 % of the Colombian species (Table 1), while the other biological groups (amphibians, insects, other invertebrates, and microorganisms) represent just around 150 species. The top-twenty families list (Table 1) includes taxa represented by 22 to 176 species, and between 43 and 927 samples. For 14 families we found recently published

Table 1. Number of samples and species per biological group and for the top 20 families represented at IAvH-CT. The proportion of Colombian species included for each group is indicated for these groups if there are numbers published recently. N.A indicates that information was not available. Data until August 2014.

Group	Number of samples at IAvH-CT	Minimun number of species at IAvH-CT	Proportion (%) of Colombian species represented at IAvH-CT	Number of species reported for Colombia	Source of the data on number of species in Colombia
Plants	7238	1139	4.1	28000	Sistema de Información sobre Biodiversidad de Colombia [SiB-Colombia] (2013), Rangel-Ch. (2006)
Birds	6634	905	47.6	1903	Donegan *et al.,* (2013)
Reptiles	819	72	12.6	571	Sistema de Información sobre Biodiversidad de Colombia [SiB-Colombia] (2013)
Fresh water fishes	454	182	12.6	1435	Maldonado-Ocampo *et al.,* (2008)
Mammals	394	77	15.7	492	Solari *et al.,*(2013)
Other invertebrates	392	3	0.5	650	Sistema de Información sobre Biodiversidad de Colombia [SiB-Colombia] (2013)
Amphibian	292	47	6.0	782	Acosta (2014)
Insects	199	56	0.5	11500	Sistema de Información sobre Biodiversidad de Colombia [SiB-Colombia] (2013) (Note: includes only the most studied taxa)
Microorganisms	46	6	N.A	N.A	None
Rubiaceae	797	176	18.1	970	Mendoza *et al.*, (2004)
Arecaceae	878	153	66.2	231	Galeano and Bernal (2010)
Melastomataceae	693	143	15.9	900	Mendoza and Ramírez (2006)
Tyrannidae	758	115	56.7	203	Salaman *et al.,* (2009)
Trochilidae	927	110	67.9	162	Salaman *et al.,* (2009)
Thraupidae	740	96	68.1	141	Salaman *et al.,* (2009)
Piperaceae	234	91	N.A.	N.A.	None
Furnariidae	617	67	60.9	110	Salaman *et al.,* (2009)
Thamnophilidae	428	59	54.6	108	Salaman *et al.,* (2009)
Asteraceae	121	45	N.A.	N.A.	None
Emberizidae	323	42	63.6	66	Salaman *et al.,* (2009)
Phyllostomidae	233	42	33.1	127	Solari *et al.,*(2013)
Scarabaeidae	126	41	N.A.	N.A.	None
Fabaceae	110	40	N.A.	N.A.	None
Psittacidae	273	37	69.8	53	Salaman *et al.,* (2009)
Parulidae	267	29	51.8	56	Salaman *et al.* ,(2009)
Characidae	43	27	6.8	399	Maldonado-Ocampo *et al.,* (2008)
Cotingidae	122	25	75.8	33	Salaman *et al.,* (2009)
Poaceae	850	23	N.A.	N.A.	None
Troglodytidae	245	22	68.8	32	Salaman *et al.*, (2009)

information about their number of species in Colombia (Table 1), and 11 of them (palms and ten bird families) have a representation of more than 50 % of its richness. Other families with large numbers of samples (more than 300) are: Fagaceae, Poaceae, Cheloniidae, and Arcidae, which reflects an intensive sampling for specific population genetic projects in a few species that harbor particular interest for some researchers.

Geographic coverage

After curating the department information for 1453 records, to complete the basic geographic information for all samples, we found that IAvH-CT includes samples from all Colombian departments (Fig. 1). However, the number of samples per department was highly variable (Fig. 1), between two and 1646, being Antioquia, Valle del Cauca, Cauca, and Vichada the most sampled departments (with more than 1000 samples each). In general, a large part of Colombian territory is relatively well sampled, particularly the Andes, northern Orinoquia, and southern Amazonia. However, there are still gaps in sampling between Amazonia-Orinoquia and in the Caribbean. Besides, it is necessary to note that samples for some departments are biased towards a few selected species. A geographical pattern of the

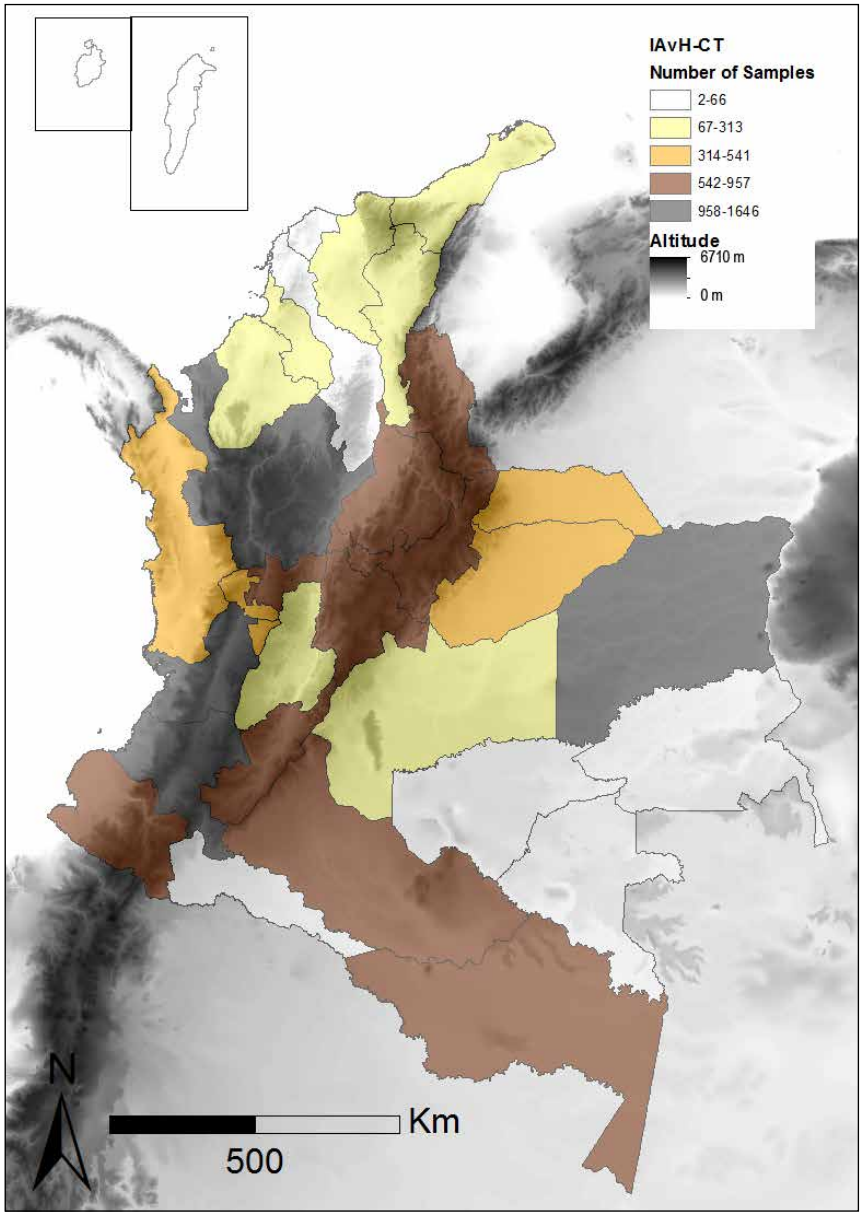

Figure 1. Geographic patterns of the number of samples, per department, housed at IAvH-CT until August 2014.

coverage of IAvH-CT in more detail could be visualized at: data.sibcolombia.net/dataset/resources/115/, and is based on a set of samples with available georeferences.

Voucher coverage

At present there are around 8900 samples at IAvH-CT for which there is information for an associated specimen voucher (*i.e.*, catalogued specimen or exsiccate) or information for the storage of such a specimen voucher in another collection specialized in the taxon. Among the almost 7500 samples without specimen voucher information, 42 % corresponds to species with numerous samples collected for population genetics projects that do not seem to have considered collection of specimens, and probably these samples will remain without voucher support.

History and management of IAvH-CT

During the establishment of IAvH in 1995, one aim was to fill in gaps on the knowledge of Colombian biodiversity. After an exploration of facilities and projects of several institutions in Colombia a gap in the genetic diversity knowledge was identified. This gap was addressed by the conformation of a national collection of tissue samples of Colombian biodiversity which was originally named *Banco de Tejidos*. This collection was an idea of the former director of IAvH, Cristián Samper, a visionary who set a keystone agenda for research on Colombian biodiversity (Samper, 1997). IAvH-CT is located, since its foundation in August 1998, in the *Centro Internacional de Agricultura Tropical* (CIAT) at Valle del Cauca, between Cali and Palmira. The CIAT has been an important partner that, in the frame of a cooperation agreement between institutions during the earlier years and a service contract during the last years, has provided the space, services, and facilities to maintain the IAvH-CT operational.

IAvH-CT is based on a vapor-phase liquid nitrogen storing system to preserve the samples below -155 °C. This system was selected following the advice of the Smithsonian Natural History Museum and the American Museum of Natural History, and consists of two cryovats (Taylor-Wharton 38K, Mark III Kseries). The liquid nitrogen is supplied by a commercial company, and one Dewar with 148 L of liquid nitrogen is necessary every 11–14 days for one cryovat. Samples are stored in boxes for 81 cryovials, which are organized in vertical racks of 13 shelves that allow storing around 60,000 samples in both cryovats. However, the capacity of IAvH-CT could be expanded to 76,700 vials if 100-vials boxes are used and if racks for 25-vial boxes are added to fill up the whole space of each cryovat. Each cryovial is marked with the IAvH-CT catalog number in its cap insert and on its lateral label, other data included are the field (or collector) number of the sample and the basic taxonomic information. In order to establish a direct link to specimen vouchers, the latest cryovials are being marked (among brackets) also with the catalogue's number of the specimen voucher (if it was already assigned) or with the collection's acronym (or the institution's name) where it was sent. Most of the cryovials are also marked with a linear barcode sticker, readable with an optic devise. This system allows recovering the information on the database for each sample by "reading" the particular vial, but by the time of writing the system is waiting to be migrated and linked to the curated database.

As noted by Palacio-Mejía (2006), the intention of IAvH in 1998 was to build up an active working tissue collection, then a basic laboratory was established in the same year with the support of the Science Funding Agency of Colombia (COLCIENCIAS) and the technical assistance of the Biotechnology Unit of CIAT. The laboratory was equipped to obtain DNA of different biological groups and to implement molecular markers based on electrophoresis banding patterns (*i.e.*, AFLPs, RAPDs, and microsatellites). Additional projects of IAvH have provided further reagents, materials, and equipment, and during recent years the change towards analyses based on DNA sequences has made it necessary to use the service of commercial companies.

Since the beginning, IAvH-CT has provided the service of storing samples for researchers. Therefore, some proportion of the samples had been deposited in custody (see below) or as donations by researchers from other institutions. However, the majority of IAvH-CT' samples have been obtained from tissues collected by the IAvH research team, sometimes in cooperation with other institutions. At least 29 institutions and around 240 collectors have been involved, in some way, in providing samples to IAvH-CT.

Issues such as sample curation, protocols for grant and deposit of samples, and the first database structure were guided by the standards set by the Smithsonian Natural History Museum and by using guidelines from Dessauer *et al.,* (1996) and Prendini *et al.*, (2002), and recently the issues discussed by Nagy (2010) have also been considered. Along the years, the database, the curation routines, and the protocols have been improved or adjusted to deal with the conditions, policies, and biodiversity of Colombia. Particularly, during the last year the database has been organized, curated (as indicated above), and migrated to Specify v. 6.4.13 (Specify Software Project, 2013) in order to share routines with other IAvH collections. The data at IAvH-CT are public and available through the SIB-Colombia and through the Global Biodiversity Information Facility (Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, 2014), and will be periodically updated. The institutional information of IAvH-CT, its policies and protocols (in Spanish) are available at http://humboldt.org.co/servicios/colecciones-biologicas/tejidos. At present, IAvH-CT is member of GGBN and is working to improve routines and sharing data and protocols to join the globally distributed databases that aim to bridge the gap between

biodiversity repositories (Droege *et al.*, 2013). In fact, the protocols for grant and deposit of samples in IAvH-CT include information of policies from other international collections (e.g., the Genetic Resource Collection of the Burke Museum, the Tissues Collection of the Ornithology Division of the Biodiversity Institute and Natural History Museum of Kansas University, the Tissue Collection of the Zoological Museum of Copenhagen, the Genetic resources of Mammals of University of Alaska, the Museum of Vertebrate Zoology of University of California, and the Frozen Tissue Collection of the Australian Museum) and fulfill several standards set in Applequist (2014).

IAvH-CT among other tissue collections

We recovered partial information for 109 non-human tissue collections around the world (data available upon request), mainly from the United States (46) and Europe (30), while Latin America included only nine. Regarding the year of foundation, indicated for 41 collections, IAvH-CT is placed among the 17 tissue collections established before 2000. Considering the storage method, only 19 collections, of 75, utilize liquid nitrogen as IAvH-CT. In relation to the number of samples we found values for 81 collections, and assuming that numbers reported for these collections correspond to single samples (not cryovials) IAvH-CT ranks 48. The data gathered also indicates that 30 collections had global or continental coverage while only ten collections were national or regional in focus as IAvH-CT. Finally, 46 collections are focused in one biological group while 38 collections included several high-rank taxa as IAvH-CT.

Bibliometric account

The compilation of studies using samples of IAvH-CT rendered a list of 48 published works (data available upon request). However, only 34 papers indicated explicitly the use of IAvH-CT samples. The remaining publications did not mention IAvH-CT, but they mentioned the work in the collection's laboratory with samples of taxa that are catalogued in IAvH-CT's database, indicated acknowledgement to IAvH-CT curators, or corresponded to works from researchers that have received grants or that have deposited samples in IAvH-CT of the same taxa included in their studies. Considering these 48 works, publications begun in 2000, had appeared in 27 journals, 23 of which (including 43 publications) had an impact factor between 0.2 and 42.4. These works included between one and 48 IAvH-CT samples, which have been used mainly to complement a larger sample obtained from other tissue collections, particularly from the United States. The class Aves represents the taxon included in most works (n=26) and reaches the high-impact journals. However, IAvH-CT samples of plants also have been included in some papers of high visibility. Those works have analyzed principally DNA sequences for one to six loci, but some studies have included other molecular markers such as AFLPs and microsatellites. Besides, IAvH-CT has provided support to at least 47 theses of pre- and postgraduate students, but a large volume of them remain as grey literature.

DISCUSSION

The goal of this paper is to present a diagnosis of IAvH-CT. This diagnosis indicates that during its history, IAvH-CT has been growing as a storage collection (around 1000 new samples per year) as well as a source of scientific information (three papers per year including information from their samples). The comparison of IAvH-CT with other collections and with the information in Zimkus and Ford (2014) and Applequist (2014), indicates that this collection fulfills several standards of international tissue collections. For instance: a broad coverage (but necessarily incomplete) of taxa and geography, a high standard cryopreservation method, public access to the information of their samples, and detailed protocols for depositing and for grant of samples. All these issues are more relevant when it is considered that IAvH-CT is focused on a megadiverse country, and that the policies of Colombia (*e.g.*, Ministerio de Ambiente y Desarrollo Sostenible, 2013a; Ministerio de Ambiente y Desarrollo Sostenible, 2013b) do not allow the existence of a similar collection in other country. In fact, the bird samples (a large part of them associated to specimen vouchers in *Colección de Aves de Colombia del Instituto Humboldt*, IAvH-A) positioned IAvH-CT among the most relevant tissue collections of birds in the world (Stoeckle and Winker, 2009), and have allowed to conduct barcoding projects (Gonzalez and Paz, 2013) and have contributed in understanding different evolutionary aspects of the Neotropical species (*e.g.*, Cadena *et al.*, 2007; Puebla-Olivares *et al.*, 2008; Mauck III and Burns, 2009; Parra *et al.*, 2009; Parra, 2010; Sedano and Burns, 2010; Chaves *et al.*, 2011; d'Horta *et al.*, 2012; Gutíerrez-Pinto *et al.*, 2012; Rheindt *et al.*, 2013; Valderrama *et al.*, 2014).

There are also issues that must be addressed in order to set higher standards for IAvH-CT and to take advantage of the space available. For instance, it is necessary to increase the representation of some regions such as Caribbean, southern Orinoquía, and Northern Amazonia as well as biological groups such as plants, invertebrates, and amphibians. Taxa such as Fungi, not represented yet in IAvH-CT, could be another target for the near future. Some of these gaps will be surely filled as a result of the ongoing and further collection work conducted by IAvH and other institutions. However, to optimize it, IAvH-CT must be involved in planning the explorations and during the field work to collect tissue samples in addition to the specimens. In fact, the relatively well sampled departments in the Andes, Orinoquía, and Amazonia reflect the intensive work conducted during several years by *Grupo de Exploración y Monitoreo Ambiental* (GEMA) of IAvH, which was assisted in this aspect from

IAvH-CT. In addition, researchers from other institutions could contribute to fill several gaps if they consider IAvH-CT as a centralized facility for Colombian biodiversity genetic resources, which could provide assistance and materials for tissue collection, and where is allowed to deposit tissue samples with exclusivity of access by up to five years.

Probably, the most pervasive weakness of IAvH-CT is the absence of vouchered specimens to support a large part of the samples stored there. Although IAvH-CT is a reference collection and since the beginning it has emphasized the association of the samples to a voucher, at present the proportion of samples for which there is information for an associated specimen in another collection is only 55%. In order to deal partially with this issue IAvH biological collections are implementing a routine to link the samples and the specimens in a synchronous curatorial process for samples collected in IAvH's recent projects, and a similar strategy could be implemented with other institutions. Besides, the protocol for samples deposited in IAvH-CT places strong emphasis on the association of each sample to some kind of evidence of the phenotype of the organism, considering not only specimens but also digital vouchers in some special cases (see Astrin *et al*., 2013). It is well known that the absence of vouchers supporting samples in tissue collections removes a large part of the scientific value of such tissues because it is not possible to confirm taxonomic identifications, nor conduct analyses of variation in phenotypic traits (*e.g.*, Ruedas *et al.*, 2000; Suarez and Tsutsui, 2004; Cuervo *et al.*, 2006; Pyke and Ehrlich, 2009; Astrin *et al*. 2013; Gaudeul and Rouhan, 2013; Rocha *et al.*, 2014)**.** Particularly, the issue of the taxonomic identification is critical in Colombia that has a high rate of description of new species, even in relatively well known groups of vertebrates (Arbeláez-Cortés, 2013a; Arbeláez-Cortés, 2013b)**.** Another deficit detected at IAvH-CT is the incongruence between taxonomic information of samples and taxonomic identity according to the molecular information obtained. This incongruence is between the molecular information and the voucher identification (not just the database information) suggesting that an error could have occurred during the sample collection. Some data obtained during different projects of IAvH indicate that this incongruence could be affecting around 5 % for bird tissues, but a detailed evaluation will be necessary to set a precise number.

As any biological collection, IAvH-CT involves a cost (around 45,000 US per year) which includes payment of the location, services, salaries, supplies, and the basic operating costs, but not research activities. This budget is similar to the one invested in other IAvH's specimen collections and is among the amount of annual budgets of international genetic resources collections (Zimkus and Ford, 2014). However, in proportion this cost is high as the number of samples is still low at IAvH-CT in comparison with other collections of IAvH, which include larger number of specimens. Considering the high capacity for storing samples of IAvH-CT the cost-benefit relationship will be balanced with the growth of this collection. However, as indicated above this growth must be planned and assisted, in order to fill up taxonomic and geographic gaps to allow a better representation of Colombian biodiversity. Additionally, it will be also necessary to assign a permanent budget to conduct the adequate scientific research allowed by such volume of samples from a megadiverse country.

Genetic diversity is an issue relatively poorly studied in Colombia (Arbeláez-Cortés, 2013b). However, it is clear that IAvH-CT has been useful to study this issue (see the bibliometric account section), and has a great potential to continue filling such a knowledge's gap, which was the original reason for its foundation. It is known that the costs of DNA analyses have dropped greatly, while fieldwork remains costly and sometimes more difficult in several areas (Applequist, 2014). Therefore, IAvH-CT rises as a keystone to support cooperative research initiatives. However, researchers must understand that Colombian policies around biodiversity and genetic resources despite being more flexible than years ago (Nemogá and Rojas, 2007; Fernández, 2011) still require some permits (*e.g.*, Ministerio de Ambiente y Desarrollo Sostenible, 2013a; Ministerio de Ambiente y Desarrollo Sostenible, 2013b). These policies are in line with the high national pride related with Colombian biodiversity, but they also render the international cooperation difficult (though not impossible), and the access to low price, or to high technology methods (when the material is sent overseas for analyses) is also slowed.

CONCLUSIONS

The diagnostic we presented for IAvH-CT clearly indicates that this collection has been an active repository of material to obtain molecular information on Colombian species (for non-commercial scientific research) as well as an important source of scientific knowledge. In spite of several gaps in geographic and taxonomic coverage, and the failure represented by the absence of voucher specimens for several samples, IAvH-CT could be considered a central repository of tissues of Colombian biodiversity, and we encourage researchers to use it and to contribute to it. The 16 years of IAvH-CT and their articulation with the other collections of the IAvH have allowed gaining experience to improve protocols and routines in order to reach higher curatorial standards. The tissues housed at IAvH-CT and their associated information must be considered as an important Colombian scientific resource, which reflect the work of many people, and are available for a broad spectrum of research possibilities. However, to understand the outstanding biota of Colombia represents a high-level scientific enterprise and its study must be conducted and encouraged, principally, as scientific cooperation.

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