RCVD MAY 11 2016

Department of the Interior
U.S. Fish and Wildlife Service

OMB No. 1018-0093
Expires 05/31/2017

Federal Fish and Wildlife Permit Application Form

Return to: U.S. Fish and Wildlife Service
Division of Management Authority (DMA)
Branch of Permits, MS: IA
5275 Leesburg Pike
Falls Church, VA 22041-3803
1-800-358-2104 or 703-358-2104

Type of Activity:
EXPORT/IMPORT/INTERSTATE AND FOREIGN COMMERCE OF NON-NATIVE PLANTS (CITES and/or ESA)
(Circle or highlight proposed activity)

Complete Sections A or B, and C, D, and E of this application. U.S. address may be required in Section C, see instructions for details.
See attached instruction pages for information on how to make your application complete and help avoid unnecessary delays.

A. Complete if applying as an individual

1.a. Last name	1.b. First name	1.c. Middle name or initial	1.d. Suffix

2. Date of birth (mm/dd/yyyy)	3. Social Security No.	4. Occupation	5. Affiliation/ Doing business as (see instructions)

6.a. Telephone number	6.b. Alternate telephone number	6.c. Fax number	6.d. E-mail address

B. Complete if applying on behalf of a business, corporation, public agency, Tribe, or institution

1.a. Name of business, agency, Tribe, or institution	1.b. Doing business as (dba)
Smithsonian Institution, National Museum of Natural	Botanical research

2. Tax identification no.	3. Description of business, agency, Tribe, or institution
53-0206027	Research and education

4.a. Principal officer Last name	4.b. Principal officer First name	4.c. Principal officer Middle name/ initial	4.d. Suffix
Wagner	Warren	[redacted]	

5. Principal officer title	6. Primary contact name
Research scientist and curator	Marc Appelhans (Smithsonian NMNH Research

7.a. Business telephone number	7.b. Alternate telephone number	7.c. Business fax number	7.d. Business e-mail address
++49 5513922220	[redacted]	++49 5513922329	mappelh@gwdg.de

C. All applicants complete address information

1.a. Physical address (Street address; Apartment #, Suite #, or Room #; no P.O. Boxes)
Smithsonian Institution; National Museum of Natural History; Department of Botany, MRC-166, 10th and Constitution

1.b. City	1.c. State	1.d. Zip code/Postal code:	1.e. County/Province	1.f. Country
Washington	DC	20560	n.a.	USA

2.a. Mailing Address (include if different than physical address; include name of contact person if applicable)
P. O. Box 37012

2.b. City	2.c. State	2.d. Zip code/Postal code:	2.e. County/Province	2.f. Country
Washington	DC	20013-7012		USA

D. All applicants MUST complete

1. Attach check or money order payable to the U.S. FISH AND WILDLIFE SERVICE **in the amount of $100 nonrefundable processing fee.** Federal, Tribal, State, and local government agencies, and those acting on behalf of such agencies, are exempt from the processing fee – ***attach documentation of fee exempt status as outlined in instructions.*** (50 CFR 13.11(d))

2. Do you currently have or have you ever had any Federal Fish and Wildlife permits?
Yes ☐ If yes, list the number of the most current permit you have held or that you are applying to renew/re-issue: _____ No ☑

3. Certification: I hereby certify that I have read and am familiar with the regulations contained in ***Title 50, Part 13 of the Code of Federal Regulations*** and the other ***applicable parts in subchapter B of Chapter I of Title 50***, and I certify that the information submitted in this application for a permit is complete and accurate to the best of my knowledge and belief. I understand that any false statement herein may subject me to the criminal penalties of 18 U.S.C. 1001.

Signature (in blue ink) of applicant/person responsible for permit (No photocopied or stamped signatures) — Date of signature (mm/dd/yyyy): 05/04/2016

E. EXPORT/IMPORT/INTERSTATE AND FOREIGN COMMERCE OF PLANTS (ESA and/or CITES)

Allow at least 90 days for the application to be processed. Applications for endangered species under the ESA s must be published in the Federal Register for a 30-day public comment period.

Complete all questions on the application. Mark questions that are not applicable with "N/A". Please use separate sheets of paper when answering this questions. On attachments or separate sheets you submit, indicate the application question number you are addressing. If you are applying for multiple species, be sure to indicate which species you are addressing in each response.

NOTE: This form should NOT be used to request authorization for commercial exports of plants that are artificially propagated in the United States. For such exports, applicants should complete form 3-200-33 (http://www.fws.gov/forms/3-200-33.pdf).

1. What activity are you requesting authorization to carry out?

EXPORT [✓] IMPORT [ ]
INTERSTATE COMMERCE [ ] FOREIGN COMMERCE [ ]

2. For EACH plant involved in the proposed activity provide:

   a. Scientific name (genus, species, and, if applicable, subspecies) and common name:

   b. Description of specimen (e.g., whole plant, cuttings, parts, products; size, height, length);

   c. Quantity of specimens;

   d. Source of specimen (wild or artificially propagated).

3. The current location of the specimens (address and country):

   Specimens will be collected in forests (Forest Reserves) on Hawai'i (Big Island), Maui, Moloka'i, O'ahu and Kaua'i

4. Recipient/Sender:
   - If **export**, provide name and address of the recipient in the foreign country.
   - If **import**, provide name and address of the exporter in the foreign country.
   - If **interstate or foreign commerce**, provide name and address of recipient.

Name: Georg-August University Goettingen
Business Name: Albrecht-von-Haller Institute for Plant Sciences
Marc Appelhans

Address:

Address: Untere Karspuele 2

City: Goettingen

State/Province: Lower Saxony

Country, Postal Code: Germany, 37073

SOURCE OF SPECIMENS (answer question 6 or 7 for each species/specimen, as appropriate):

5. For plants **taken from the wild,** provide the following for each species/specimen collected:
   a. Scientific name;
   b. Number and size class of specimens collected (e.g., 100 juveniles; 50 mature);
   c. Specific location and date of collection for each specimen;
   d. Who (name and address) collected the specimens;
   e. Copies of documents that indicates that the plants were legally collected (e.g., State permits or licenses, landowner's permission, collection permits). Be sure to correlate each document to the corresponding specimen;
   f. Approximate density (e.g., number of plants per acre) and distribution of the species at the collection site(s);
   g. Collection methodology (e.g., whether the specimens were removed from an area of few to several patches of plants, percentage of specimens removed at a specific location); AND
   h. Estimate the number of plants collected to how many plants remain at the location.
   i. Describe efforts made to utilize artificially propagated specimens in lieu of taking plants from the wild.
   j. If applicant did not collect specimens, provide the invoice or other chain of custody documentation that shows the name, address and telephone number of the person from whom you obtained the plants and the date of acquisition of the specimen. Documentation should trace back to the original collector.

6. For **artificially propagated** plants, provide documentation, such as receipts, showing the name, address and telephone number of the person from whom you purchased the plants and the date(s) of acquisition of each specimen and a statement, preferable from the propagator, on how the specimens were propagated (e.g., description of the nursery, propagation method, source and location of parental stock).

7. Provide a full statement justifying the proposed activity (e.g., export, import, interstate commerce, foreign commerce), including the following details:

   a. Describe the purpose of your proposed activity. For example, if the purpose is scientific research, attach a copy of your research proposal outlining the purpose, objectives, methods (e.g., specific information on survey/collection methods, sampling regime, equipment to be used), and whether similar work has already been done or is currently being done. If the purpose is conservation education, provide copies of educational materials (e.g., handouts, text of signage or public presentations), and include the purpose and objectives of the proposed activity. If the purpose is for propagation for conservation purposes, provide a description of how the species will be propagated, disposition of progeny, and cooperative agreements that are/will be established for re-introduction.
   b. Describe the technical expertise of each person as it relates to the proposed activities.
   c. If the species is listed as endangered under the ESA, describe how the activities will enhance or benefit the wild population.
   d. If the requested activity involves native species, provide information to show that the activity is consistent with any recovery plan for the species.
   e. Provide copies of contracts or agreements or other permits that identify persons involved and dates of activities for which the permit is sought.

8. If the proposed activity includes propagating or maintaining live plants at your facility, provide the following:

   a. Approximate number of specimens you currently maintain for each species requested.
   b. Describe the propagation method (e.g., seed, cutting, mericlone) used.
   c. Describe the conditions where the plants are grown and provide photographs of your facilities.
   d. Describe your background and experience working with this or similar species, including
      (i) the number of years each species has been cultivated by you; and
      (ii) the number of plants successfully propagated annually.
   e. Discuss your willingness to participate in a cooperative propagation program and maintain or contribute data regarding your propagation success with the species.
   f. Provide a copy of your State license and U.S. Department of Agriculture General permit, as appropriate.

9. If **import** or **export**, provide:
   a. Copy of any required foreign permits (for CITES Appendix-I plants provide a copy of import permit or evidence a permit will be issued). If plant is to be taken from the wild, provide documentation from the foreign government approving the action.
   b. Describe: (i) the type, size, and construction of shipping containers and (ii) the arrangements for watering and caring for the specimens during transportation.
   c. A statement on the disposition of all imported plants, plant material, and progeny, if produced.

10. Name and address where you wish permit mailed, if different from page 1 (All permits will be mailed via the U.S. Postal Service, unless you identify an alternative means below):

11. If you wish the permit to be delivered by means other than USPS regular mail, provide an air bill, pre-paid envelope, or billing information. If you do not have a pre-paid envelope or air bill and wish to pay for a courier service with your credit card, please check the box below. Please DO NOT include credit card number or other information; you will be contacted for this information.

    ☑ If a permit is issued, please send it via a courier service to the address on page 1 or question 9. I understand that you will contact me for my credit card information once the application has been processed.

12. Who should we contact if we have questions about the application? (Include name, phone number, and email):

    Marc Appelhans, ++49 5513922220, mappelh@gwdg.de

13. **Disqualification Factor**. A conviction, or entry of a plea of guilty or nolo contendere, for a felony violation of the Lacey Act, the Migratory Bird Treaty Act, or the Bald and Golden Eagle Protection Act disqualifies any such person from receiving or exercising the privileges of a permit, unless such disqualification has been expressly waived by the Service Director in response to a written petition. (50 CFR 13.21(c)) Have you or any of the owners of the business, if applying as a business, been convicted, or entered a plea of guilty or nolo contendere, forfeited collateral, or are currently under charges for any violations of the laws mentioned above?

    ☐ Yes ☑ No If you answered "Yes" provide: a) the individual's name, b) date of charge, c) charge(s), d) location of incident, e) court, and f) action taken for each violation.

Appendix to form 3-200-36

Section E:

2a: Scientific and common names of all species.

Not all species have a common name, and common names are often in Hawaiian language. Melicope species are often called 'alani' on Hawaii, and another English name (used only in Australia) is 'doughwood'. Myrsine species are known as 'colicwood', and Zanthoxylum species are known as 'prickly ash' or 'Hercules club', and 'A'e' in Hawaiian. The Hawaiian name for Platydesma is 'Pilo kea'.

We intend to collect the following species of the genera Melicope, Platydesma and Zanthoxylum (all Rutaceae family) and Myrsine (Primulaceae family) on Hawaii. [scientific names in italics; common names in brackets]

Melicope adscendens (auwahi melicope), *Melicope anisata* (mokihana), *Melicope balloui* (Ballou's melicope), *Melicope barbigera* (uahiapele), *Melicope christophersenii* (Waianae Range melicope), *Melicope cinerea* (manena), *Melicope clusiifolia* (kukaemoa, kolokolo mokihana), *Melicope cruciata* (Cross-bearing Melicope, pilo 'ula), *Melicope degeneri* (Kokee Stream melicope), *Melicope elliptica* (leiohi'iaka), *Melicope feddei*, *Melicope haleakalae* (Haleakala melicope), *Melicope haupuensis* (Haupa Mountain melicope), *Melicope hawaiensis* (mokihana kukae moa), *Melicope hiiakae*, *Melicope hosakae*, *Melicope kaalaensis* (Kaala melicope), *Melicope kavaiensis* (pilo 'ula), *Melicope knudsenii* (Olokele Valley melicope), *Melicope lydgatei* (Koolau Range melicope), *Melicope macropus* (Kaholuamanu melicope), *Melicope makahae* (Makaha Valley melicope), *Melicope molokaiensis*, *Melicope mucronulata*, *Melicope munroi* (lanahale), *Melicope nealae*, *Melicope oahuensis*, *Melicope obovata* (Makawao melicope), *Melicope orbicularis* (Honokahua melicope), *Melicope ovalis* (Hana melicope), *Melicope ovata*, *Melicope pallida* (pale melicope), *Melicope paniculata* (Lihue melicope), *Melicope peduncularis*, *Melicope pseudoanisata*, *Melicope puberula* (hairy melicope), *Melicope quadrangularis* (four angle melicope), *Melicope radiata*, *Melicope reflexa* (lava melicope), *Melicope rotundifolia*, *Melicope saint-johnii* (St. John's melicope), *Melicope sandwicensis* (Mt. Kaala melicope), *Melicope sessilis*, *Melicope volcanica*, *Melicope waialealae* (Alani wai), *Melicope wailauensis*, *Melicope wawraeana* (Monoa melicope), *Melicope zahlbruckneri* (Zahlbruckner's melicope, kipuka piaula) → 48 species

Myrsine alyxifolia, *Myrsine degeneri* (summit colicwood), *Myrsine denticulata*, *Myrsine fernseei* (streambank colicwood), *Myrsine fosbergii* (Koolau Range colicwood), *Myrsine helleri* ('Oliko), *Myrsine juddii* (cloudswept colicwood), *Myrsine kauaiensis*, *Myrsine knudsenii* (Kokee colicwood), *Myrsine lanaiensis*, *Myrsine lessertiana* (Kolea lau nui), *Myrsine linearifolia* (narrowleaf colicwood), *Myrsine mezii* (Hanapepe River colicwood), *Myrsine petiolata* (swamp colicwood), *Myrsine pukooensis*, *Myrsine punctata*, *Myrsine sandwicensis* (Kolea lau li'i), *Myrsine vaccinioides*, *Myrsine wawraea* → 19 species

Platydesma cornuta, Platydesma remyi (Hawai'i pilo kea), *Platydesma rostrata* (Pilo kea lau li'i), *Platydesma spathulata* (Pilo kea) → 4 species

Zanthoxylum dipetalum (Kawa'u), *Zanthoxylum hawaiiense* (A'e, Hawai'i prickly ash), *Zanthoxylum kauaense* (A'e, Kaua'i prickly ash), *Zanthoxylum oahuense* (A'e, O'ahu prickly ash) → 4 species

2b: Description of specimen

Several of the above-mentioned species are federally listed as endangered. These are:

Melicope adscendens, Melicope balloui, Melicope christophersenii, Melicope cinerea, Melicope cruciata, Melicope degeneri, Melicope haleakalae, Melicope haupuensis, Melicope hawaiensis, Melicope hiiakae, Melicope knudsenii, Melicope lydgatei, Melicope macropus, Melicope makahae, Melicope mucronulata, Melicope munroi, Melicope nealae, Melicope obovata, Melicope ovalis, Melicope pallida, Melicope paniculata, Melicope puberula, Melicope quadrangularis, Melicope reflexa, Melicope saint-johnii, Melicope sandwicensis, Melicope wailauensis, Melicope zahlbruckneri → 28 species

Myrsine fosbergii, Myrsine juddii, Myrsine knudsenii, Myrsine linearifolia, Myrsine mezii, Myrsine vaccinioides → 6 species

Platydesma cornuta, Platydesma remyi, Platydesma rostrata → 3 species

Zanthoxylum dipetalum, Zanthoxylum hawaiiense, Zanthoxylum oahuense → 3 species

For these species, we would like to collect a single leaf from one individual tree and export it to Germany. We are also planning to collect one herbarium specimen (a small twig) from one individual tree per species, but the herbarium specimen will stay in Hawaii and will be deposited at Bishop Museum. The leaf will be used for molecular lab work and the herbarium specimen will serve as a reference, so that future generations can still check if the species identification was correct.

For the more common and non-endangered species, we intend to collect material from two trees per species. In case a species grows on more than one island, we would like to sample from two individual trees per island. The material collected consists of a single leaf and four herbarium vouchers (four small twigs) and five (Myrsine) to ten

(Rutaceae genera) viable seeds (seeds will be taken from only one tree per species). We intend to collect four herbarium vouchers, and distribute them to the following herbaria: Bishop Museum (Honolulu; BISH), National Tropical Botanical Garden (Kalaeo, Kaua'i; PTBG), Smithsonian Institution (Washington DC; US) and Goettingen University (Goettingen, Germany; GOET). Four herbarium vouchers need to be collected so that all co-operation partners (Warren Wagner, Smithsonian Institution; Kenneth Wood, National Tropical Botanical Garden; Marc Appelhans at Goettingen) have a complete set of specimens. In addition, the Hawaiian regulations require depositing one herbarium voucher at Bishop Museum. Since only one of these institutions is outside of the USA, only the export of one herbarium voucher per tree is requested. The seeds are needed for cultivation in the Botanical Garden in Goettingen.

2c: Quantity of specimens

For the protected species (40 species in 4 genera in total; see 2b), only a single leaf needs to be exported. Since material from only one tree per species will be collected, the total number of specimens (single leaves) from the protected species will be 40.

The remaining 35 species are not federally listed and we would like to collect material from two individual trees per species per island. Since most species are endemic to a single island, but some species are found on all islands, the numbers of specimens per species differ, and we are listing the numbers of specimens for each species accordingly:

Melicope anisata: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope barbigera: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope clusiifolia: Hawai'i, Maui, Moloka'i, O'ahu, Kaua'i → 10 specimens = 10 leaves, 10 herbarium vouchers, 10 seeds
Melicope elliptica: Maui, Moloka'i, O'ahu → 6 specimens = 6 leaves, 6 herbarium vouchers, 10 seeds
Melicope feddei: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope hosakae: O'ahu → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope kaalaensis: O'ahu → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope kavaiensis: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope molokaiensis: Maui, Moloka'i → 4 specimens = 4 leaves, 4 herbarium vouchers, 10 seeds
Melicope oahuensis: O'ahu → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope orbicularis: Maui → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope ovata: O'ahu, Kaua'i → 4 specimens = 4 leaves, 4 herbarium vouchers, 10 seeds
Melicope peduncularis: Maui, Moloka'i, O'ahu, Kaua'i → 8 specimens = 8 leaves, 8 herbarium vouchers, 10 seeds

Melicope pseudoanisata: Hawai'i, Maui → 4 specimens = 4 leaves, 4 herbarium vouchers, 10 seeds
Melicope radiata: Hawai'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope rotundifolia: O'ahu → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope sessilis: Maui, Moloka'i → 4 specimens = 4 leaves, 4 herbarium vouchers, 10 seeds
Melicope volcanica: Hawai'i, Maui, Moloka'i → 6 specimens = 6 leaves, 6 herbarium vouchers, 10 seeds
Melicope waialealae: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope wawraeana: O'ahu, Kaua'i → 4 specimens = 4 leaves, 4 herbarium vouchers, 10 seeds

Myrsine alyxifolia: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 5 seeds
Myrsine degeneri: O'ahu → 2 specimens = 2 leaves, 2 herbarium vouchers, 5 seeds
Myrsine denticulata: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 5 seeds
Myrsine fernseei: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 5 seeds
Myrsine helleri: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 5 seeds
Myrsine kauaiensis: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 5 seeds
Myrsine lanaiensis: Hawai'i, Maui, Moloka'i, O'ahu, Kaua'i → 10 specimens = 10 leaves, 10 herbarium vouchers, 5 seeds
Myrsine lessertiana: Hawai'i, Maui, Moloka'i, O'ahu, Kaua'i → 10 specimens = 10 leaves, 10 herbarium vouchers, 5 seeds
Myrsine petiolata: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 5 seeds
Myrsine pukooensis: Maui, Moloka'i, O'ahu → 6 specimens = 6 leaves, 6 herbarium vouchers, 5 seeds
Myrsine punctata: O'ahu, Kaua'i → 4 specimens = 4 leaves, 4 herbarium vouchers, 5 seeds
Myrsine sandwicensis: Hawai'i, Maui, Moloka'i, O'ahu → 8 specimens = 8 leaves, 8 herbarium vouchers, 5 seeds
Myrsine wawraea: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 5 seeds

Platydesma spathulata: Hawai'i, Maui, O'ahu, Kaua'i → 8 specimens = 8 leaves, 8 herbarium vouchers, 10 seeds

Zanthoxylum kauaense: Hawai'i, Maui, Moloka'i, O'ahu, Kaua'i → 10 specimens = 10 leaves, 10 herbarium vouchers, 10 seeds

2d: Source of specimen

The specimens will be collected in the wild. We have already applied for the required permits to collect plants (both endangered and not endangered species) from Forest Reserves in all four Hawaiian counties.

We have applied for the following permits:
-Research/Collection/Possession Permit For Hawaii Threatened & Endangered Plant Species

-Access/Research/Botanical Collection Permit For Kaua'i Forest Reserves
-Access/Research/Botanical Collection Permit For O'ahu Forest Reserves
-Access/Research/Botanical Collection Permit For Hawai'i Forest Reserves
-Access/Research/Botanical Collection Permit For Maui Nui Forest Reserves (Maui & Moloka'i)

5a: Scientific name for each species
→ Scientific names for all species are listed under 2a

5b: Number and size class of specimens collected
→ 40 single leaves will be collected from endangered plants. These leaves will be dried in silica gel.
→ 144 single leaves, 144 herbarium vouchers (each consisting of one twig of about the size of the US letter paper size) and 285 seeds will be collected from non-endangered plants. Additional herbarium vouchers will be collected for US institutions (Bishop Museum, National Tropical Botanical Garden, Smithsonian Institution). The 144 herbarium vouchers are the ones that will be exported to Goettingen/Germany.
→ See 2c for the amount of specimens per species.

5c: Specific location and date of collection for each specimen

A five week field trip to Hawaii is planned in summer/fall 2016 and all specimens will be collected during this field trip.

The locations of the specimens are Forest Reserves on the Hawaiian island Kaua'i, O'ahu, Maui, Moloka'i and Hawai'i (Big Island). The names of the Forest Reserves are as follows:

Kaua'i: Na Pali-Kona Forest Reserve, Pu'u Ka Pele Forest Reserve, Lihue-Koloa Forest Reserve, Kalepa Forest Reserve, Nounou Forest Reserve

O'ahu: Waianae Kai Forest Reserve, Mokuleia Forest Reserve, Nanakuli Forest Reserve, Makua Kea'au Forest Reserve, Kuaokala Forest Reserve, Ewa Forest Reserve, Honululu Watershed Forest Reserve, Kuliouou Forest Reserve, Kaipapau Forest Reserve

Maui: Koolau Forest Reserve, Makawao Forest Reserve, Hanawi Natural Forest Reserve, Hana Forest Reserve, Kipahulu Forest Reserve, West Maui Forest Reserve

Moloka'i: Moloka'i Forest Reserve

Big Island: Puna Forest Reserve, Hilo Forest Reserve, Upper Waiakea Forest Reserve, Kau Forest Reserve, Kohala Forest Reserve, Manowaialee Forest Reserve

5d: Who collected the specimens

Marc Appelhans (Georg-August University Goettingen, Albrecht-von-Haller Institute for Plant Sciences, Untere Karspuele 2, Goettingen, Germany, 37073; Research Associate at the Smithsonian Institution, National Museum of Natural History, PO Box 37012, Washington, DC 20013-7012) will collect the specimens together with a graduate student (Claudia Paetzold; Georg-August University Goettingen, Albrecht-von-Haller Institute for Plant Sciences, Untere Karspuele 2, Goettingen, Germany, 37073). We are working together with colleagues from Hawaii and will collect endangered species together with them. Our contacts for each Hawaiian county are:

Hawai'I (Big Island)
Lyman Perry
Hawaii District Botanist
Division of Forestry and Wildlife
19 E Kawili St
Hilo, HI 96720
(808) 938-7795
Lyman.Perry@hawaii.gov

Maui Nui (Maui, Moloka'i)
Lance K. De Silva
Forest Management Supervisor I
State of HI - DLNR
Division of Forestry & Wildlife - Maui Nui
1955 Main St . Suite 400
Wailuku, HI 96793
(808) 873-3980
Lance.K.DeSilva@hawaii.gov

O'ahu
Ryan Keala Ishima Peralta
Forest Management Supervisor I
State of Hawaii
Department of Land and Natural Resources
Division of Forestry and Wildlife
2135 Makiki Heights Drive
Honolulu, Hawaii 96822
(808) 292-5645
Ryan.K.Peralta@hawaii.gov

Lara Reynolds
Oahu District Botanist
Division of Forestry and Wildlife
Department of Land & Natural Resources
2551 Waimano Home Road, Bldg. #202
Pearl City, HI 96782
(808) 342-3081
Lara.S.Reynolds@hawaii.gov

Kaua'i

Kenneth R. Wood
Research Biologist
National Tropical Botanical Garden
3530 Papalina Road
Kalaheo HI 96741
(808) 332-7324
kwood@ntbg.org

Adam Williams
Kaua'i District Botanist
State Division of Forestry and
Wildlife
3060 Eiwa St. Rm 306
Lihue, Kauai 96766
(808) 421-9091
Adam.M.Williams@hawaii.gov

5e: Copies of documents that indicates that the plants were legally collected

We have applied for the following collecting permits from the Department of Land and Natural Resources - Division of Forestry and Wildlife:

-Research/Collection/Possession Permit For Hawaii Threatened & Endangered Plant Species
-Access/Research/Botanical Collection Permit For Kaua'i Forest Reserves
-Access/Research/Botanical Collection Permit For O'ahu Forest Reserves
-Access/Research/Botanical Collection Permit For Hawai'i Forest Reserves
-Access/Research/Botanical Collection Permit For Maui Nui Forest Reserves (Maui & Moloka'i)

The permit applications are currently being reviewed and we will notify the U.S. Fish and Wildlife Service once a decision is made and we will send copies of the collection permits.

5f: Approximate density and distribution of the species

Non-endangered species of Melicope and Myrsine form a common component of Hawaiian plant communities. Seventy-five percent of the species are endemic to single islands, and have >500 individuals per sq km where they are locally common. The density of some species is difficult to assess with some of them are found only in a single forest with low numbers of individuals. These species are:

Melicope adscendens, Melicope degeneri, Melicope haupuensis, Melicope knudsenii, Melicope lydgatei, Melicope mucronulata, Melicope quadrangularis, Melicope wailauensis, Melicope zahlbruckneri, Myrsine knudsenii, Myrsine mezii, Platydesma remyi, Zanthoxylum hawaiiense

Most species occur on only one Hawaiian island. The distribution for each species is shown in the following table:

Genus	Species	Kaua'i	O'ahu	Moloka'i	Lana'i	Maui	Hawai'i (Big island)
Myrsine	alyxifolia	X					
Myrsine	degeneri		X				
Myrsine	denticulata	X					
Myrsine	emarginata		X		X	X	
Myrsine	fernseei	X					
Myrsine	fosbergii	X	X				
Myrsine	helleri	X					
Myrsine	juddii		X				
Myrsine	kauaiensis	X					
Myrsine	knudsenii	X					
Myrsine	lanaiensis	X	X	X	X	X	X
Myrsine	lessertiana	X	X	X	X	X	X
Myrsine	linearifolia	X					
Myrsine	mezii	X					
Myrsine	petiolata	X					
Myrsine	pukooensis		X	X	X	X	
Myrsine	punctata	X	X				
Myrsine	sandwicensis		X	X	X	X	X
Myrsine	vaccinioides					X	
Myrsine	wawraea	X					
Melicope	adscendens					X	
Melicope	anisata	X					
Melicope	balloui					X	
Melicope	barbigera	X					
Melicope	christophersenii		X				
Melicope	cinerea		X				
Melicope	clusiifolia	X	X	X	X	X	X
Melicope	cruciata	X					
Melicope	degeneri	X					
Melicope	elliptica		X	X		X	
Melicope	feddei	X					
Melicope	haleakalae					X	
Melicope	haupuensis	X					
Melicope	hawaiensis			X	X	X	X
Melicope	hiiakae		X				
Melicope	hosakae		X				
Melicope	kaalaensis		X				
Melicope	kavaiensis	X					

Melicope	knudsenii	X				X	
Melicope	lydgatei		X				
Melicope	macropus	X					
Melicope	makahae		X				
Melicope	molokaiensis			X		X	
Melicope	mucronulata			X		X	
Melicope	munroi			X	X		
Melicope	nealae	X					
Melicope	oahuensis		X				
Melicope	obovata					X	
Melicope	orbicularis					X	
Melicope	ovalis					X	
Melicope	ovata	X	X				
Melicope	pallida	X	X				
Melicope	paniculata	X					
Melicope	peduncularis	X	X	X		X	
Melicope	pseudoanisata					X	X
Melicope	puberula	X					
Melicope	quadrangularis	X					
Melicope	radiata						X
Melicope	reflexa			X			
Melicope	rotundifolia		X				
Melicope	saint-johnii		X				
Melicope	sandwicensis		X				
Melicope	sessilis			X		X	
Melicope	volcanica			X	X	X	X
Melicope	waialealae	X					
Melicope	wailauensis			X			
Melicope	wawraeana	X	X				
Melicope	zahlbruckneri						X
Platydesma	cornuta		X				
Platydesma	cornuta		X				
Platydesma	remyi						X
Platydesma	rostrata	X					
Platydesma	spathulata	X	X			X	X
Zanthoxylum	dipetalum	X	X	X			X
Zanthoxylum	dipetalum						X
Zanthoxylum	hawaiiense	X		X	X	X	X
Zanthoxylum	kauaense	X	X	X	X	X	X
Zanthoxylum	oahuense		X				

5g: Collection methodology

All plant species which we would like to collect and export are trees or shrubs. Since we plan to collect single leaves, herbarium vouchers and seeds, no individual plants will be killed.

Specimens will be taken from one individual tree per species for the protected species (see 2b), and from two individual trees per species per island for the unprotected species (see 2b).

5h: Plants remaining at the location

Since only fragments of the plants will be taken, all sampled plants will remain alive and all plants will remain at the locations.

5i: Efforts made to utilize artificially propagated specimens

It is important for the project to make field observation regarding the ecology and environment of the specimens and therefore artificially propagated specimens are not suited.

5j: Original collector of additional specimens

N/A. All specimens will be collected by Marc Appelhans and Claudia Paetzold, as indicated in section 5d.

6: Artificially propagated specimens

N/A.

7a: Purpose of the proposed activity

The planned research is about plant systematics, evolution and biogeography. Systematics is the very basis of any biodiversity related topic. Without the proper naming, description, establishing species-boundaries and encripting the evolutionary history of a species, further studies are not possible. The PI of this project (Marc Appelhans) has worked with Warren Wagner (Smithsonian Institution, McBride chair at NTBG) and Ken Wood (NTBG) on the systematics and biogeography of Hawaiian

Melicope (which includes Platydesma) before. During these studies, we found out that the species Melicope knudsenii, which is known from Kaua'i and Maui, is in fact not "a good species" since it consists of three independent evolutionary lineages that should be regarded as three different species. One of these species, which we are currently formally describing, is endemic to Kaua'i and it is know from a handful of individual trees only. Thus, by using molecular tools to establish species-boundaries, we can identify new species and find out which lineages need special attention and a conservation plan.

In the past 20 years, plant systematics mainly relied on the sequencing of few DNA regions such as single genes, introns or spacer regions. The advent of Next-Generation Sequencing methods has been a major revolution in the field, and it enables researchers to sequence large parts of the genomes. Especially (geologically) young speciation events can be studied in great detail using these new methods, since closely related species are highly similar in their genetic information and a large quantity and quality of information is needed to detect the differences among these close relatives. The plant radiations on the Hawaiian Islands are prime examples for such young speciation events. So far, no study based on Hawaiian plants has been carried out using our methodology, so that our project will be a prime example in Hawaiian biogeography and evolution.

The proposed study has been financed by the DFG (Deutsche Forschungsgemeinschaft), which is the German equivalent of NSF, and we are attaching the grant application, which contains detailed information about the background, methodology and preliminary findings.

7b: Technical expertise

The researchers involved in this project have excellent expertise to ensure the success of the proposed study. The study requires expertise in molecular labwork, bioinformatics and deep knowledge in recognizing and identifying the species.

The PI of this project (Dr. Marc Appelhans; Goettingen University; Research associate Smithsonian Institution, Washington, DC) has been working on the systematics, phylogeny, taxononmy and biogeography of Hawaiian Rutaceae (Citrus family) since his postdoctoral studies at the Smithsonian Institution in Washington DC in 2014. He has published five scientific articles about the Rutaceae genus Melicope with his supervisors from the Smithsonian (Warren Wagner, Jun Wen) since 2014 and three additional papers are currently in preparation or submitted. Mr. Appelhans is teaching a MSc course about Next-Generation Sequencing at Goettingen University and is the co-editor of a recently published book about the use of Next-Generation Sequencing in plant systematics (Hörandl E, Appelhans MS (eds.): Regnum Vegetabile 158. Next Generation Sequencing in Plant Systematics. Koeltz Scientific Books, Königstein.).

Dr. Warren Wagner (Smithsonian Institution, Washington, DC) is a world-leading expert on the flora and biogeography of the Hawaiian Islands. He has been working on Hawaiian plants for decades and holds the position "McBryde Chair for Hawaiian and Pacific Plant Studies" at the National Tropical Botanical Garden in Kalaheo/Kaua'i. Dr. Wagner was Dr. Appelhans' postdoc-supervisor and both conceived the ideas for this new project.

Kenneth Wood is a research biologist at the National Tropical Botanical Garden in Kalaheo/Kaua'i. He is an expert in recognizing Hawaiian plants, especially Melicope and Myrsine (main target genera of this project). A major interest of his work is the conservation of the Hawaiian flora, a topic on which he has published several scientific articles. His expert knowledge regarding species identification and the locations are invaluable for the project.

Claudia Paetzold (Goettingen University) will be involved in the project as a graduate/doctoral student. The project will be her first contact with Hawaiian plants. The position in the project has been advertised internationally, and she has been selected because of her excellent knowledge regarding DNA sequencing and Next-Generation Sequencing related bioinformatics.

7c: Endangered species - Benefits for wild populations

As outlined in section 7a, plant systematic studies are the basis for proper conservation plans of endangered species. Our (Appelhans, Wagner, Wood) previous research has shown that the species Melicope knudsenii consists of three independent evolutionary lineages and therefore represents three different species, which are all critically endangered. Describing these differences, establishing DNA-based species boundaries, and proposing names for species new to science is therefore an essential foundation for protecting these species. Like it was the case for Melicope knudsenii, we expect that several other "species" that occur on more than one Hawaiian island might represent more than one species. Our research will show how many species there are and will show which species and populations exhibit unique genetic information and need to be protected.

7d: Consistency of activity with recovery plan for species.

All targeted species are native to the Hawaiian Islands. Only fragments of the plants will be collected and no plants will be killed, so that no recovery plans are violated. Endangered species will be collected together with Hawaiian colleagues (Plant Extinction Prevention program; Division of Forestry & Wildlife), who will ensure that the impact on individual trees will be minimal.

7e: Persons involved and dates of activities

Researchers involved in this project are Marc Appelhans (mappelh@gwdg.de), Warren Wagner (wagnerw@si.edu), Kenneth Wood (kwood@ntbg.org) and Claudia Paetzold (paetzold@gwdg.de). We did not make special contracts or agreements, but we have worked and published together since 2012 and there is a strong wish from all sides to continue to work on this project together. Appelhans, Wagner and Wood have written the grant application together.

The planned field work on Hawaii is planned for summer/fall 2016 and the duration will be five weeks.

8a: Number of specimens we currently maintain.

Currently no specimens of Hawaiian Melicope, Platydesma, Zanthoxylum and Myrsine are maintained at Goettingen University. We would like to collect seeds of 35 unprotected species listed in section 2c for cultivation at the Botanical Garden of Goettingen University.

8b: Propagation method

In all cases plants will be grown from seeds.

8c: Growing conditions

The Botanical Garden of Goettingen University is equipped with state of the art greenhouses and equipment to cultivate (sub)tropical plants. Climate chambers will guarantee constant environmental conditions for the germination phase. After the seedling stage, plants will be cultivated in different greenhouses depending on their ecological niche. Species from wet montane forests and bogs will be cultivated in our tropical fern greenhouse; species from mesic forests will be cultivated in our tropical greenhouse, and species from drier habitats will be grown together with our collections from Australia, South Africa and the Mediterranean.

Climate chamber with controlled temperature, humidity and light intensity.

Overview of several greenhouses of the Botanical Garden in Goettingen.

8d: Cultivation plants: background and experience

The botanical garden of Goettingen University was established in 1736. There has been a long tradition in the cultivation of tropical plants in greenhouses and the gardeners have extensive knowledge to ensure that the plants will be taken care of professionally.

8e: Participation in a cooperative propagation program

We are of course willing to participate in any cooperative propagation program that will be beneficial for the surviving of the species. We are in close contact with researchers from the National Tropical Botanical Garden on Kaua'i and we will share all data regarding growing conditions of successfully cultivated plants with them.

8f: State license and U.S. Department of Agriculture General permit

N/A.

9a: Copy of any required foreign permits.

None of the targeted species of this study are CITES-listed, so that only phytosanitary restrictions need to be taken into account for the import of specimens to Germany. We have contacted the "Landwirtschaftskammer Niedersachsen" (Department of Agriculture for the state Lower Saxony), who attest that no special permit is needed for the four targeted species (genera Melicope, Platydesma, Zanthoxylum, Myrsine). A letter from this institution is attached to this appendix.

9b: Type, size, and construction of shipping containers

The material will be sent in standard FedEx paper boxes. Herbarium specimens are stored in folded newspaper sheets, seeds and dried leaves will be deposited in ziplock bags. In total, all specimens from one island will be put into a single box, so that five boxes will be exported.

Seeds will be potted in the Botanical Garden of Goettingen University, so that no watering and caring for the specimens during transportation need to be arranged.

9c: Statement on the disposition of all imported plants

The imported herbarium sheets will be deposited at the herbarium of Goettingen University. Dried leaves will be used for DNA extraction and will be used up for these studies. DNA extracts will be stored at -80°C in the labs of Goettingen University. Seeds will be used to grow plants, and root tips from those plants will be used for microscopy to count chromosome numbers and determine ploidy levels, and fresh leaves will be used for flow cytometry in order to determine genome sizes. The plants will be cultivated in the greenhouses of the Botanical Garden Goettingen.

Department of the Interior
U.S. Fish and Wildlife Service

OMB No. 1018-0093
Expires 05/31/2017

Federal Fish and Wildlife Permit Application Form

Return to: U.S. Fish and Wildlife Service
Division of Management Authority (DMA)
Branch of Permits, MS: IA
5275 Leesburg Pike
Falls Church, VA 22041-3803
1-800-358-2104 or 703-358-2104

Type of Activity:
EXPORT/IMPORT/INTERSTATE AND FOREIGN COMMERCE OF NON-NATIVE PLANTS (CITES and/or ESA)

(Circle or highlight proposed activity)

Complete Sections A or B, and C, D, and E of this application. U.S. address may be required in Section C, see instructions for details.
See attached instruction pages for information on how to make your application complete and help avoid unnecessary delays.

A.	**Complete if applying as an individual**		
1.a. Last name	1.b. First name	1.c. Middle name or initial	1.d. Suffix

2. Date of birth (mm/dd/yyyy)	3. Social Security No.	4. Occupation	5. Affiliation/ Doing business as (see instructions)

6.a. Telephone number	6.b. Alternate telephone number	6.c. Fax number	6.d. E-mail address

B.	**Complete if applying on behalf of a business, corporation, public agency, Tribe, or institution**		
1.a. Name of business, agency, Tribe, or institution		1.b. Doing business as (dba)	
Smithsonian Institution, National Museum of Natural		Botanical research	
2. Tax identification no.	3. Description of business, agency, Tribe, or institution		
53-0206027	Research and education		
4.a. Principal officer Last name	4.b. Principal officer First name	4.c. Principal officer Middle name/ initial	4.d. Suffix
Wagner	Warren	[redacted]	
5. Principal officer title	6. Primary contact name		
Research scientist and curator	Marc Appelhans (Smithsonian NMNH Research		
7.a. Business telephone number	7.b. Alternate telephone number	7.c. Business fax number	7.d. Business e-mail address
++49 5513922220	[redacted]	++49 5513922329	mappelh@gwdg.de

C.	**All applicants complete address information**			
1.a. Physical address (Street address; Apartment #, Suite #, or Room #; no P.O. Boxes)				
Smithsonian Institution; National Museum of Natural History; Department of Botany, MRC-166, 10th and Constitution				
1.b. City	1.c. State	1.d. Zip code/Postal code:	1.e. County/Province	1.f. Country
Washington	DC	20560	n.a.	USA
2.a. Mailing Address (include if different than physical address; include name of contact person if applicable)				
P. O. Box 37012				
2.b. City	2.c. State	2.d. Zip code/Postal code:	2.e. County/Province	2.f. Country
Washington	DC	20013-7012		USA

D.	**All applicants MUST complete**
1.	Attach check or money order payable to the U.S. FISH AND WILDLIFE SERVICE **in the amount of $100 nonrefundable processing fee.** Federal, Tribal, State, and local government agencies, and those acting on behalf of such agencies, are exempt from the processing fee – ***attach documentation of fee exempt status as outlined in instructions.*** (50 CFR 13.11(d))
2.	Do you currently have or have you ever had any Federal Fish and Wildlife permits? Yes ☐ If yes, list the number of the most current permit you have held or that you are applying to renew/re-issue: _____ No ☑
3.	Certification: I hereby certify that I have read and am familiar with the regulations contained in ***Title 50, Part 13 of the Code of Federal Regulations*** and the other ***applicable parts in subchapter B of Chapter I of Title 50***, and I certify that the information submitted in this application for a permit is complete and accurate to the best of my knowledge and belief. I understand that any false statement herein may subject me to the criminal penalties of 18 U.S.C. 1001. _____ 05/04/2016 Signature (**in blue ink**) of applicant/person responsible for permit (No photocopied or stamped signatures) — Date of signature (mm/dd/yyyy)

E. EXPORT/IMPORT/INTERSTATE AND FOREIGN COMMERCE OF PLANTS (ESA and/or CITES)

Allow at least 90 days for the application to be processed. Applications for endangered species under the ESA s must be published in the Federal Register for a 30-day public comment period.

Complete all questions on the application. Mark questions that are not applicable with "N/A". Please use separate sheets of paper when answering this questions. On attachments or separate sheets you submit, indicate the application question number you are addressing. If you are applying for multiple species, be sure to indicate which species you are addressing in each response.

NOTE: This form should NOT be used to request authorization for commercial exports of plants that are artificially propagated in the United States. For such exports, applicants should complete form 3-200-33 (http://www.fws.gov/forms/3-200-33.pdf).

1. What activity are you requesting authorization to carry out?

EXPORT [✓] IMPORT [ ]
INTERSTATE COMMERCE [ ] FOREIGN COMMERCE [ ]

2. For EACH plant involved in the proposed activity provide:

   a. Scientific name (genus, species, and, if applicable, subspecies) and common name:

   b. Description of specimen (e.g., whole plant, cuttings, parts, products; size, height, length);

   c. Quantity of specimens;

   d. Source of specimen (wild or artificially propagated).

3. The current location of the specimens (address and country):

   Specimens will be collected in forests (Forest Reserves) on Hawai'i (Big Island), Maui, Moloka'i, O'ahu and Kaua'i

4. Recipient/Sender:
   - If **export**, provide name and address of the recipient in the foreign country.
   - If **import**, provide name and address of the exporter in the foreign country.
   - If **interstate or foreign commerce**, provide name and address of recipient.

Name:	Georg-August University Goettingen Albrecht-von-Haller Institute for Plant Sciences
Business Name:	Marc Appelhans
Address:	
Address:	Untere Karspuele 2
City:	Goettingen
State/Province:	Lower Saxony
Country, Postal Code:	Germany, 37073

SOURCE OF SPECIMENS (answer question 6 or 7 for each species/specimen, as appropriate):

5. For plants **taken from the wild**, provide the following for each species/specimen collected:
   a. Scientific name;
   b. Number and size class of specimens collected (e.g., 100 juveniles; 50 mature);
   c. Specific location and date of collection for each specimen;
   d. Who (name and address) collected the specimens;
   e. Copies of documents that indicates that the plants were legally collected (e.g., State permits or licenses, landowner's permission, collection permits). Be sure to correlate each document to the corresponding specimen;
   f. Approximate density (e.g., number of plants per acre) and distribution of the species at the collection site(s);
   g. Collection methodology (e.g., whether the specimens were removed from an area of few to several patches of plants, percentage of specimens removed at a specific location); AND
   h. Estimate the number of plants collected to how many plants remain at the location.
   i. Describe efforts made to utilize artificially propagated specimens in lieu of taking plants from the wild.
   j. If applicant did not collect specimens, provide the invoice or other chain of custody documentation that shows the name, address and telephone number of the person from whom you obtained the plants and the date of acquisition of the specimen. Documentation should trace back to the original collector.

6. For **artificially propagated** plants, provide documentation, such as receipts, showing the name, address and telephone number of the person from whom you purchased the plants and the date(s) of acquisition of each specimen and a statement, preferable from the propagator, on how the specimens were propagated (e.g., description of the nursery, propagation method, source and location of parental stock).

7. Provide a full statement justifying the proposed activity (e.g., export, import, interstate commerce, foreign commerce), including the following details:

   a. Describe the purpose of your proposed activity. For example, if the purpose is scientific research, attach a copy of your research proposal outlining the purpose, objectives, methods (e.g., specific information on survey/collection methods, sampling regime, equipment to be used), and whether similar work has already been done or is currently being done. If the purpose is conservation education, provide copies of educational materials (e.g., handouts, text of signage or public presentations), and include the purpose and objectives of the proposed activity. If the purpose is for propagation for conservation purposes, provide a description of how the species will be propagated, disposition of progeny, and cooperative agreements that are/will be established for re-introduction.
   b. Describe the technical expertise of each person as it relates to the proposed activities.
   c. If the species is listed as endangered under the ESA, describe how the activities will enhance or benefit the wild population.
   d. If the requested activity involves native species, provide information to show that the activity is consistent with any recovery plan for the species.
   e. Provide copies of contracts or agreements or other permits that identify persons involved and dates of activities for which the permit is sought.

8. If the proposed activity includes propagating or maintaining live plants at your facility, provide the following:

   a. Approximate number of specimens you currently maintain for each species requested.
   b. Describe the propagation method (e.g., seed, cutting, mericlone) used.
   c. Describe the conditions where the plants are grown and provide photographs of your facilities.
   d. Describe your background and experience working with this or similar species, including
      (i) the number of years each species has been cultivated by you; and
      (ii) the number of plants successfully propagated annually.
   e. Discuss your willingness to participate in a cooperative propagation program and maintain or contribute data regarding your propagation success with the species.
   f. Provide a copy of your State license and U.S. Department of Agriculture General permit, as appropriate.

9. If **import** or **export**, provide:
   a. Copy of any required foreign permits (for CITES Appendix-I plants provide a copy of import permit or evidence a permit will be issued). If plant is to be taken from the wild, provide documentation from the foreign government approving the action.
   b. Describe: (i) the type, size, and construction of shipping containers and (ii) the arrangements for watering and caring for the specimens during transportation.
   c. A statement on the disposition of all imported plants, plant material, and progeny, if produced.

10. Name and address where you wish permit mailed, if different from page 1 (All permits will be mailed via the U.S. Postal Service, unless you identify an alternative means below):

11. If you wish the permit to be delivered by means other than USPS regular mail, provide an air bill, pre-paid envelope, or billing information. If you do not have a pre-paid envelope or air bill and wish to pay for a courier service with your credit card, please check the box below. Please DO NOT include credit card number or other information; you will be contacted for this information.

    ☑ If a permit is issued, please send it via a courier service to the address on page 1 or question 9. I understand that you will contact me for my credit card information once the application has been processed.

12. Who should we contact if we have questions about the application? (Include name, phone number, and email):

    Marc Appelhans, ++49 5513922220, mappelh@gwdg.de

13. **Disqualification Factor.** A conviction, or entry of a plea of guilty or nolo contendere, for a felony violation of the Lacey Act, the Migratory Bird Treaty Act, or the Bald and Golden Eagle Protection Act disqualifies any such person from receiving or exercising the privileges of a permit, unless such disqualification has been expressly waived by the Service Director in response to a written petition. (50 CFR 13.21(c)) Have you or any of the owners of the business, if applying as a business, been convicted, or entered a plea of guilty or nolo contendere, forfeited collateral, or are currently under charges for any violations of the laws mentioned above?

    ☐ Yes ☑ No If you answered "Yes" provide: a) the individual's name, b) date of charge, c) charge(s), d) location of incident, e) court, and f) action taken for each violation.

Landwirtschaftskammer Niedersachsen

Landwirtschaftskammer Niedersachsen • Wunstorfer Landstr. 9 • 30453 Hannover

Pflanzenschutzamt
Fachbereich 3.7
Wunstorfer Landstraße 9
30453 Hannover
Telefon: 0511 4005-0
Telefax: 0511 4005-2215

Internet: www.lwk-niedersachsen.de

Bankverbindung
Landessparkasse zu Oldenburg
BLZ 280 501 00 | Kto 000-199 4599

To whom it may concern

Ihr Zeichen	Unser Zeichen	Ansprechpartner	In	Durchwahl	E-Mail	Datum
	SG 3.7.2	Brunhild Köhler		-2201	Brunhild.Koehler@LWK-Niedersachsen.de	05.04.2016

Information to the responsible Plant Protection Office about the import of seeds / dried plants into an EU-Member State:
Seeds from: *Melicope*, *Platydesma und Zanthoxylum* (Rutaceae), *Myrsine* (Primulaceae) and various dried plant samples

Importer:
Dr. Marc Appelhans, Curator of the Herbarium
University of Goettingen
Albrecht-von-Haller Institute for Plant Sciences Department of Systematic Botany
Untere Karspuele 2, 37073 Goettingen, Germany

This is to certify that the import of the **above mentioned seed species and of dried plants in general** into the Europian Union from third countries is allowed without phytosanitary measures or restrictions.

Kind regards

Brunhild Köhler
Plant Health
Import, Export, EU-Single Market

Deutscher Pflanzengesundheitsdienst • Pflanzengesundheitszentrale • Amtliche • NI 01

Project Description – Project Proposals

Principal investigator:
Dr. Marc S. Appelhans

Georg-August-Universität Göttingen, Albrecht-von-Haller-Institute for Plant Sciences, Department of Systematics, Biodiversity and Evolution of Plants, Untere Karspüle 2, 37073 Göttingen

Phone: ++49 (0) 551 3922220
Email: marc.appelhans@biologie.uni-goettingen.de

Title: Biogeography and evolution of the largest adaptive radiation of woody plants (*Melicope*, Rutaceae) on the Hawaiian Islands.

Project Description

1 State of the art and preliminary work

The proposed study aims at deciphering the evolutionary history, phylogeography and putative hybridization events of Hawaiian *Melicope* (*Citrus*- or Rue-family; Rutaceae) as an example of a recent and species-rich radiation of flowering plants.

The Hawaiian Islands are a perfect "natural laboratory" to study recent and species-rich radiations. With approximately 5 MA (millions of years) Kaua'i and Ni'ihau are the oldest of the current main islands and the archipelago ranks among the most isolated landmasses on the planet (Price & Elliott-Fisk, 2004). Due to their recent age and their isolated geography, only relatively few organismal lineages successfully colonized the archipelago and some of them underwent adaptive radiations that lead to great numbers of species (Funk & Wagner, 1995; Price & Wagner, 2004; Givnish *et al.*, 2009). There are often vast morphological differences between those species, which is usually contrasted by very low genetic variation (Baldwin & Sanderson, 1998; Cronk *et al.*, 2005; Knope *et al.*, 2012). The *Melicope* lineage is a very good example for this phenomenon as it shows a wide array of morphological variation (e.g., growth form, connation of carpels, leaf shape, indumentum), but low genetic variation (Wagner *et al.*, 1990; Harbaugh *et al.*, 2009; Appelhans *et al.*, 2014a, b). *Melicope* constitutes one of the most species-rich plant lineages on the Hawaiian archipelago and, with a total number of 52 species, it accounts for the largest lineage of woody plants (Wagner *et al.*, 1990). The bird-dispersed genus *Melicope* also occurs on other Pacific Islands, Australasia, Malesia, S Asia, Madagascar and the Mascarene Islands. Altogether, with about 230 species, *Melicope* is the largest genus of Rutaceae (Hartley, 2001). In the course of a postdoctoral project at the Smithsonian Institution (Washington DC, USA), the applicant studied the phylogeny and biogeography of *Melicope* and related genera. Six nuclear and plastid markers were sequenced using Sanger sequencing. The phylogenetic trees inferred from this dataset were highly supported and well resolved for most branches, but the Hawaiian clade showed relatively poor resolution (Fig. 1). Similar results are reported for other Hawaiian lineages (Baldwin & Sanderson, 1998; Cronk *et al.*, 2005; Knope *et al.*, 2012). Until the advent of Next Generation Sequencing (NGS) techniques, studies on recent and species-rich radiations were often hampered by the low quantity of DNA sequence information, which did not deliver enough variable sites to untangle the evolutionary histories of these rapid radiations (Maddison & Knowles, 2006; Rubin *et al.*, 2012; Eaton & Ree, 2013). Phylogenetic and biogeographic studies mostly do not require the sequencing of a complete and annotated genome or transcriptome. Instead, a sufficiently large number of single nucleotide polymorphisms (SNP's), which carry useful phylogenetic information, is needed. Restriction site associated DNA sequencing (RADseq; Baird *et al.*, 2008) is a very cost-effective NGS method to recover large amounts of SNP data across the genome. Because not the whole genome is sequenced using this method, several samples can be multiplexed and sequenced together on a single Illumina lane, which makes the method very time- and cost-effective (Baird *et al.*, 2008; Eaton & Ree, 2013). It is well suited for non-model taxa for which no

fully sequenced close relative is present, because it does not involve the assembly of whole genomes. So far, RADseq has mainly been used for population genetic studies (e.g., Narum *et al.*, 2013), but has recently shown its utility in resolving phylogenetic relationships at and below species-level and even postglacial radiations as well as the radiation of cichlid fishes in Lake Victoria – "the fastest known vertebrate species radiation" – have been untangled using this method (Emerson *et al.*, 2010; Rubin *et al.*, 2012; Cariou *et al.*, 2013; Eaton & Ree, 2013; Wagner *et al.*, 2013; Cruaud *et al.*, 2014; Hipp *et al.*, 2014; Ebel *et al.*, 2015; Herrera & Shank, 2015). At the time of submitting this proposal, no RADseq study based on Hawaiian plants has been published, so that the proposed study will serve as a case study in Hawaiian biogeography.

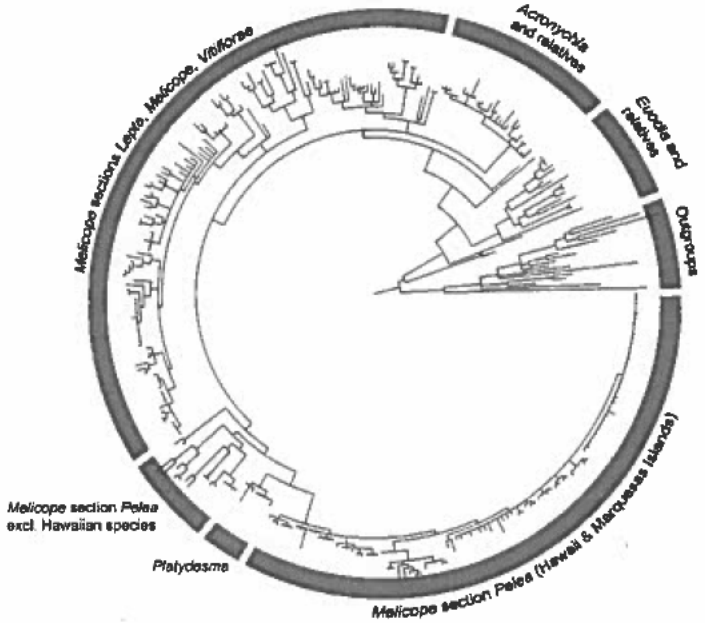

Fig.1: Bayesian consensus tree of *Melicope* and related genera based on Sanger sequencing of six chloroplast and nuclear markers. Most branches of the tree are well resolved and supported, except for the clade that contains the Hawaiian radiation (=*Melicope* section *Pelea* (Hawaii & Marquesas Islands) (adapted from Appelhans *et al.*, 2014b).

In the course of preparing this project proposal, we tested the suitability of the RADseq method to untangle phylogenetic relationships among Hawaiian *Melicope* species. In total 23 samples – 12 Hawaiian and 11 non-Hawaiian *Melicope* – were sequenced on one Illumina HiSeq 2500 using *Pst*I as restriction enzyme. All Hawaiian material used in our previous publications (Appelhans *et al.*, 2014a, b) did not deliver the sufficient DNA quantity needed for RADseq, and our collaboration partner Kenneth Wood (National Tropical Botanical Garden, Kalaheo, Hawaii/USA) sent us newly collected silica-material. Non-Hawaiian samples were taken from cultivated plants or used from recently collected material. This demonstrates the need for recently collected material for RADseq. Library preparation and sequencing were carried out by Eurofins MWG and we analyzed the DNA sequence reads with the program pyRAD (see "Lab work and data analyses" under 2.3 for details). A total of 78,614 RAD loci that included 75,233 phylogenetically informative sites were detected, and used for phylogenetic reconstruction using MrBayes 3.2.5 (Ronquist *et al.*, 2012). The resulting consensus tree showed very high resolution and support (Fig. 2). All except one node in the tree were highly supported (>0.95 posterior probability, pp). The only node that was not supported (0.77pp) was part of the backbone phylogeny suggesting that the RADseq method might not be suited to resolve deeper nodes due to problems with homology assessment of RAD loci. All nodes within the Hawaiian clade were strongly supported and the two species (*M. barbigera*, *M. degeneri*) of which more than one sample was sequenced were monophyletic (Fig. 2).

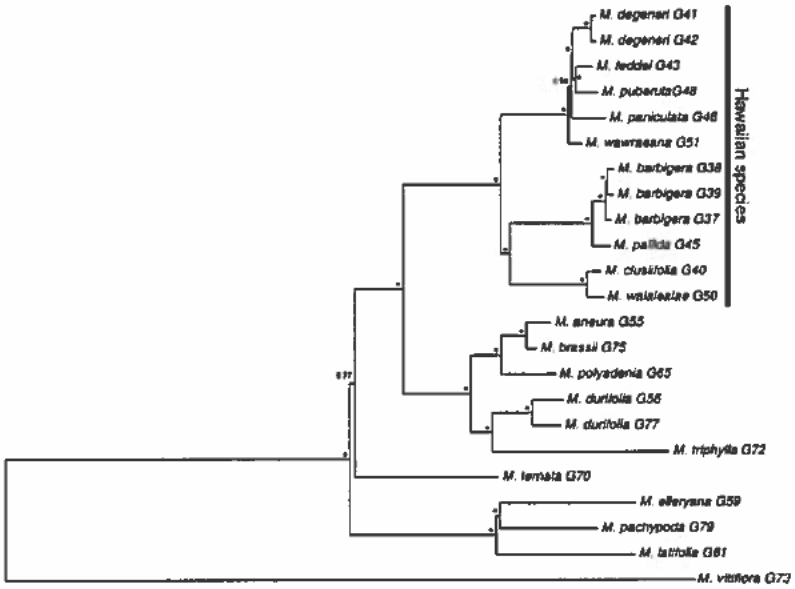

Fig.2: Preliminary RADseq results. Bayesian consensus tree of 23 *Melicope* specimens including 12 specimens from the Hawaiian Islands based on 75,233bp. The phylogenetic tree is completely resolved and all but one basal node are statistically supported (≥0.95pp). Nodes with maximum support (1.00pp) are marked with an asterisk (*). *M.* = *Melicope*. (Appelhans, unpublished results).

A resolved phylogeny of the large radiation of Hawaiian *Melicope* is the starting point of a series of analyses to answer biogeographic and evolutionary questions. The location of the Hawaiian Islands on a moving volcanic hotspot has created an archipelago in which islands are arranged in sequence with geological age (Price & Elliott-Fisk, 2004). Our preliminary molecular dating results (BEAST software; Drummond & Rambaut, 2007) inferred from our Sanger sequencing dataset point to a relatively old age of the Hawaiian *Melicope* lineage (Appelhans *et al.*, in preparation). We assume that the lineage was an early colonizer of the archipelago at a time when Nihoa and Necker (now small, rocky islands without forests) were established, but the oldest current main islands Kaua'i and Ni'ihau were still submerged. With each newly emerging island, *Melicope* could have colonized the archipelago in a stepwise manner (rule of progression; Funk & Wagner, 1995). The islands Maui, Lanai, Moloka'i and Kaho'olawe were merged into one island (Maui Nui) until Moloka'i started to separate at around 0.6 MA ago and the remaining islands were probably connected until the Last Glacial Maximum during periods with low sea level (Price & Elliot-Fisk, 2004). Because of alternating periods of low and high sea levels, islands were separated by the ocean repeatedly, and a vicariance pattern might be expected for species endemic to these islands (Funk & Wagner, 1995).

A common problem for plants colonizing new and distant areas is the small size of the founder population reflecting a low genetic diversity. Inbreeding depression, loss of genetic variation and accumulation of deleterious mutations are connected to higher extinction rates on island systems (Frankham, 1998). Polyploid taxa are thought to be better suited to colonize new areas than diploid taxa due to their higher genetic diversity (Seehausen, 2004; te Beest *et al.*, 2012). This pattern is very obvious in the Hawaiian flora, which has the highest incidence of polyploidy worldwide (Carr, 1998). However, not much is known about hybridization and ploidy levels in *Melicope*. Based on intermediate morphological characters, the New Zealand endemic *Melicope mantellii* is often considered to be a hybrid of the other two species (*M. simplex* & *M. ternata*) from New Zealand (Hartley, 2001). However, genetic studies verifying this hypothesis are still lacking. Hybridization in *Acronychia*, a very close relative of *Melicope* (Fig. 1; Holzmeyer *et al.*, 2015), has been observed (Rossetto, 2005). Chromosome numbers have been counted for only 21 out of about 230 *Melicope* species including nine Hawaiian species (Hartley, 2001; Kiehn, 2005, unpublished). With the data at hand it seems likely that the genus is diploid with a basic chromosome number of x=18 (2*n*=36).

Interestingly, one of the measured Hawaiian species shows a tetraploid pattern, which suggests that at least some of the Hawaiian species are polyploid. Up to seven species of *Melicope* are reported to grow in sympatry on the Hawaiian Islands (Wagner *et al.*, 1990), which suggests that there is a potential for hybridization. The indication that several of the measured Hawaiian *Melicope* species are diploid suggests that the earliest colonizing populations might have been diploid and that allopolyploid hybridization occurred later on during the radiation of the genus. A contrasting pattern of hybridization has been observed in other Hawaiian lineages, in which the hybridization events usually preceded the colonization events (Carr, 1998; Barrier *et al.*, 1999; Lindqvist & Albert, 2002; Lindqvist *et al.*, 2003; Soltis *et al.*, 2009; Baldwin & Wagner, 2010; Marcussen *et al.*, 2012; Roy *et al.*, 2013).
In the course of this project, we will determine the hybrid origin of a taxon by comparing the RADseq data (Partitioned D statistics test; see 2.3) with our results from flow cytometry and chromosomal counts. Prof. Dr. Michael Kiehn (University of Vienna) is a specialist on both Hawaiian plants and chromosome counting and will be involved in the chromosomal counting. He has already provided six unpublished chromosomal counts for Hawaiian *Melicope* and will be involved in measuring more species from plant material that will be collected during fieldwork and then cultivated in the Göttingen Botanical Garden. We tested if silica gel dried plant material of *Melicope* is suited for flow cytometry by measuring fresh material and one month old silica dried material obtained from the same *Melicope triphylla* plant cultivated at Göttingen Botanical Garden. Our measurements are highly congruent and ascertain that the species is diploid with a C-value of about 0.90pg. We also tested older (2007-2010) silica-dried material of Hawaiian species and these samples did not deliver clear results, thus we confirm the findings by Suda & Trávníček (2006), who do not recommend using material older than 20 months. Our measurements will be based on the silica gel material and plants grown from seeds collected in the course of this study.

1.1 Project-related publications

Holzmeyer L, Duretto M, Crayn D, Hörandl E, Heslewood M, Jayanthan J, **Appelhans MS. 2015**. Phylogeny of *Acronychia* (Rutaceae) and first insights into its historical biogeography and evolution of fruit characters. *PLoS ONE* **10:** e0136296.

Appelhans MS, Wen J, Wagner WL. **2014**. A molecular phylogeny of *Acronychia*, *Euodia*, *Melicope* and relatives (Rutaceae) reveals polyphyletic genera and key innovations for species richness. *Molecular Phylogenetics and Evolution* **79:** 54–68.

Appelhans MS, Wen J, Wood KR, Allan GJ, Zimmer EA, Wagner WL. **2014.** Molecular phylogenetic analysis of Hawaiian Rutaceae (*Melicope*, *Platydesma* and *Zanthoxylum*) and their different colonisation patterns. *Botanical Journal of the Linnean Society* **174:** 425–448.

Appelhans MS, Wagner WL, Wood KR. **2014**. *Melicope balgooyi* Appelhans,W.L.Wagner & K.R.Wood, a new species and new record in *Melicope* section *Melicope* (Rutaceae) for the Austral Islands. *PhytoKeys* **39:** 77–86.

Appelhans MS, Janssens SB, Smets E, Razafimandimbison SG, Keßler PJA. **2012.** Age and historical biogeography of the pantropically distributed Spathelioideae (Rutaceae, Sapindales). *Journal of Biogeography* **39:** 1235–1250.

Appelhans MS, Van Heuven BJ, Lens F, Baas P. **2012.** Phylogenetic and ecological signals in the wood of the Spathelioideae (Rutaceae). *IAWA Journal* 33: 337-353.

Appelhans MS, Smets E, Razafimandimbison SG, Haevermans T, van Marle EJ, Rabarison H, Couloux A, Randrianarivelojosia M, Keßler PJA. **2011.** Phylogeny, evolutionary trends, and classification of the *Spathelia* / *Ptaeroxylon* clade: morphological and molecular insights. *Annals of Botany* **107:** 1259–1277.

Razafimandimbison SG, **Appelhans MS**, Rabarison H, Haevermans T, Rakotondrafara A, Rakotonandrasana SR, Ratsimbason M, Labat J-N, Keßler PJA, Smets E, Cruaud C, Couloux A, Randrianarivelojosia M. **2010**. Implications of a molecular phylogenetic study of the Malagasy genus *Cedrelopsis* and its relatives (Ptaeroxylaceae). *Molecular Phylogenetics and Evolution* 57: 258-265.

Appelhans MS, Smets E, Baas P, Keßler PJA. **2010.** *Cneorum* (Rutaceae) in Cuba? The solution to a 150 year old mystery. *Taxon* 59: 1126-1134.

Appelhans M, Weber HC, Imhof S. **2008.** Rutaceae sampled from Germany, Malta, and Mallorca (Spain) are associated with AMF clustering with *Glomus hoi* Berch & Trappe. *Mycorrhiza* 18: 263-268.

2 Objectives and work programme

2.1 Anticipated total duration of the project

The project is designed for a total duration of three years. See time schedule in 2.3.

2.2 Objectives

Within the proposed project we will study the evolutionary history of Hawaiian *Melicope* as an example of a recent and species-rich plant radiation. The main bottleneck until now has been the lack of phylogenetic resolution (Appelhans *et al.*, 2014a), which hindered the understanding of mechanisms of speciation and colonization. RADseq is a promising approach to better resolve the phylogeny and our preliminary study has proven that it is perfectly suited for Hawaiian *Melicope* (Fig. 2). RADseq opens the door to more integrative studies on species radiations on the Hawaiian Islands. The proposed project involves RADseq of 120 specimens of the genus *Melicope* with subsequent phylogenetic reconstruction, biogeographical analyses and hybridization/introgression studies. The latter will also involve flow cytometry and chromosome counting. Our results will deliver new and important insights into island biogeography, hybridization and the question of how to tackle lineages with low genetic variation. The project will be among the first plant radiations studied with RADseq and the very first for the Hawaiian Islands. The exploration of RADseq data for molecular dating and diversification analyses is at the very beginning (Cavender-Bares *et al.*, 2015), so that our study will be among the very first case studies using RADseq in biogeographical analyses.

Our particular research questions and hypotheses are as follows:

1. RADseq method & Phylogenetic reconstruction
Previous molecular phylogenetic studies on Hawaiian *Melicope* (Harbaugh *et al.*, 2009; Appelhans *et al.*, 2014a, b) were successful in resolving the major clades and showed that the Hawaiian Islands were not a dead-end for dispersal. However, a resolution at species-level was not possible using Sanger sequencing. Our preliminary RADseq dataset containing 12 Hawaiian and 11 non-Hawaiian *Melicope* specimens delivered 75,233 SNPs and the resulting phylogenetic tree was completely resolved and statistically supported for the Hawaiian taxa (Fig. 2). This means a nearly 100-fold increase in variable characters as compared to the six-marker dataset used by Appelhans et al. (2014a; 768 variable characters of which 430 were parsimony-informative).
A resolved phylogeny is the first goal of the proposed study and questions related to the monophyly of sections and species will be answered. The resolved phylogeny will also be the basis for the following questions. The proposed study will be the first NGS project about a major adaptive radiation on the Hawaiian Islands and it will serve as a case study for future studies on Hawaiian taxa and studies in other geologically young areas or young species groups in which Sanger Sequencing does not provide sufficient data quantity.

2. (Hawaiian) Biogeography
When and where did the ancestor of Hawaiian *Melicope* arise, when was the archipelago colonized and how did *Melicope* spread over the Hawaiian Islands?
Our hypotheses based on preliminary results (Appelhans *et al.*, in preparation) are that the ancestors of Hawaiian *Melicope* stem form New Guinea and/or nearby islands and that the Hawaiian lineage is slightly older than the current islands, suggesting that older islands of the Hawaiian-Emperor seamount chain such as Nihoa and Necker were colonized first. From there,

Kaua'i and the younger islands were colonized. Judging from the high number of species and the fact that all four sections of Hawaiian *Melicope* are present on Kaua'i (Wagner *et al.*, 1990), an initial radiation on this island seems likely. With the emergence of the younger islands, new areas became available for colonization and our hypothesis is that they were colonized in a stepwise manner mainly from the older to the younger islands.

Our existing Sanger sequencing data (Appelhans *et al.*, 2014a) does not allow many biogeographical conclusions, but the habitat preferences of Hawaiian *Melicope* shows an interesting pattern. Most Hawaiian *Melicope* species (79%) are single-island endemics and many of these species are restricted to wet and bog forest habitats. The few multi-island species (species that occur on more than one Hawaiian Island) of *Melicope* are found also in dry and mesic forest. Hawaiian lobeliads (Campanulaceae) show a similar pattern (Givnish *et al.*, 2009). Givnish *et al.* (2009) argued that forest-interior bird species, which disperse the diaspores, often baulk at habitat barriers. The same could be true for the wet-forest species of the bird-dispersed *Melicope*. Dispersal by very stationary birds would mean very restricted dispersal capabilities, so that dry and mesic ancestors might account for most inter-island dispersal with repeated dispersal into wet-forest habitats. Figure 3 pictures this scenario and shows the placement of wet- and dry-habitat species in a phylogenetic tree. The rare occasions in which a wet-forest species disperses to an adjacent island would immediately lead to a genetic barrier with a potential of subsequent speciation. In this case, the phylogenetic trees would show wet-forest species (or species groups) from adjacent islands as sister groups.

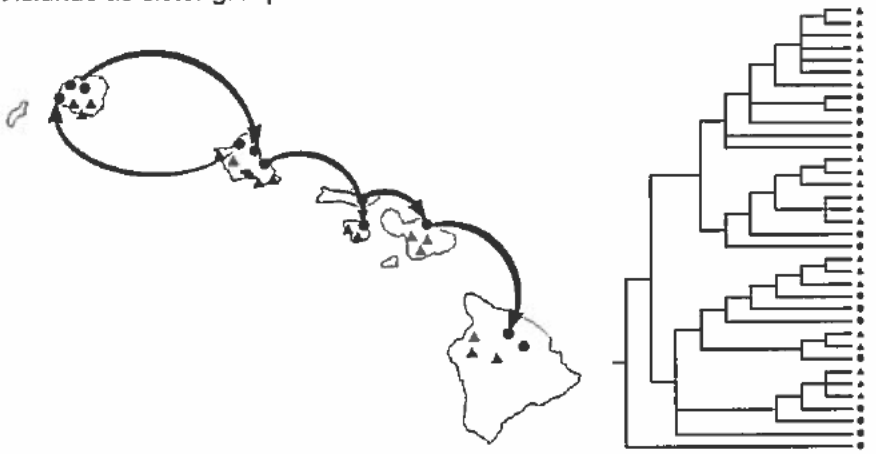

Fig.3: A possible colonization scenario of Hawaiian *Melicope*. Taxa that are adapted to dry or mesic habitats (black dots) account for the inter-island colonization (black arrows; mainly from older to younger islands) while wet-forest taxa (gray triangles) descended from dry or mesic taxa and hardly cross the barrier to another island. In a phylogenetic tree, dry or mesic taxa would mainly represent early diverging lineages that are sister to clusters of wet-forest taxa.

The major modes of speciation of Hawaiian *Melicope* might be geographic isolation (relatively rare inter-island colonizations) and ecological isolation (adaptation to different habitats [e.g. moist forest habitats]). An additional factor, especially in areas where several *Melicope* species co-occur, could be hybridization, a topic that will be discussed in the following paragraph.

3. Hybridization & Species-richness

Do several Hawaiian *Melicope* species have a hybrid origin? Did this hybridization happen before or after the colonization of the archipelago? Did the hybridization event(s) have an influence on the net diversification rates (hybridization as driver of adaptive radiation)? Are homoploid hybrids more common than allopolyploid hybrids?

The available information on chromosome numbers and ploidy levels in Hawaiian *Melicope* (Hartley, 2001; Kiehn, 2005, unpublished), suggests that the ancestor of Hawaiian *Melicope* was diploid and that polyploidization occurred at least once on the Hawaiian Islands. The tetraploid species *M. wawraeana* belongs to the most species-rich clade of Hawaiian *Melicope*, which

includes about 30 species (Appelhans *et al.*, 2014a). The species has been confused with *M. oahuensis* and *M. peduncularis* and its fruits sometimes approach the features of both species (Wagner *et al.*, 1990). We will test the hypothesis that *M. oahuensis* and *M. peduncularis* are the potential parent species of *M. wawraeana*. In addition to the tetraploid *M. wawraeana*, chromosome counts for three more species of the species-rich clade are known (Kiehn, 2005, unpublished). The three species are diploid ($2n$=36) and our hypothesis is that the ancestor of the clade was diploid and that allopolyploid hybridization occurred at least once in this clade.
Allopolyploidy is the result of a meiotic mismatch that might be expected when two less closely related species hybridize (Seehausen, 2004; Buggs *et al.*, 2009; Paun *et al.*, 2009). The Hawaiian radiation of *Melicope* is relatively young and genetic divergence is probably low. One might therefore not expect meiotic mismatches during a putative hybridization event in this case, and hybrid species would have the same chromosome number as the parent species. Our hypothesis is that homoploid hybrids might exist in Hawaiian *Melicope* and that they are more common than polyploid hybrids. Analysis of the RADseq data will directly enable us to differentiate between homoploid hybrids and their parent species, and also between allopolyploid and autopolyploid species (see paragraph about the Partitioned D-statistics test in 2.3). Up to seven species of *Melicope* occur sympatrically on the Hawaiian Islands (Wagner *et al.*, 1990) so that an important requirement for hybridization exists. Another important requirement for hybrid speciation is genetic isolation between the hybrid and its parental taxa. In allopolyploids this is directly achieved through reproductive isolation caused by the different chromosome numbers of gametes. Seehausen (2004) argued that some hybrid genotypes might be better adapted in extreme habitats and novel niches compared to their parent taxa, while at the same time showing lower fitness under parental ecological conditions. Transgressive segregation in F2 and later hybrid generations is the putative mechanism behind this type of hybrid speciation (Rieseberg & Willis, 2007). In this way, hybrids would be ecologically isolated from the parents and might develop into new species. Seehausen (2004) hypothesized that hybridization might be a driver of adaptive radiation in unstable or new environments. *Melicope* probably arrived very early in the history of the Hawaiian Islands (Appelhans *et al.*, in preparation). Molecular dating of most other Hawaiian plant lineages suggests a younger age than the oldest of the current main islands (Lindqvist & Albert, 2002; Clark *et al.*, 2009; Havran *et al.*, 2009; Keeley & Funk, 2011; Knope *et al.*, 2012; Sebastian *et al.*, 2012) so that one might expect *Melicope* to be among the first colonizers of the then underutilized and developing ecological niches on the single islands. Many Hawaiian plant lineages are paleopolyploids (Carr, 1998; Barrier *et al*, 1999; Lindqvist & Albert, 2002; Lindqvist *et al.*, 2003; Soltis *et al.*, 2009; Baldwin & Wagner, 2010; Marcussen *et al.*, 2012; Roy *et al.*, 2013) and *Melicope* is among the few Hawaiian lineages that show different ploidy levels. *Melicope* therefore is a perfect model taxon to test Seehausen's (2004) theories with modern NGS methods.

2.3 Work programme incl. proposed research methods

Study taxa
Melicope is an ideal model taxon to study island biogeography. Of the total +/- 230 species in this genus, 52 are endemic to the Hawaiian Islands making *Melicope* the largest Hawaiian radiation of woody plants (Hartley, 2001; Wagner *et al.*, 1990). The species grow at a number of different habitats including bog forests, wet forests, mesic forests and dry forests. About 79% of the species, especially those adapted to very humid areas (wet forests and bogs), are single island endemics. The patchy information on chromosome numbers suggests that at least some Hawaiian species are polyploid (Hartley, 2001; Kiehn, 2005, unpublished). Wagner *et al.* (1990) highlight that as many as seven species of *Melicope* occur sympatrically in some areas.

Fieldwork on the Hawaiian Islands
The samples for this project have to be collected in the wild. RADseq requires high quality and quantity of DNA and four weeks of fieldwork are therefore planned in the first year of the project. Due to the high proportion of single-island endemics, collecting on all larger islands (Kaua'i, O'ahu, Maui, Moloka'i and Hawai'i) is necessary in order to obtain a sufficiently high taxon sampling. The

fieldwork will be carried out by the principal investigator and the PhD student. Kenneth Wood (National Tropical Botanical Garden [NTBG], Kalaheo, Kaua'i) has agreed to assist during the fieldwork and Warren Wagner (Washington DC) will help to assemble a list of locations that will be visited. Several collection and export permits are needed (separate permit for each National Park). We will apply for these permits prior to the start of the project together with our counterparts from NTBG (Kenneth Wood, David Lorence) as well as Warren Wagner and Michael Kiehn, who are both affiliated to NTBG (Wagner: McBryde Chair of Hawaiian Plant Studies; Kiehn: Research Associate).
During the fieldwork, about 120 specimens of most Hawaiian *Melicope* species will be collected. Some species are regarded as (possibly) extinct, so that our taxon sampling will not reach 100% of the described species. Our goal is to sample most species with two or three specimens and to include up to five samples for species that either occur on several islands (e.g., *M. clusiifolia*, *M. hawaiiensis*, *M. peduncularis*, *M. volcanica*), that are very variable (e.g., *M. clusiifolia*, *M. hawaiiensis*, *M. knudsenii*) or for which the delimitation towards other species is not clear (e.g., *M. elliptica* species complex, *M. kavaiensis* species complex, *M. oahuensis*/*M. anisata*, *M. wawraeana*).
Seeds, needed for germination and chromosome counting on root tips, will be collected for cultivation at Göttingen Botanical Garden. Leaf material for RADseq and flow cytometry will be collected in silica gel. Three duplicates of herbarium vouchers will be collected and one set of specimens will be sent to Göttingen University, Washington DC (Smithsonian Institution), and NTBG (Kalaheo, Kaua'i/ Hawaii) each, so that the involved cooperation partners will have access to the collections and can help with the identification of specimens. We will digitize the Göttingen specimens and make the scans and metadata available through the webpage of the Göttingen herbarium, so that researchers worldwide have the possibility to review and verify the species identifications.

Lab work and data analyses

Lab work will be carried out by the PhD student and a technical assistant (Jennifer Krüger).

Sequencing will be performed on 120 samples (40 species, 3 individuals per species) collected during our field trip as well as five non-Hawaiian *Melicope* species as outgroups. Outgroup-samples have been sequenced in the course of generating preliminary data and sequencing can be repeated if necessary from *Melicope* species cultivated in the Botanical Gardens in Göttingen (*Melicope elleryana*, *M. triphylla*, *M. ternata*), Leiden (Netherlands; *M. denhamii*) and NTBG (*M. latifolia*).
Total DNA will be extracted using the DNeasy Plant Mini kit (Qiagen, Hilden, Germany). We will do two extractions per sample in order to assure that the high DNA quantity needed for RADseq (~5µg) is provided. DNA quantification will be measured using a Qubit 3.0 fluorometer (Life Technologies, Darmstadt, Germany). The actual library preparation and sequencing will be outsourced to Eurofins MWG Operon (Ebersberg, Germany; http://www. eurofinsgenomics.eu/), which is – to our knowledge – the only sequencing company in Germany that offers RADseq service. DNA will be digested with the 6-base cutter restriction enzyme *Pst*I, which has a large number of cutting sites in the genomes of both animals and plants (Davey & Blaxter, 2011; Eaton & Ree, 2013; Wagner *et al.*, 2013) and yielded more than 75,000 RAD loci in our preliminary study. A barcoded P1 adapter (Primer site & adapter that binds to Illumina flow cell & barcode for individual samples so that several samples can be multiplexed) will be ligated to the ends of the digested DNA. The barcoded DNA samples will be sheared by sonication in order to generate fragments of a length of several hundred basepairs (bp). For size selection, the multiplexed samples will be run on an electrophoresis gel and fragments of approximately 300-500bp will be isolated by gel extraction. We expect tetraploid and hybrid species in Hawaiian *Melicope*, for which high coverage (at least 50 fold) is essential to identify heterozygous SNPs (Hirsch & Buell, 2013). Multiplexing of 23 samples on one lane (23 samples plus one internal control) will be appropriate for this coverage (see cost estimation by Eurofins MWG GmbH). A P2 adapter, which carries the reverse primer sequence, will finally be ligated to the fragments, which will then be ready for single-end

sequencing using an Illumina HiSeq 2500 system (Etter *et al.*, 2011; read length 100bp; 150-180 million reads per lane).
A free software pipeline (*pyRAD*; http://pyrad.googlecode.com) for processing Illumina FASTQ sequence files into a multi-sequence alignment has been developed by Dr. Deren Eaton (Eaton & Ree, 2013; Eaton, 2014), who will be involved in our project as a collaborator. *pyRAD* will be used to de-multiplex the Illumina sequence data, trimming barcodes, restriction sites and adapters as well as removing low quality sequence reads. Sequences of each de-multiplexed sample are clustered by similarity using USEARCH (Edgar, 2010) and clusters with low coverage will be deleted. The remaining clusters will be used for SNP calling and to calculate consensus sequences for each sample using the MUSCLE algorithm (Edgar, 2004). Multi-sequence alignments will be generated from the consensus sequences using MUSCLE and used for further analysis.
In addition to *pyRAD*, which we used for our preliminary data analysis, we will explore other analysis software such as stacks (Catchen *et al.*, 2011), AftrRAD (Sovic *et al.*, 2015) and the CLC genomics workbench (Aarhus, Denmark). RADseq datasets often contain a larger amount of missing data because some RAD loci will not be sequenced for all samples (most likely due to mutations in the cutting sites). The effect of missing data has been explored by creating different data matrices that consisted of as many RAD loci as possible and allowing RAD loci to be missing in several samples, versus including only RAD loci that were available for most samples (trade-off: number of included RAD loci vs. amount of missing data for each RAD locus) (Eaton & Ree, 2013; Wagner *et al.*, 2013; Chattopadhyay *et al.*, 2014). We will assemble and analyze different datasets accordingly to find a balance between missing data and number of loci included. A known problem with RADseq is the orthology assessment of RAD loci (Rubin *et al.*, 2012; Leaché *et al.*, 2015). Low orthology of RAD loci is correlated with mutations in the cutting sites of restriction enzymes and are thus more problematic with higher genetic variation and old clade ages (>50MA; Rubin *et al.*, 2012). In a study (Cavender-Bares *et al.*, 2015) focusing on a group of oak species that were slightly older than the estimates for Hawaiian *Melicope* (Appelhans *et al.*, in preparation), homology assessment proved not to be problematic. This, together with the results of our preliminary study, indicates that homology assessment will most probably not be a critical point for the planned study.
Phylogenetic analyses of the preliminary RADseq dataset were done using MrBayes 3.2.4 (Ronquist *et al.*, 2012). However, the final datasets will contain much more data, so that phylogenetic analyses will be carried out using mainly fast maximum likelihood (ML) algorithms implemented in programs such as RAxML (Stamatakis, 2006) and GARLI (Zwickl, 2006). We are working together with the *Gesellschaft für wissenschaftliche Datenverarbeitung mbH Göttingen* (GWDG) who are maintaining high-performance computer clusters on which we will run the analyses. We will further explore the feasibility of Bayesian Inference using MrBayes (Ronquist *et al.*, 2012), Phycas (Lewis *et al.*, 2015) and BUCKy (Larget *et al.*, 2010) using partial datasets (e.g. using only variable sites). Hybridization networks as implemented in SplitsTree 4.0 (Huson & Bryant 2006) will be used to study reticulate evolution and to reconstruct hybrid-parent relationships (Pirie *et al.*, 2009; Pellino *et al.*, 2013).

We will explore the use of RADseq for molecular dating and will also carry out molecular dating analyses based on the already existing Sanger sequencing data (Appelhans *et al.*, 2014b) for comparison. No fossils of Hawaiian *Melicope* are known, but a root age of the lineage can be taken from our biogeographical work on the whole genus (Appelhans *et al.*, in preparation). In a second approach we will use the age of islands as calibration points for dating. Molecular dating analyses will be performed using the BEAST package (Drummond & Rambaut, 2007). Additionally, we will perform diversification analyses using BAMM (Rabosky, 2014), MEDUSA (Alfaro *et al.*, 2009) and BayesRate (Silvestro *et al.*, 2011). A particular interesting feature of the BAMM package is its ability to account for missing taxa and the non-random distribution of missing taxa in the phylogeny. Net diversification rates between different subclades will be compared and a specific hypothesis that will be tested is that hybridization events with subsequent speciation might have caused diversification rate changes.

In order to trace back inter-island colonization patterns, we will perform ancestral area analyses (AAR) using the programs BioGeoBEARS (Matzke, 2013) and Lagrange (Ree *et al.*, 2005; Ree & Smith, 2008). An advantage of both programs in comparison with other AAR methods is the

possibility to exclude certain areas during certain geological epochs and to allow the dispersal probabilities between two areas to change through time. In our case, the age/emergence of each Hawaiian island (and its availability for colonization) can be incorporated into the analyses. BioGeoBEARS also features a model that accounts for founder-event speciation ("jump-dispersal"), which is an important model in islands systems and which has not been implemented in other AAR programs so far. In addition, ecological parameters such as habitat preferences and altitudinal ranges will be plotted on the phylogenetic trees using Mesquite (Maddison & Maddison, 2011) in order to shed further light on the colonization and occupation of ecological niches of species.

Screening for hybridization and polyploidy will involve three aspects. To test for polyploid taxa, we will carry out chromosome counts based on root tips from germinated seeds and we will determine genome sizes and ploidy levels using flow cytometry. Flow cytometry will be done using silica gel dried leaves and seedlings on a Partec CyFlow Space flow cytometer (Münster, Germany) with the DAPI (4', 6-diamidino-2-phenylindole) fluorochrome applying a standard protocol (Hojsgaard *et al.*, 2012). The differentiation between auto- and allopolyploid taxa and also the differentiation between parent species and homoploid hybrids will be done using the RADseq data. Hybrid taxa are characterized by high levels of heterozygosity because alleles were inherited by parents that belong to different species. The *pyRAD* package contains the Partitioned D-statistics test, which detects introgression and the directionality of gene flow, based on the occurrence of heterozygosity in the RADseq data (Eaton & Ree, 2013). Based on five-taxon subsets of a defined tree topology (from the phylogenetic analyses), the test monitors alleles (SNPs) that are incongruent with the tree topology. There are six possibilities of how an incongruency can be distributed in a five-taxon tree (Fig. 4, right side). If one incongruency pattern is dominant across all alleles, the incongruence is asymmetric. While a symmetric incongruence would be a signal of stochastic lineage sorting, asymmetric incongruence points to introgression.

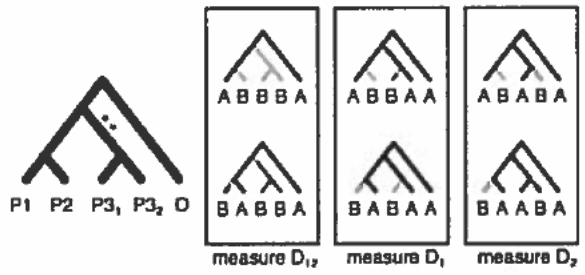

Fig.4: Principle of the Partitioned D statistics test. A derived character (symbol B) is shared by $P3_1$ and/or $P3_2$ with one of the two taxa P1 and P2 (patterns: ABBBA, BABBA, ABBAA, BABAA, ABABA, BAABA). The distribution of that particular character is incongruent with the fixed tree topology. If the overall frequency of one of the patterns across the genome (=across the RAD loci, as they represent a random subset of the whole genome) is higher than the frequency of the other patterns, the incongruence is asymmetric which is a clear indicator of introgression. In case ABBBA is the dominant pattern, there would have been introgression between P2 and the ancestor of $P3_1$ and $P3_2$. Figure adapted from Eaton & Ree (2013).

During the proposed study, we will perform the Partitioned D statistics test based on a series of five-taxon subsets. We will focus on taxa for which chromosomal counts and flow cytometric measurements show differing ploidy levels, taxa growing in sympatry (Wagner *et al.*, 1990), and species-complexes that are morphologically difficult to untangle or show intermediate characters (Wagner at al., 1990; i.e. *M. elliptica* species complex, *M. kavaiensis* species complex, *M. oahuensis*/*M. anisata*). The results of the hybridization studies will be compared with the phylogenetic analyses, the BEAST analyses, and the diversification rate analyses in order to evaluate the time of origin(s), the number of hybridization events and the effect of hybridization on net diversification.

Time schedule

	First year				Second year				Third year			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
Fieldwork Hawaii												
Preparation	X											
Collection of plant material		X										
Lab work & analyses												
RADseq (DNA extraction and QC; further steps done by company)		X	X	X								
Seed germination		X	X	X								
Flow cytometry				X	X							
Chromosome counting				X								
Data analysis					X	X	X	X	X	X		
Writing manuscripts							X			X	X	X
Conferences							X				X	

The application for collecting permits will be handled prior to the start of the project and will be done in cooperation with Warren Wagner (Smithsonian Institution, Washington DC/USA) and Kenneth Wood (National Tropical Botanical Gardens; Kalaheo, Hawaii/USA).

2.4 Data handling

The sequence data will be stored on servers of Göttingen University and published sequence data will be uploaded to EMBL/Genbank. Microscopic images and flow cytometric histograms will be saved and stored in Göttingen. Silica-dried leaf samples and DNA extracts will be kept at -80°C at Göttingen University. Herbarium specimens (3 duplicates of each collection) will be stored in the herbaria of Göttingen University (GOET), the Smithsonian Institution (US) and NTBG (PTBG). In order to provide direct access to the collected herbarium vouchers for researchers worldwide, we will digitize (scanning and databasing) the GOET specimens and make them available through our webpage. Living plants from collected seeds will be kept for further cultivation at the Botanical Garden of Göttingen University.

2.7 Information on scientific and financial involvement of international cooperation partners

See 5.4.1 for a list of cooperation partners and their scientific involvement in the project. The two main cooperation partners will be Dr. Warren Wagner and Dr. Jun Wen (both Smithsonian Institution, Washington DC, USA). Dr. Wagner has worked on the Hawaiian flora and biogeography for many years. He is an expert on Hawaiian *Melicope* and he will be participating in many parts of the project, e.g. assembling a list of locations that will be visited during the fieldwork, identifying species, discussing the results and writing manuscripts. Dr. Wen is an expert in the fields of phylogenetics and biogeography. She leads ongoing projects using several NGS methods including among others RADseq. Dr. Wen has been involved in the study design (e.g., choice of restriction enzyme, number of multiplexing) and she will participate in data analysis, discussing results and writing manuscripts.
Dr. Deren Eaton (Yale University) is a specialist in the field of Next Generation Sequencing and he developed and maintains the software pipeline *pyRAD*. He will be involved in data analysis. Prof. Dr. Michael Kiehn from the University of Vienna is an expert on chromosome counting and he has extensive knowledge about the Hawaiian flora. He will be involved especially in chromosome counting and discussing hybridization and polyploidization. Kenneth Wood is a research botanist at NTBG and has excellent knowledge on the Hawaiian flora and *Melicope*. He will be involved in the application for collection permits, the field trip and the identification of specimens. We are also in

contact with other researchers from Hawai'i (Dr. Marian Chau [University of Hawai'i at Manoa], Dr. Shelley James [Bishop Museum, Honolulu] Dr. David Lorence [NTBG], Hank Oppenheimer [Maui Nui Plant Extinction Prevention Program, Maui], Lara Reynolds [Department of Land & Natural Resources, Pearl City/O'ahu]) and these contacts will be helpful during the fieldwork.
No financial involvement of the international cooperation partners is intended.

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4 Requested modules/funds

4.1 Basic Module

Total amount (staff, consumable, travel, publication costs) requested: 166,985.50€

4.1.1 Funding for Staff

Salary for a PhD student (N.N., 65% of TV-L, E13) is requested for three years.
Quantity: 0,65
Sum per year: 39,390.00€ (according to DFG Personnel Rates for 2015 [65% of 60,600.00€])
Total: 118,170.00€

Student assistant (N.N.) for the digitization of herbarium specimens: 50h in the first year of the project. The hourly rate from Göttingen University for a student assistant with a BSc degree is 14.91€ (including taxes and employer´s share of social security contributions).
Total: 745.50€

4.1.2 Direct Project Costs

In addition to the funding for staff, we are requesting funds for lab work, travel (fieldwork, conferences) and publication costs.

4.1.2.1 Equipment up to €10,000, Software and Consumables

Total amount requested: 27,900.00€

Consumables	
Description	**Amount**
Fieldwork Hawaii	
Permits, sending costs, silica gel, bags, herbarium sheets, paper tags	500.00€
Cultivation of plants	
Seed germination (pots, substrates, labels)	250.00€
RADseq	
DNA extraction (DNeasy Plant Mini Kit, 120 samples, double extraction; 682.20€ plus VAT)	811.82€
DNA quality and quantity check (240 samples) using the Qubit 3.0 Fluorometer (Qubit dsDNA HS Assay Kit, 250.64€ plus VAT)	298.26€
Spot quality assessment/quality control at the sequencing company (Eurofins MWG) (220€ plus VAT)*	261.80€
DNA normalization (Eurofins MWG) (1000€ plus VAT)*	1,190.00€
RADseq library preparation (Eurofins MWG) (120 samples à 90€ plus VAT)*	12,852.00€
Sequencing costs (Eurofins MWG; single-end Illumina HiSeq) (120 samples multiplexed on five channels à 1920€ plus VAT)*	11,424.00€
Data management fee (Eurofins MWG) (200€ plus VAT)*	238.00€

Flow cytometry	
Flow cytometric measurement (120 samples à 0.30€) and glassware	74.12€
Total	27,900.00€

*See the attached price offer by Eurofins MWG GmbH.

4.1.2.2 Travel Expenses

Total amount requested: 17,920.00€

Fieldwork to collect plant material is needed in the first year of the project.

We request support for participation in international conferences for the applicant (principal investigator) and the PhD student in the second and third year of the project (1500€ per person per conference visit). We are envisaging the "XIX International Botanical Congress" in Shenzen (China) in 2017 and the North American "Botany 2018" meeting (organized among others by ASPT [American Society of Plant Taxonomists]; venue not announced yet).

Travel expenses First year	
Description	**Amount**
Fieldwork in Hawaii	
2x airfare Frankfurt - Honolulu	3.000€ (2x 1.500€)
2x airfare between Hawaiian Islands	1.400€ (2x 700€)
Car rental and gasoline (24 days x 80€)	1.920€
Accommodation for Principal Investigator and PhD student (28 days x 60€ per person)	3.360€
Per diem (28 days x 40€ per person)	2.240€
Total	11.920€

Travel expenses Second year	
Description	**Amount**
Conference	
2x travel, accommodation and conference fee (Principal Investigator and PhD student)	3000€ (2x 1500€)
Total	3000€

Travel expenses Third year	
Description	**Amount**
Conference	
2x travel, accommodation and conference fee (Principal Investigator and PhD student)	3000€ (2x 1500€)
Total	3000€

4.1.2.6 Project-related publication expenses

750€ are requested per year (2250€ in total). We will publish our results in ISI ranked, peer-reviewed journals within the field of plant systematics, biogeography and hybridization (e.g., Journal of Biogeography, Molecular Phylogenetics and Evolution, Annals of Botany, Systematic Biology, Evolution).

5 Project requirements

5.1 Employment status information

Appelhans, Marc S., Dr; Scientific staff and curator of the university herbarium (until 3/2019), University of Göttingen

5.2 First-time proposal data

Appelhans, Marc S., Dr. (see 5.1)

5.3 Composition of the project group

Scientific group members

Appelhans, Marc S., Dr.; see above (5.1); *Principal investigator*

Hörandl, Elvira, Univ. Prof., Dr.; Professor for Systematics, Biodiversity and Evolution of Plants (permanent position), University of Göttingen; *Polyploidy & Hybridization, supervisor of PhD student*

Hojsgaard, Diego, PhD; Assistant position in the Department of Systematics, Biodiversity and Evolution of Plants (until 12/2019), University of Göttingen; *Polyploidy, Chromosome counting & Flow cytometry techniques*

Non-scientific group members

Krüger, Jennifer; Lab technician in the Department of Systematics, Biodiversity and Evolution of Plants (permanent 100%), University of Göttingen; *Assisting molecular lab work and preparation of samples for Flow Cytometry*

Friedrichs, Sylvia, Schmidt Sabine; Gardeners in the Department of Systematics, Biodiversity and Evolution of Plants (both permanent 50%), University of Göttingen; *Cultivation of collected seeds form field trip to Hawaii in greenhouses of the Botanical Garden*

Freundt, Heike; Secretary in the Department of Systematics, Biodiversity and Evolution of Plants (permanent 50%), University of Göttingen; *Help with administration of the project*

Liessmann, Gabriele; Herbarium assistant in the Department of Systematics, Biodiversity and Evolution of Plants (permanent 50%), University of Göttingen; *Preparing and mounting of herbarium specimens*

5.4 Cooperation with other researchers

5.4.1 Researchers with whom you have agreed to cooperate on this project

Wagner, Warren L., PhD; Research Scientist, Curator & Chair of Botany, Smithsonian Institution, Washington DC/ USA & McBryde Chair of Hawaiian Plant Studies, National Tropical Botanical Garden, Kalaheo, Hawaii/USA; *Hawaiian biogeography, Hawaiian flora, Melicope*

Wen, Jun, PhD; Research Scientist & Curator, Smithsonian Institution, Washington DC/ USA; *Phylogeny & Biogeography, NGS methods*

Eaton, Deren, PhD; Postdoc, Yale University, New Haven, Connecticut/USA; *Bioinformatics (especially pyRAD package), NGS methods, Introgression and hybridization analyses*

Kiehn, Michael, Prof. Dr.; Director of the Botanical Garden of the University of Vienna/ Austria & Research Associate National Tropical Botanical Garden, Kalaheo, Hawaii/USA; *Chromosome counts, Hawaiian flora*

Wood, Kenneth, MSc; Research Biologist, National Tropical Botanical Garden, Kalaheo, Hawaii/USA; *Field trip to Hawaii, Collection permits, Plant Identification*

Bohrer, Rainer, Dr.; Staff member at the *Gesellschaft fur wissenschaftliche Datenverarbeitung mbH Göttingen* (GWDG); *Bioinformatics software, Computational resources*

5.4.2 Researchers with whom you have collaborated scientifically within the past three years

Marc Appelhans:
Steven **Janssens** (University of Leuven, Belgium), Sylvain **Razafimandimbison** (Bergianska Institute Stockholm, Sweden), Erik **Smets**, Paul **Keßler**, Pieter **Baas**, Frederic **Lens** (all Leiden University, Netherlands), **Nina Stock**, Matthias **Niedrig** (Robert Koch-Institute Berlin, Germany), Laurence **Dorr****, Vicki **Funk****, Warren **Wagner**, Jun **Wen**, Elizabeth **Zimmer** (all Smithsonian Institution, Washington DC/USA), Kenneth **Wood** (National Tropical Botanical Garden, Kalaheo, Hawaii/USA), Gerard **Allan** (Northern Arizona University, Flagstaff, Arizona/USA), Timothy **Sharbel***, Marco **Pellino*** (both Leipniz Institute of Plant Genetics and Crop Plant Research Gatersleben, Germany), Darren **Crayn** (Australian Tropical Herbarium, Cairns, Australia), Marco **Duretto** (Royal Botanic Garden Sydney, Australia), Elvira **Hörandl**, Laura **Holzmeyer**, Bastian **Steudel**** (Göttingen University), Stefanie **Ickert-Bond**** (University of Alaska, Fairbanks, USA)
**Manuscripts in preparation*, ***Manuscripts have been submitted.*
Joint publications with all other persons have been published.

5.5 Scientific equipment

The department of Systematics, Biodiversity and Evolution of Plants is equipped with a modern molecular laboratory that will be used to extract DNA and prepare the samples for the outsourced RADseq. A Qubit 3.0 Fluorometer, needed for the quantification of DNA extracts, is also present in our laboratories. A flow cytometer (Partec Flow Cytometer CyFlow Space with High-speed Autoloader) used to measure genome sizes and ploidy levels as well as microscopic equipment for chromosome counting are available in the department.

In terms of computer facilities, we work together with the *Gesellschaft für wissenschaftliche Datenverarbeitung mbH Göttingen* (GWDG), who provide the computing capacity (computer cluster) needed for the computationally elaborate bioinformatic analyses. The needed software packages mentioned in the methods section are available. Commercial software packages such as

the CLC genomics workbench and Geneious, which might be needed in addition to the *pyRAD* package, are available in the department. In addition to computer clusters at the GWDG, a high-performance computer with 16 cores (2x8) and 64GB-RAM is available in the department.

All equipment needed for the digitization of the herbarium specimens ("HerbScan" [a large-format scanner especially designed for herbarium specimens], PC, barcodes) is available in our department. The Göttingen herbarium is currently migrating its databases to the DiversityWorkbench system (http://diversityworkbench.net) and the digitization will be done using this system.

Cogliano, Mary <mary_cogliano@fws.gov>

Re: Status of processing request for Permit No. "96221B""

1 message

Cogliano, Mary <mary_cogliano@fws.gov> Wed, Aug 17, 2016 at 3:39 PM
To: "Wagner, Warren" <WAGNERW@si.edu>
Cc: mappelh@gwdg.de

Dear Mr. Wagner,

I'm contacting you to follow up on your permit application for export of plant specimens collected from Hawaii. First, I want to let you know that since the plants on your application are not CITES-listed, you only need an export permit for the Federally Threatened and Endangered Species. Concerning collection of specimens of these species that are either Threatened or Endangered under the U.S. Endangered Species Act, please provide me with copies of all the required collection permits. Your permit application indicated that you have applied for these collection permits.

In addition, concerning *Platydesma cornuta*, please clarify whether you intend to export var. cornuta or var. decurrens or both.

Please be aware that if the requested information is not received by this office within 45 days, your application will be abandoned and administratively closed. Once a file is closed, you will need to submit a new application and all required fees for the Service to consider your proposed activity.

Thank you,

Mary Cogliano

On Mon, Jul 11, 2016 at 11:06 AM, Wagner, Warren <WAGNERW@si.edu> wrote:

> Good morning ms. Cogliano, I am writing to further query the status of our permit request. It would be most helpful to our project if you could let me know the status of the request. Thanks,
>
> Warren
>
> ---
>
> Warren [redacted] Wagner
>
> Curator of Pacific Botany
>
> Department of Botany, MRC-166
>
> Smithsonian Institution
>
> P. O. Box 37012
>
> Washington, DC 20013-7012
>
> ph 202-633-0968
>
> e-mail wagnerw@si.edu

See Pacific floras and Onagraceae under research at http://botany.si.edu/

From: E-Mail Sys#5 [permits@fws.gov]

Sent: Thursday, May 12, 2016 4:00 PM

To: Appelhans, Marc

Cc: permits@fws.gov

Subject: May 12 2016 10:00:00; Acknowledgement letter for Permit No. "96221B""

SMITHSONIAN INSTITUTION, NATIONAL MUSEUM OF NATURAL 10TH & CONSTITUTION AVENUE, NW PO BOX 37012 , NHB ROOM 85 - MRC 106-107 WASHINGTON, DC 20560 U.S.A.

Thank you for submitting an application to the U.S. Fish and Wildlife Service. The application was received by the Division of Management Authority on 05/11/2016; check number "NOT AVAILABLE" accompanied the application.

Your application has been assigned the following PRT identification number: **US96221B/9

While processing time may be less, you should anticipate a minimum of 30 days to process your request, with many requests averaging between 60 to 90 days due to some requests which need to be published in the Federal Register and/or be reviewed by other Service offices.

From: Vargas, Darcy [mailto:darcy_vargas@fws.gov]
Sent: Wednesday, July 06, 2016 9:41 AM
To: Wagner, Warren <WAGNERW@si.edu>
Cc: Mary Cogliano <mary_cogliano@fws.gov>; Amneris Siaca <amneris_siaca@fws.gov>
Subject: Re:

Good morning Mr. Wagner,

It was nice meeting you also!

We have 2 applications pending from the Simthsonian pending:

- Amneris Siaca is currently working on the National Zoological Park injurious request; 99423B

- Mary Cogliano is assigned the National Museum of Natural History's request to export specimens to Germany; 96221B

I have "cc" Amneris and Mary so that they may provide a status update.

Respectfully,

Darcy Vargas
Biologist

US Fish and Wildlife Service

MS: IA

5275 Leesburg Pike

Falls Church, VA 22041-3803

www.fws.gov
www.cites.org

***Sign up* for our e-newsletter to learn how we're working around the globe to protect species and their habitats!**

If you'd like to personalize your own sentence w/ hyperlink, here's the full link: http://visitor.r20.constantcontact.com/manage/optin?v=0016mDWXmlC-eCNJ4wf_4IA3WaTa8IjzcuPb8jWWJtQIDE8kRHO2RaQ17v2A6OUJgeCSOjzrh7ruV2Nz76Ues6ALGcio28DZ6UAnX5e55gpAO4%3D

On Wed, Jul 6, 2016 at 8:03 AM, Wagner, Warren <WAGNERW@si.edu> wrote:

> Hi Darcy,
>
> Great meeting you at the symposium in May. My colleague, Marc Applehans, asked me if I had heard anything about our permit application. I thought I would just send a quick email to check if it was processing and where it was in the pipeline. I am asking primarily since he needs to begin planning the actual field trips soon. Thanks very much,
>
> Best,
>
> Warren
>
> _____
>
> Warren Wagner
>
> Curator of Pacific Botany
>
> Department of Botany, MRC-166

Smithsonian Institution

P. O. Box 37012

Washington, DC 20013-7012

ph 202-633-0968

e-mail wagnerw@si.edu

See Pacific floras and Onagraceae under research at http://botany.si.edu/

_____

--
Mary Cogliano, Ph.D.
Supervisory Biologist & Policy Specialist
Division of Management Authority
U.S. Fish and Wildlife Service
5275 Leesburg Pike, MS: IA
Falls Church, VA 22041-3803
Phone: (703) 358-1991

Cogliano, Mary <mary_cogliano@fws.gov>

Export Permit Application

1 message

Cogliano, Mary <mary_cogliano@fws.gov> Wed, Aug 17, 2016 at 3:52 PM
To: grant_canterbury@fws.gov

Hi Grant,

We have received a permit application to export Threatened and Endangered plant specimens of species native to Hawaii for purposes of scientific research. I'm forwarding you the application for your review and comment. Attached is the application file. Please provide me with any comments by COB August 26th.

Please let me know if you are not the appropriate contact, and direct me in the right direction if possible.

Best regards,

Mary

--
Mary Cogliano, Ph.D.
Supervisory Biologist & Policy Specialist
Division of Management Authority
U.S. Fish and Wildlife Service
5275 Leesburg Pike, MS: IA
Falls Church, VA 22041-3803
Phone: (703) 358-1991

96221b appl to reviewers.pdf
1560K

RCVD MAY 11 2016

Department of the Interior
U.S. Fish and Wildlife Service

OMB No. 1018-0093
Expires 05/31/2017

Federal Fish and Wildlife Permit Application Form

Return to: U.S. Fish and Wildlife Service
Division of Management Authority (DMA)
Branch of Permits, MS: IA
5275 Leesburg Pike
Falls Church, VA 22041-3803
1-800-358-2104 or 703-358-2104

Type of Activity:
EXPORT/IMPORT/INTERSTATE AND FOREIGN COMMERCE OF NON-NATIVE PLANTS (CITES and/or ESA)

(Circle or highlight proposed activity)

Complete Sections A or B, and C, D, and E of this application. U.S. address may be required in Section C, see instructions for details.
See attached instruction pages for information on how to make your application complete and help avoid unnecessary delays.

A. Complete if applying as an individual

1.a. Last name	1.b. First name	1.c. Middle name or initial	1.d. Suffix

2. Date of birth (mm/dd/yyyy)	3. Social Security No.	4. Occupation	5. Affiliation/ Doing business as (see instructions)

6.a. Telephone number	6.b. Alternate telephone number	6.c. Fax number	6.d. E-mail address

B. Complete if applying on behalf of a business, corporation, public agency, Tribe, or institution

1.a. Name of business, agency, Tribe, or institution	1.b. Doing business as (dba)
Smithsonian Institution, National Museum of Natural	Botanical research

2. Tax identification no.	3. Description of business, agency, Tribe, or institution
53-0206027	Research and education

4.a. Principal officer Last name	4.b. Principal officer First name	4.c. Principal officer Middle name/ initial	4.d. Suffix
Wagner	Warren	[redacted]	

5. Principal officer title	6. Primary contact name
Research scientist and curator	Marc Appelhans (Smithsonian NMNH Research

7.a. Business telephone number	7.b. Alternate telephone number	7.c. Business fax number	7.d. Business e-mail address
++49 5513922220	[redacted]	++49 5513922329	mappelh@gwdg.de

C. All applicants complete address information

1.a. Physical address (Street address; Apartment #, Suite #, or Room #; no P.O. Boxes)
Smithsonian Institution; National Museum of Natural History; Department of Botany, MRC-166, 10th and Constitution

1.b. City	1.c. State	1.d. Zip code/Postal code:	1.e. County/Province	1.f. Country
Washington	DC	20560	n.a.	USA

2.a. Mailing Address (include if different than physical address; include name of contact person if applicable)
P. O. Box 37012

2.b. City	2.c. State	2.d. Zip code/Postal code:	2.e. County/Province	2.f. Country
Washington	DC	20013-7012		USA

D. All applicants MUST complete

1. Attach check or money order payable to the U.S. FISH AND WILDLIFE SERVICE **in the amount of $100 nonrefundable processing fee.** Federal, Tribal, State, and local government agencies, and those acting on behalf of such agencies, are exempt from the processing fee – ***attach documentation of fee exempt status as outlined in instructions.*** (50 CFR 13.11(d))

2. Do you currently have or have you ever had any Federal Fish and Wildlife permits?
Yes ☐ If yes, list the number of the most current permit you have held or that you are applying to renew/re-issue: _____ No ☑

3. Certification: I hereby certify that I have read and am familiar with the regulations contained in ***Title 50, Part 13 of the Code of Federal Regulations*** and the other ***applicable parts in subchapter B of Chapter I of Title 50***, and I certify that the information submitted in this application for a permit is complete and accurate to the best of my knowledge and belief. I understand that any false statement herein may subject me to the criminal penalties of 18 U.S.C. 1001.

Signature (in blue ink) of applicant/person responsible for permit (No photocopied or stamped signatures) — Date of signature (mm/dd/yyyy): 05/04/2016

E. EXPORT/IMPORT/INTERSTATE AND FOREIGN COMMERCE OF PLANTS (ESA and/or CITES)

Allow at least 90 days for the application to be processed. Applications for endangered species under the ESA s must be published in the Federal Register for a 30-day public comment period.

Complete all questions on the application. Mark questions that are not applicable with "N/A". Please use separate sheets of paper when answering this questions. On attachments or separate sheets you submit, indicate the application question number you are addressing. If you are applying for multiple species, be sure to indicate which species you are addressing in each response.

NOTE: This form should NOT be used to request authorization for commercial exports of plants that are artificially propagated in the United States. For such exports, applicants should complete form 3-200-33 (http://www.fws.gov/forms/3-200-33.pdf).

1. What activity are you requesting authorization to carry out?

EXPORT [✓] IMPORT [ ]
INTERSTATE COMMERCE [ ] FOREIGN COMMERCE [ ]

2. For EACH plant involved in the proposed activity provide:

   a. Scientific name (genus, species, and, if applicable, subspecies) and common name:

   b. Description of specimen (e.g., whole plant, cuttings, parts, products; size, height, length);

   c. Quantity of specimens;

   d. Source of specimen (wild or artificially propagated).

3. The current location of the specimens (address and country):

   Specimens will be collected in forests (Forest Reserves) on Hawai'i (Big Island), Maui, Moloka'i, O'ahu and Kaua'i

4. Recipient/Sender:
   - If **export**, provide name and address of the recipient in the foreign country.
   - If **import**, provide name and address of the exporter in the foreign country.
   - If **interstate or foreign commerce**, provide name and address of recipient.

Name: Georg-August University Goettingen
Business Name: Albrecht-von-Haller Institute for Plant Sciences
Marc Appelhans

Address:

Address: Untere Karspuele 2

City: Goettingen

State/Province: Lower Saxony

Country, Postal Code: Germany, 37073

SOURCE OF SPECIMENS (answer question 6 or 7 for each species/specimen, as appropriate):

5. For plants **taken from the wild,** provide the following for each species/specimen collected:
   a. Scientific name;
   b. Number and size class of specimens collected (e.g., 100 juveniles; 50 mature);
   c. Specific location and date of collection for each specimen;
   d. Who (name and address) collected the specimens;
   e. Copies of documents that indicates that the plants were legally collected (e.g., State permits or licenses, landowner's permission, collection permits). Be sure to correlate each document to the corresponding specimen;
   f. Approximate density (e.g., number of plants per acre) and distribution of the species at the collection site(s);
   g. Collection methodology (e.g., whether the specimens were removed from an area of few to several patches of plants, percentage of specimens removed at a specific location); AND
   h. Estimate the number of plants collected to how many plants remain at the location.
   i. Describe efforts made to utilize artificially propagated specimens in lieu of taking plants from the wild.
   j. If applicant did not collect specimens, provide the invoice or other chain of custody documentation that shows the name, address and telephone number of the person from whom you obtained the plants and the date of acquisition of the specimen. Documentation should trace back to the original collector.

6. For **artificially propagated** plants, provide documentation, such as receipts, showing the name, address and telephone number of the person from whom you purchased the plants and the date(s) of acquisition of each specimen and a statement, preferable from the propagator, on how the specimens were propagated (e.g., description of the nursery, propagation method, source and location of parental stock).

7. Provide a full statement justifying the proposed activity (e.g., export, import, interstate commerce, foreign commerce), including the following details:

   a. Describe the purpose of your proposed activity. For example, if the purpose is scientific research, attach a copy of your research proposal outlining the purpose, objectives, methods (e.g., specific information on survey/collection methods, sampling regime, equipment to be used), and whether similar work has already been done or is currently being done. If the purpose is conservation education, provide copies of educational materials (e.g., handouts, text of signage or public presentations), and include the purpose and objectives of the proposed activity. If the purpose is for propagation for conservation purposes, provide a description of how the species will be propagated, disposition of progeny, and cooperative agreements that are/will be established for re-introduction.
   b. Describe the technical expertise of each person as it relates to the proposed activities.
   c. If the species is listed as endangered under the ESA, describe how the activities will enhance or benefit the wild population.
   d. If the requested activity involves native species, provide information to show that the activity is consistent with any recovery plan for the species.
   e. Provide copies of contracts or agreements or other permits that identify persons involved and dates of activities for which the permit is sought.

8. If the proposed activity includes propagating or maintaining live plants at your facility, provide the following:

   a. Approximate number of specimens you currently maintain for each species requested.
   b. Describe the propagation method (e.g., seed, cutting, mericlone) used.
   c. Describe the conditions where the plants are grown and provide photographs of your facilities.
   d. Describe your background and experience working with this or similar species, including
      (i) the number of years each species has been cultivated by you; and
      (ii) the number of plants successfully propagated annually.
   e. Discuss your willingness to participate in a cooperative propagation program and maintain or contribute data regarding your propagation success with the species.
   f. Provide a copy of your State license and U.S. Department of Agriculture General permit, as appropriate.

9. If **import** or **export**, provide:
   a. Copy of any required foreign permits (for CITES Appendix-I plants provide a copy of import permit or evidence a permit will be issued). If plant is to be taken from the wild, provide documentation from the foreign government approving the action.
   b. Describe: (i) the type, size, and construction of shipping containers and (ii) the arrangements for watering and caring for the specimens during transportation.
   c. A statement on the disposition of all imported plants, plant material, and progeny, if produced.

10. Name and address where you wish permit mailed, if different from page 1 (All permits will be mailed via the U.S. Postal Service, unless you identify an alternative means below):

11. If you wish the permit to be delivered by means other than USPS regular mail, provide an air bill, pre-paid envelope, or billing information. If you do not have a pre-paid envelope or air bill and wish to pay for a courier service with your credit card, please check the box below. Please DO NOT include credit card number or other information; you will be contacted for this information.

    ☑ If a permit is issued, please send it via a courier service to the address on page 1 or question 9. I understand that you will contact me for my credit card information once the application has been processed.

12. Who should we contact if we have questions about the application? (Include name, phone number, and email):

    Marc Appelhans, ++49 5513922220, mappelh@gwdg.de

13. **Disqualification Factor**. A conviction, or entry of a plea of guilty or nolo contendere, for a felony violation of the Lacey Act, the Migratory Bird Treaty Act, or the Bald and Golden Eagle Protection Act disqualifies any such person from receiving or exercising the privileges of a permit, unless such disqualification has been expressly waived by the Service Director in response to a written petition. (50 CFR 13.21(c)) Have you or any of the owners of the business, if applying as a business, been convicted, or entered a plea of guilty or nolo contendere, forfeited collateral, or are currently under charges for any violations of the laws mentioned above?

    ☐ Yes ☑ No If you answered "Yes" provide: a) the individual's name, b) date of charge, c) charge(s), d) location of incident, e) court, and f) action taken for each violation.

Appendix to form 3-200-36

Section E:

2a: Scientific and common names of all species.

Not all species have a common name, and common names are often in Hawaiian language. Melicope species are often called 'alani' on Hawaii, and another English name (used only in Australia) is 'doughwood'. Myrsine species are known as 'colicwood', and Zanthoxylum species are known as 'prickly ash' or 'Hercules club', and 'A'e' in Hawaiian. The Hawaiian name for Platydesma is 'Pilo kea'.

We intend to collect the following species of the genera Melicope, Platydesma and Zanthoxylum (all Rutaceae family) and Myrsine (Primulaceae family) on Hawaii. [scientific names in italics; common names in brackets]

Melicope adscendens (auwahi melicope), *Melicope anisata* (mokihana), *Melicope balloui* (Ballou's melicope), *Melicope barbigera* (uahiapele), *Melicope christophersenii* (Waianae Range melicope), *Melicope cinerea* (manena), *Melicope clusiifolia* (kukaemoa, kolokolo mokihana), *Melicope cruciata* (Cross-bearing Melicope, pilo 'ula), *Melicope degeneri* (Kokee Stream melicope), *Melicope elliptica* (leiohi'iaka), *Melicope feddei*, *Melicope haleakalae* (Haleakala melicope), *Melicope haupuensis* (Haupa Mountain melicope), *Melicope hawaiensis* (mokihana kukae moa), *Melicope hiiakae*, *Melicope hosakae*, *Melicope kaalaensis* (Kaala melicope), *Melicope kavaiensis* (pilo 'ula), *Melicope knudsenii* (Olokele Valley melicope), *Melicope lydgatei* (Koolau Range melicope), *Melicope macropus* (Kaholuamanu melicope), *Melicope makahae* (Makaha Valley melicope), *Melicope molokaiensis*, *Melicope mucronulata*, *Melicope munroi* (lanahale), *Melicope nealae*, *Melicope oahuensis*, *Melicope obovata* (Makawao melicope), *Melicope orbicularis* (Honokahua melicope), *Melicope ovalis* (Hana melicope), *Melicope ovata*, *Melicope pallida* (pale melicope), *Melicope paniculata* (Lihue melicope), *Melicope peduncularis*, *Melicope pseudoanisata*, *Melicope puberula* (hairy melicope), *Melicope quadrangularis* (four angle melicope), *Melicope radiata*, *Melicope reflexa* (lava melicope), *Melicope rotundifolia*, *Melicope saint-johnii* (St. John's melicope), *Melicope sandwicensis* (Mt. Kaala melicope), *Melicope sessilis*, *Melicope volcanica*, *Melicope waialealae* (Alani wai), *Melicope wailauensis*, *Melicope wawraeana* (Monoa melicope), *Melicope zahlbruckneri* (Zahlbruckner's melicope, kipuka piaula) → 48 species

Myrsine alyxifolia, *Myrsine degeneri* (summit colicwood), *Myrsine denticulata*, *Myrsine fernseei* (streambank colicwood), *Myrsine fosbergii* (Koolau Range colicwood), *Myrsine helleri* ('Oliko), *Myrsine juddii* (cloudswept colicwood), *Myrsine kauaiensis*, *Myrsine knudsenii* (Kokee colicwood), *Myrsine lanaiensis*, *Myrsine lessertiana* (Kolea lau nui), *Myrsine linearifolia* (narrowleaf colicwood), *Myrsine mezii* (Hanapepe River colicwood), *Myrsine petiolata* (swamp colicwood), *Myrsine pukooensis*, *Myrsine punctata*, *Myrsine sandwicensis* (Kolea lau li'i), *Myrsine vaccinioides*, *Myrsine wawraea* → 19 species

Platydesma cornuta, Platydesma remyi (Hawai'i pilo kea), *Platydesma rostrata* (Pilo kea lau li'i), *Platydesma spathulata* (Pilo kea) → 4 species

Zanthoxylum dipetalum (Kawa'u), *Zanthoxylum hawaiiense* (A'e, Hawai'i prickly ash), *Zanthoxylum kauaense* (A'e, Kaua'i prickly ash), *Zanthoxylum oahuense* (A'e, O'ahu prickly ash) → 4 species

2b: Description of specimen

Several of the above-mentioned species are federally listed as endangered. These are:

Melicope adscendens, Melicope balloui, Melicope christophersenii, Melicope cinerea, Melicope cruciata, Melicope degeneri, Melicope haleakalae, Melicope haupuensis, Melicope hawaiensis, Melicope hiiakae, Melicope knudsenii, Melicope lydgatei, Melicope macropus, Melicope makahae, Melicope mucronulata, Melicope munroi, Melicope nealae, Melicope obovata, Melicope ovalis, Melicope pallida, Melicope paniculata, Melicope puberula, Melicope quadrangularis, Melicope reflexa, Melicope saint-johnii, Melicope sandwicensis, Melicope wailauensis, Melicope zahlbruckneri → 28 species

Myrsine fosbergii, Myrsine juddii, Myrsine knudsenii, Myrsine linearifolia, Myrsine mezii, Myrsine vaccinioides → 6 species

Platydesma cornuta, Platydesma remyi, Platydesma rostrata → 3 species

Zanthoxylum dipetalum, Zanthoxylum hawaiiense, Zanthoxylum oahuense → 3 species

For these species, we would like to collect a single leaf from one individual tree and export it to Germany. We are also planning to collect one herbarium specimen (a small twig) from one individual tree per species, but the herbarium specimen will stay in Hawaii and will be deposited at Bishop Museum. The leaf will be used for molecular lab work and the herbarium specimen will serve as a reference, so that future generations can still check if the species identification was correct.

For the more common and non-endangered species, we intend to collect material from two trees per species. In case a species grows on more than one island, we would like to sample from two individual trees per island. The material collected consists of a single leaf and four herbarium vouchers (four small twigs) and five (Myrsine) to ten

(Rutaceae genera) viable seeds (seeds will be taken from only one tree per species). We intend to collect four herbarium vouchers, and distribute them to the following herbaria: Bishop Museum (Honolulu; BISH), National Tropical Botanical Garden (Kalaeo, Kaua'i; PTBG), Smithsonian Institution (Washington DC; US) and Goettingen University (Goettingen, Germany; GOET). Four herbarium vouchers need to be collected so that all co-operation partners (Warren Wagner, Smithsonian Institution; Kenneth Wood, National Tropical Botanical Garden; Marc Appelhans at Goettingen) have a complete set of specimens. In addition, the Hawaiian regulations require depositing one herbarium voucher at Bishop Museum. Since only one of these institutions is outside of the USA, only the export of one herbarium voucher per tree is requested. The seeds are needed for cultivation in the Botanical Garden in Goettingen.

2c: Quantity of specimens

For the protected species (40 species in 4 genera in total; see 2b), only a single leaf needs to be exported. Since material from only one tree per species will be collected, the total number of specimens (single leaves) from the protected species will be 40.

The remaining 35 species are not federally listed and we would like to collect material from two individual trees per species per island. Since most species are endemic to a single island, but some species are found on all islands, the numbers of specimens per species differ, and we are listing the numbers of specimens for each species accordingly:

Melicope anisata: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope barbigera: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope clusiifolia: Hawai'i, Maui, Moloka'i, O'ahu, Kaua'i → 10 specimens = 10 leaves, 10 herbarium vouchers, 10 seeds
Melicope elliptica: Maui, Moloka'i, O'ahu → 6 specimens = 6 leaves, 6 herbarium vouchers, 10 seeds
Melicope feddei: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope hosakae: O'ahu → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope kaalaensis: O'ahu → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope kavaiensis: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope molokaiensis: Maui, Moloka'i → 4 specimens = 4 leaves, 4 herbarium vouchers, 10 seeds
Melicope oahuensis: O'ahu → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope orbicularis: Maui → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope ovata: O'ahu, Kaua'i → 4 specimens = 4 leaves, 4 herbarium vouchers, 10 seeds
Melicope peduncularis: Maui, Moloka'i, O'ahu, Kaua'i → 8 specimens = 8 leaves, 8 herbarium vouchers, 10 seeds

Melicope pseudoanisata: Hawai'i, Maui → 4 specimens = 4 leaves, 4 herbarium vouchers, 10 seeds
Melicope radiata: Hawai'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope rotundifolia: O'ahu → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope sessilis: Maui, Moloka'i → 4 specimens = 4 leaves, 4 herbarium vouchers, 10 seeds
Melicope volcanica: Hawai'i, Maui, Moloka'i → 6 specimens = 6 leaves, 6 herbarium vouchers, 10 seeds
Melicope waialealae: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 10 seeds
Melicope wawraeana: O'ahu, Kaua'i → 4 specimens = 4 leaves, 4 herbarium vouchers, 10 seeds

Myrsine alyxifolia: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 5 seeds
Myrsine degeneri: O'ahu → 2 specimens = 2 leaves, 2 herbarium vouchers, 5 seeds
Myrsine denticulata: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 5 seeds
Myrsine fernseei: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 5 seeds
Myrsine helleri: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 5 seeds
Myrsine kauaiensis: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 5 seeds
Myrsine lanaiensis: Hawai'i, Maui, Moloka'i, O'ahu, Kaua'i → 10 specimens = 10 leaves, 10 herbarium vouchers, 5 seeds
Myrsine lessertiana: Hawai'i, Maui, Moloka'i, O'ahu, Kaua'i → 10 specimens = 10 leaves, 10 herbarium vouchers, 5 seeds
Myrsine petiolata: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 5 seeds
Myrsine pukooensis: Maui, Moloka'i, O'ahu → 6 specimens = 6 leaves, 6 herbarium vouchers, 5 seeds
Myrsine punctata: O'ahu, Kaua'i → 4 specimens = 4 leaves, 4 herbarium vouchers, 5 seeds
Myrsine sandwicensis: Hawai'i, Maui, Moloka'i, O'ahu → 8 specimens = 8 leaves, 8 herbarium vouchers, 5 seeds
Myrsine wawraea: Kaua'i → 2 specimens = 2 leaves, 2 herbarium vouchers, 5 seeds

Platydesma spathulata: Hawai'i, Maui, O'ahu, Kaua'i → 8 specimens = 8 leaves, 8 herbarium vouchers, 10 seeds

Zanthoxylum kauaense: Hawai'i, Maui, Moloka'i, O'ahu, Kaua'i → 10 specimens = 10 leaves, 10 herbarium vouchers, 10 seeds

2d: Source of specimen

The specimens will be collected in the wild. We have already applied for the required permits to collect plants (both endangered and not endangered species) from Forest Reserves in all four Hawaiian counties.

We have applied for the following permits:
-Research/Collection/Possession Permit For Hawaii Threatened & Endangered Plant Species

-Access/Research/Botanical Collection Permit For Kaua'i Forest Reserves
-Access/Research/Botanical Collection Permit For O'ahu Forest Reserves
-Access/Research/Botanical Collection Permit For Hawai'i Forest Reserves
-Access/Research/Botanical Collection Permit For Maui Nui Forest Reserves (Maui & Moloka'i)

5a: Scientific name for each species
→ Scientific names for all species are listed under 2a

5b: Number and size class of specimens collected
→ 40 single leaves will be collected from endangered plants. These leaves will be dried in silica gel.
→ 144 single leaves, 144 herbarium vouchers (each consisting of one twig of about the size of the US letter paper size) and 285 seeds will be collected from non-endangered plants. Additional herbarium vouchers will be collected for US institutions (Bishop Museum, National Tropical Botanical Garden, Smithsonian Institution). The 144 herbarium vouchers are the ones that will be exported to Goettingen/Germany.
→ See 2c for the amount of specimens per species.

5c: Specific location and date of collection for each specimen

A five week field trip to Hawaii is planned in summer/fall 2016 and all specimens will be collected during this field trip.

The locations of the specimens are Forest Reserves on the Hawaiian island Kaua'i, O'ahu, Maui, Moloka'i and Hawai'i (Big Island). The names of the Forest Reserves are as follows:

Kaua'i: Na Pali-Kona Forest Reserve, Pu'u Ka Pele Forest Reserve, Lihue-Koloa Forest Reserve, Kalepa Forest Reserve, Nounou Forest Reserve

O'ahu: Waianae Kai Forest Reserve, Mokuleia Forest Reserve, Nanakuli Forest Reserve, Makua Kea'au Forest Reserve, Kuaokala Forest Reserve, Ewa Forest Reserve, Honululu Watershed Forest Reserve, Kuliouou Forest Reserve, Kaipapau Forest Reserve

Maui: Koolau Forest Reserve, Makawao Forest Reserve, Hanawi Natural Forest Reserve, Hana Forest Reserve, Kipahulu Forest Reserve, West Maui Forest Reserve

Moloka'i: Moloka'i Forest Reserve

Big Island: Puna Forest Reserve, Hilo Forest Reserve, Upper Waiakea Forest Reserve, Kau Forest Reserve, Kohala Forest Reserve, Manowaialee Forest Reserve

5d: Who collected the specimens

Marc Appelhans (Georg-August University Goettingen, Albrecht-von-Haller Institute for Plant Sciences, Untere Karspuele 2, Goettingen, Germany, 37073; Research Associate at the Smithsonian Institution, National Museum of Natural History, PO Box 37012, Washington, DC 20013-7012) will collect the specimens together with a graduate student (Claudia Paetzold; Georg-August University Goettingen, Albrecht-von-Haller Institute for Plant Sciences, Untere Karspuele 2, Goettingen, Germany, 37073). We are working together with colleagues from Hawaii and will collect endangered species together with them. Our contacts for each Hawaiian county are:

Hawai'I (Big Island)
Lyman Perry
Hawaii District Botanist
Division of Forestry and Wildlife
19 E Kawili St
Hilo, HI 96720
(808) 938-7795
Lyman.Perry@hawaii.gov

Maui Nui (Maui, Moloka'i)
Lance K. De Silva
Forest Management Supervisor I
State of HI - DLNR
Division of Forestry & Wildlife - Maui Nui
1955 Main St . Suite 400
Wailuku, HI 96793
(808) 873-3980
Lance.K.DeSilva@hawaii.gov

O'ahu
Ryan Keala Ishima Peralta
Forest Management Supervisor I
State of Hawaii
Department of Land and Natural Resources
Division of Forestry and Wildlife
2135 Makiki Heights Drive
Honolulu, Hawaii 96822
(808) 292-5645
Ryan.K.Peralta@hawaii.gov

Lara Reynolds
Oahu District Botanist
Division of Forestry and Wildlife
Department of Land & Natural Resources
2551 Waimano Home Road, Bldg. #202
Pearl City, HI 96782
(808) 342-3081
Lara.S.Reynolds@hawaii.gov

Kaua'i

Kenneth R. Wood
Research Biologist
National Tropical Botanical Garden
3530 Papalina Road
Kalaheo HI 96741
(808) 332-7324
kwood@ntbg.org

Adam Williams
Kaua'i District Botanist
State Division of Forestry and
Wildlife
3060 Eiwa St. Rm 306
Lihue, Kauai 96766
(808) 421-9091
Adam.M.Williams@hawaii.gov

5e: Copies of documents that indicates that the plants were legally collected

We have applied for the following collecting permits from the Department of Land and Natural Resources - Division of Forestry and Wildlife:

-Research/Collection/Possession Permit For Hawaii Threatened & Endangered Plant Species
-Access/Research/Botanical Collection Permit For Kaua'i Forest Reserves
-Access/Research/Botanical Collection Permit For O'ahu Forest Reserves
-Access/Research/Botanical Collection Permit For Hawai'i Forest Reserves
-Access/Research/Botanical Collection Permit For Maui Nui Forest Reserves (Maui & Moloka'i)

The permit applications are currently being reviewed and we will notify the U.S. Fish and Wildlife Service once a decision is made and we will send copies of the collection permits.

5f: Approximate density and distribution of the species

Non-endangered species of Melicope and Myrsine form a common component of Hawaiian plant communities. Seventy-five percent of the species are endemic to single islands, and have >500 individuals per sq km where they are locally common. The density of some species is difficult to assess with some of them are found only in a single forest with low numbers of individuals. These species are:

Melicope adscendens, Melicope degeneri, Melicope haupuensis, Melicope knudsenii, Melicope lydgatei, Melicope mucronulata, Melicope quadrangularis, Melicope wailauensis, Melicope zahlbruckneri, Myrsine knudsenii, Myrsine mezii, Platydesma remyi, Zanthoxylum hawaiiense

Most species occur on only one Hawaiian island. The distribution for each species is shown in the following table:

Genus	Species	Kaua'i	O'ahu	Moloka'i	Lana'i	Maui	Hawai'i (Big island)
Myrsine	alyxifolia	X					
Myrsine	degeneri		X				
Myrsine	denticulata	X					
Myrsine	emarginata		X		X	X	
Myrsine	fernseei	X					
Myrsine	fosbergii	X	X				
Myrsine	helleri	X					
Myrsine	juddii		X				
Myrsine	kauaiensis	X					
Myrsine	knudsenii	X					
Myrsine	lanaiensis	X	X	X	X	X	X
Myrsine	lessertiana	X	X	X	X	X	X
Myrsine	linearifolia	X					
Myrsine	mezii	X					
Myrsine	petiolata	X					
Myrsine	pukooensis		X	X	X	X	
Myrsine	punctata	X	X				
Myrsine	sandwicensis		X	X	X	X	X
Myrsine	vaccinioides					X	
Myrsine	wawraea	X					
Melicope	adscendens					X	
Melicope	anisata	X					
Melicope	balloui					X	
Melicope	barbigera	X					
Melicope	christophersenii		X				
Melicope	cinerea		X				
Melicope	clusiifolia	X	X	X	X	X	X
Melicope	cruciata	X					
Melicope	degeneri	X					
Melicope	elliptica		X	X		X	
Melicope	feddei	X					
Melicope	haleakalae					X	
Melicope	haupuensis	X					
Melicope	hawaiensis			X	X	X	X
Melicope	hiiakae		X				
Melicope	hosakae		X				
Melicope	kaalaensis		X				
Melicope	kavaiensis	X					

Melicope	knudsenii	X				X	
Melicope	lydgatei		X				
Melicope	macropus	X					
Melicope	makahae		X				
Melicope	molokaiensis			X		X	
Melicope	mucronulata			X		X	
Melicope	munroi			X	X		
Melicope	nealae	X					
Melicope	oahuensis		X				
Melicope	obovata					X	
Melicope	orbicularis					X	
Melicope	ovalis					X	
Melicope	ovata	X	X				
Melicope	pallida	X	X				
Melicope	paniculata	X					
Melicope	peduncularis	X	X	X		X	
Melicope	pseudoanisata					X	X
Melicope	puberula	X					
Melicope	quadrangularis	X					
Melicope	radiata						X
Melicope	reflexa			X			
Melicope	rotundifolia		X				
Melicope	saint-johnii		X				
Melicope	sandwicensis		X				
Melicope	sessilis			X		X	
Melicope	volcanica			X	X	X	X
Melicope	waialealae	X					
Melicope	wailauensis			X			
Melicope	wawraeana	X	X				
Melicope	zahlbruckneri						X
Platydesma	cornuta		X				
Platydesma	cornuta		X				
Platydesma	remyi						X
Platydesma	rostrata	X					
Platydesma	spathulata	X	X			X	X
Zanthoxylum	dipetalum	X	X	X			X
Zanthoxylum	dipetalum						X
Zanthoxylum	hawaiiense	X		X	X	X	X
Zanthoxylum	kauaense	X	X	X	X	X	X
Zanthoxylum	oahuense		X				

5g: Collection methodology

All plant species which we would like to collect and export are trees or shrubs. Since we plan to collect single leaves, herbarium vouchers and seeds, no individual plants will be killed.

Specimens will be taken from one individual tree per species for the protected species (see 2b), and from two individual trees per species per island for the unprotected species (see 2b).

5h: Plants remaining at the location

Since only fragments of the plants will be taken, all sampled plants will remain alive and all plants will remain at the locations.

5i: Efforts made to utilize artificially propagated specimens

It is important for the project to make field observation regarding the ecology and environment of the specimens and therefore artificially propagated specimens are not suited.

5j: Original collector of additional specimens

N/A. All specimens will be collected by Marc Appelhans and Claudia Paetzold, as indicated in section 5d.

6: Artificially propagated specimens

N/A.

7a: Purpose of the proposed activity

The planned research is about plant systematics, evolution and biogeography. Systematics is the very basis of any biodiversity related topic. Without the proper naming, description, establishing species-boundaries and encripting the evolutionary history of a species, further studies are not possible. The PI of this project (Marc Appelhans) has worked with Warren Wagner (Smithsonian Institution, McBride chair at NTBG) and Ken Wood (NTBG) on the systematics and biogeography of Hawaiian

Melicope (which includes Platydesma) before. During these studies, we found out that the species Melicope knudsenii, which is known from Kaua'i and Maui, is in fact not "a good species" since it consists of three independent evolutionary lineages that should be regarded as three different species. One of these species, which we are currently formally describing, is endemic to Kaua'i and it is know from a handful of individual trees only. Thus, by using molecular tools to establish species-boundaries, we can identify new species and find out which lineages need special attention and a conservation plan.

In the past 20 years, plant systematics mainly relied on the sequencing of few DNA regions such as single genes, introns or spacer regions. The advent of Next-Generation Sequencing methods has been a major revolution in the field, and it enables researchers to sequence large parts of the genomes. Especially (geologically) young speciation events can be studied in great detail using these new methods, since closely related species are highly similar in their genetic information and a large quantity and quality of information is needed to detect the differences among these close relatives. The plant radiations on the Hawaiian Islands are prime examples for such young speciation events. So far, no study based on Hawaiian plants has been carried out using our methodology, so that our project will be a prime example in Hawaiian biogeography and evolution.

The proposed study has been financed by the DFG (Deutsche Forschungsgemeinschaft), which is the German equivalent of NSF, and we are attaching the grant application, which contains detailed information about the background, methodology and preliminary findings.

7b: Technical expertise

The researchers involved in this project have excellent expertise to ensure the success of the proposed study. The study requires expertise in molecular labwork, bioinformatics and deep knowledge in recognizing and identifying the species.

The PI of this project (Dr. Marc Appelhans; Goettingen University; Research associate Smithsonian Institution, Washington, DC) has been working on the systematics, phylogeny, taxononmy and biogeography of Hawaiian Rutaceae (Citrus family) since his postdoctoral studies at the Smithsonian Institution in Washington DC in 2014. He has published five scientific articles about the Rutaceae genus Melicope with his supervisors from the Smithsonian (Warren Wagner, Jun Wen) since 2014 and three additional papers are currently in preparation or submitted. Mr. Appelhans is teaching a MSc course about Next-Generation Sequencing at Goettingen University and is the co-editor of a recently published book about the use of Next-Generation Sequencing in plant systematics (Hörandl E, Appelhans MS (eds.): Regnum Vegetabile 158. Next Generation Sequencing in Plant Systematics. Koeltz Scientific Books, Königstein.).

Dr. Warren Wagner (Smithsonian Institution, Washington, DC) is a world-leading expert on the flora and biogeography of the Hawaiian Islands. He has been working on Hawaiian plants for decades and holds the position "McBryde Chair for Hawaiian and Pacific Plant Studies" at the National Tropical Botanical Garden in Kalaheo/Kaua'i. Dr. Wagner was Dr. Appelhans' postdoc-supervisor and both conceived the ideas for this new project.

Kenneth Wood is a research biologist at the National Tropical Botanical Garden in Kalaheo/Kaua'i. He is an expert in recognizing Hawaiian plants, especially Melicope and Myrsine (main target genera of this project). A major interest of his work is the conservation of the Hawaiian flora, a topic on which he has published several scientific articles. His expert knowledge regarding species identification and the locations are invaluable for the project.

Claudia Paetzold (Goettingen University) will be involved in the project as a graduate/doctoral student. The project will be her first contact with Hawaiian plants. The position in the project has been advertised internationally, and she has been selected because of her excellent knowledge regarding DNA sequencing and Next-Generation Sequencing related bioinformatics.

7c: Endangered species - Benefits for wild populations

As outlined in section 7a, plant systematic studies are the basis for proper conservation plans of endangered species. Our (Appelhans, Wagner, Wood) previous research has shown that the species Melicope knudsenii consists of three independent evolutionary lineages and therefore represents three different species, which are all critically endangered. Describing these differences, establishing DNA-based species boundaries, and proposing names for species new to science is therefore an essential foundation for protecting these species. Like it was the case for Melicope knudsenii, we expect that several other "species" that occur on more than one Hawaiian island might represent more than one species. Our research will show how many species there are and will show which species and populations exhibit unique genetic information and need to be protected.

7d: Consistency of activity with recovery plan for species.

All targeted species are native to the Hawaiian Islands. Only fragments of the plants will be collected and no plants will be killed, so that no recovery plans are violated. Endangered species will be collected together with Hawaiian colleagues (Plant Extinction Prevention program; Division of Forestry & Wildlife), who will ensure that the impact on individual trees will be minimal.

7e: Persons involved and dates of activities

Researchers involved in this project are Marc Appelhans (mappelh@gwdg.de), Warren Wagner (wagnerw@si.edu), Kenneth Wood (kwood@ntbg.org) and Claudia Paetzold (paetzold@gwdg.de). We did not make special contracts or agreements, but we have worked and published together since 2012 and there is a strong wish from all sides to continue to work on this project together. Appelhans, Wagner and Wood have written the grant application together.

The planned field work on Hawaii is planned for summer/fall 2016 and the duration will be five weeks.

8a: Number of specimens we currently maintain.

Currently no specimens of Hawaiian Melicope, Platydesma, Zanthoxylum and Myrsine are maintained at Goettingen University. We would like to collect seeds of 35 unprotected species listed in section 2c for cultivation at the Botanical Garden of Goettingen University.

8b: Propagation method

In all cases plants will be grown from seeds.

8c: Growing conditions

The Botanical Garden of Goettingen University is equipped with state of the art greenhouses and equipment to cultivate (sub)tropical plants. Climate chambers will guarantee constant environmental conditions for the germination phase. After the seedling stage, plants will be cultivated in different greenhouses depending on their ecological niche. Species from wet montane forests and bogs will be cultivated in our tropical fern greenhouse; species from mesic forests will be cultivated in our tropical greenhouse, and species from drier habitats will be grown together with our collections from Australia, South Africa and the Mediterranean.

Climate chamber with controlled temperature, humidity and light intensity.

Overview of several greenhouses of the Botanical Garden in Goettingen.

8d: Cultivation plants: background and experience

The botanical garden of Goettingen University was established in 1736. There has been a long tradition in the cultivation of tropical plants in greenhouses and the gardeners have extensive knowledge to ensure that the plants will be taken care of professionally.

8e: Participation in a cooperative propagation program

We are of course willing to participate in any cooperative propagation program that will be beneficial for the surviving of the species. We are in close contact with researchers from the National Tropical Botanical Garden on Kaua'i and we will share all data regarding growing conditions of successfully cultivated plants with them.

8f: State license and U.S. Department of Agriculture General permit

N/A.

9a: Copy of any required foreign permits.

None of the targeted species of this study are CITES-listed, so that only phytosanitary restrictions need to be taken into account for the import of specimens to Germany. We have contacted the "Landwirtschaftskammer Niedersachsen" (Department of Agriculture for the state Lower Saxony), who attest that no special permit is needed for the four targeted species (genera Melicope, Platydesma, Zanthoxylum, Myrsine). A letter from this institution is attached to this appendix.

9b: Type, size, and construction of shipping containers

The material will be sent in standard FedEx paper boxes. Herbarium specimens are stored in folded newspaper sheets, seeds and dried leaves will be deposited in ziplock bags. In total, all specimens from one island will be put into a single box, so that five boxes will be exported.

Seeds will be potted in the Botanical Garden of Goettingen University, so that no watering and caring for the specimens during transportation need to be arranged.

9c: Statement on the disposition of all imported plants

The imported herbarium sheets will be deposited at the herbarium of Goettingen University. Dried leaves will be used for DNA extraction and will be used up for these studies. DNA extracts will be stored at -80°C in the labs of Goettingen University. Seeds will be used to grow plants, and root tips from those plants will be used for microscopy to count chromosome numbers and determine ploidy levels, and fresh leaves will be used for flow cytometry in order to determine genome sizes. The plants will be cultivated in the greenhouses of the Botanical Garden Goettingen.

Department of the Interior
U.S. Fish and Wildlife Service

OMB No. 1018-0093
Expires 05/31/2017

Federal Fish and Wildlife Permit Application Form

Return to: U.S. Fish and Wildlife Service
Division of Management Authority (DMA)
Branch of Permits, MS: IA
5275 Leesburg Pike
Falls Church, VA 22041-3803
1-800-358-2104 or 703-358-2104

Type of Activity:
EXPORT/IMPORT/INTERSTATE AND FOREIGN COMMERCE OF NON-NATIVE PLANTS (CITES and/or ESA)

(Circle or highlight proposed activity)

Complete Sections A or B, and C, D, and E of this application. U.S. address may be required in Section C, see instructions for details.
See attached instruction pages for information on how to make your application complete and help avoid unnecessary delays.

A.	**Complete if applying as an individual**		
1.a. Last name	1.b. First name	1.c. Middle name or initial	1.d. Suffix

2. Date of birth (mm/dd/yyyy)	3. Social Security No.	4. Occupation	5. Affiliation/ Doing business as (see instructions)

6.a. Telephone number	6.b. Alternate telephone number	6.c. Fax number	6.d. E-mail address

B.	**Complete if applying on behalf of a business, corporation, public agency, Tribe, or institution**		
1.a. Name of business, agency, Tribe, or institution		1.b. Doing business as (dba)	
Smithsonian Institution, National Museum of Natural		Botanical research	
2. Tax identification no.	3. Description of business, agency, Tribe, or institution		
53-0206027	Research and education		
4.a. Principal officer Last name	4.b. Principal officer First name	4.c. Principal officer Middle name/ initial	4.d. Suffix
Wagner	Warren	[redacted]	
5. Principal officer title	6. Primary contact name		
Research scientist and curator	Marc Appelhans (Smithsonian NMNH Research		

7.a. Business telephone number	7.b. Alternate telephone number	7.c. Business fax number	7.d. Business e-mail address
++49 5513922220	[redacted]	++49 5513922329	mappelh@gwdg.de

C.	**All applicants complete address information**			
1.a. Physical address (Street address; Apartment #, Suite #, or Room #; no P.O. Boxes)				
Smithsonian Institution; National Museum of Natural History; Department of Botany, MRC-166, 10th and Constitution				
1.b. City	1.c. State	1.d. Zip code/Postal code:	1.e. County/Province	1.f. Country
Washington	DC	20560	n.a.	USA
2.a. Mailing Address (include if different than physical address; include name of contact person if applicable)				
P. O. Box 37012				
2.b. City	2.c. State	2.d. Zip code/Postal code:	2.e. County/Province	2.f. Country
Washington	DC	20013-7012		USA

D.	**All applicants MUST complete**
1.	Attach check or money order payable to the U.S. FISH AND WILDLIFE SERVICE **in the amount of $100 nonrefundable processing fee.** Federal, Tribal, State, and local government agencies, and those acting on behalf of such agencies, are exempt from the processing fee – ***attach documentation of fee exempt status as outlined in instructions.*** (50 CFR 13.11(d))
2.	Do you currently have or have you ever had any Federal Fish and Wildlife permits? Yes ☐ If yes, list the number of the most current permit you have held or that you are applying to renew/re-issue: _____ No ☑
3.	Certification: I hereby certify that I have read and am familiar with the regulations contained in ***Title 50, Part 13 of the Code of Federal Regulations*** and the other ***applicable parts in subchapter B of Chapter I of Title 50***, and I certify that the information submitted in this application for a permit is complete and accurate to the best of my knowledge and belief. I understand that any false statement herein may subject me to the criminal penalties of 18 U.S.C. 1001. _____ 05/04/2016 Signature (**in blue ink**) of applicant/person responsible for permit (No photocopied or stamped signatures) — Date of signature (mm/dd/yyyy)

E. EXPORT/IMPORT/INTERSTATE AND FOREIGN COMMERCE OF PLANTS (ESA and/or CITES)

Allow at least 90 days for the application to be processed. Applications for endangered species under the ESA s must be published in the Federal Register for a 30-day public comment period.

Complete all questions on the application. Mark questions that are not applicable with "N/A". Please use separate sheets of paper when answering this questions. On attachments or separate sheets you submit, indicate the application question number you are addressing. If you are applying for multiple species, be sure to indicate which species you are addressing in each response.

NOTE: This form should NOT be used to request authorization for commercial exports of plants that are artificially propagated in the United States. For such exports, applicants should complete form 3-200-33 (http://www.fws.gov/forms/3-200-33.pdf).

1. What activity are you requesting authorization to carry out?

EXPORT [✓] IMPORT [ ]
INTERSTATE COMMERCE [ ] FOREIGN COMMERCE [ ]

2. For EACH plant involved in the proposed activity provide:

   a. Scientific name (genus, species, and, if applicable, subspecies) and common name:

   b. Description of specimen (e.g., whole plant, cuttings, parts, products; size, height, length);

   c. Quantity of specimens;

   d. Source of specimen (wild or artificially propagated).

3. The current location of the specimens (address and country):

   Specimens will be collected in forests (Forest Reserves) on Hawai'i (Big Island), Maui, Moloka'i, O'ahu and Kaua'i

4. Recipient/Sender:
   - If **export**, provide name and address of the recipient in the foreign country.
   - If **import**, provide name and address of the exporter in the foreign country.
   - If **interstate or foreign commerce**, provide name and address of recipient.

Name:	Georg-August University Goettingen Albrecht-von-Haller Institute for Plant Sciences
Business Name:	Marc Appelhans
Address:	
Address:	Untere Karspuele 2
City:	Goettingen
State/Province:	Lower Saxony
Country, Postal Code:	Germany, 37073

SOURCE OF SPECIMENS (answer question 6 or 7 for each species/specimen, as appropriate):

5. For plants **taken from the wild**, provide the following for each species/specimen collected:
   a. Scientific name;
   b. Number and size class of specimens collected (e.g., 100 juveniles; 50 mature);
   c. Specific location and date of collection for each specimen;
   d. Who (name and address) collected the specimens;
   e. Copies of documents that indicates that the plants were legally collected (e.g., State permits or licenses, landowner's permission, collection permits). Be sure to correlate each document to the corresponding specimen;
   f. Approximate density (e.g., number of plants per acre) and distribution of the species at the collection site(s);
   g. Collection methodology (e.g., whether the specimens were removed from an area of few to several patches of plants, percentage of specimens removed at a specific location); AND
   h. Estimate the number of plants collected to how many plants remain at the location.
   i. Describe efforts made to utilize artificially propagated specimens in lieu of taking plants from the wild.
   j. If applicant did not collect specimens, provide the invoice or other chain of custody documentation that shows the name, address and telephone number of the person from whom you obtained the plants and the date of acquisition of the specimen. Documentation should trace back to the original collector.

6. For **artificially propagated** plants, provide documentation, such as receipts, showing the name, address and telephone number of the person from whom you purchased the plants and the date(s) of acquisition of each specimen and a statement, preferable from the propagator, on how the specimens were propagated (e.g., description of the nursery, propagation method, source and location of parental stock).

7. Provide a full statement justifying the proposed activity (e.g., export, import, interstate commerce, foreign commerce), including the following details:

   a. Describe the purpose of your proposed activity. For example, if the purpose is scientific research, attach a copy of your research proposal outlining the purpose, objectives, methods (e.g., specific information on survey/collection methods, sampling regime, equipment to be used), and whether similar work has already been done or is currently being done. If the purpose is conservation education, provide copies of educational materials (e.g., handouts, text of signage or public presentations), and include the purpose and objectives of the proposed activity. If the purpose is for propagation for conservation purposes, provide a description of how the species will be propagated, disposition of progeny, and cooperative agreements that are/will be established for re-introduction.
   b. Describe the technical expertise of each person as it relates to the proposed activities.
   c. If the species is listed as endangered under the ESA, describe how the activities will enhance or benefit the wild population.
   d. If the requested activity involves native species, provide information to show that the activity is consistent with any recovery plan for the species.
   e. Provide copies of contracts or agreements or other permits that identify persons involved and dates of activities for which the permit is sought.

8. If the proposed activity includes propagating or maintaining live plants at your facility, provide the following:

   a. Approximate number of specimens you currently maintain for each species requested.
   b. Describe the propagation method (e.g., seed, cutting, mericlone) used.
   c. Describe the conditions where the plants are grown and provide photographs of your facilities.
   d. Describe your background and experience working with this or similar species, including
      (i) the number of years each species has been cultivated by you; and
      (ii) the number of plants successfully propagated annually.
   e. Discuss your willingness to participate in a cooperative propagation program and maintain or contribute data regarding your propagation success with the species.
   f. Provide a copy of your State license and U.S. Department of Agriculture General permit, as appropriate.

9. If **import** or **export**, provide:
   a. Copy of any required foreign permits (for CITES Appendix-I plants provide a copy of import permit or evidence a permit will be issued). If plant is to be taken from the wild, provide documentation from the foreign government approving the action.
   b. Describe: (i) the type, size, and construction of shipping containers and (ii) the arrangements for watering and caring for the specimens during transportation.
   c. A statement on the disposition of all imported plants, plant material, and progeny, if produced.

10. Name and address where you wish permit mailed, if different from page 1 (All permits will be mailed via the U.S. Postal Service, unless you identify an alternative means below):

11. If you wish the permit to be delivered by means other than USPS regular mail, provide an air bill, pre-paid envelope, or billing information. If you do not have a pre-paid envelope or air bill and wish to pay for a courier service with your credit card, please check the box below. Please DO NOT include credit card number or other information; you will be contacted for this information.

    ☑ If a permit is issued, please send it via a courier service to the address on page 1 or question 9. I understand that you will contact me for my credit card information once the application has been processed.

12. Who should we contact if we have questions about the application? (Include name, phone number, and email):

    Marc Appelhans, ++49 5513922220, mappelh@gwdg.de

13. **Disqualification Factor.** A conviction, or entry of a plea of guilty or nolo contendere, for a felony violation of the Lacey Act, the Migratory Bird Treaty Act, or the Bald and Golden Eagle Protection Act disqualifies any such person from receiving or exercising the privileges of a permit, unless such disqualification has been expressly waived by the Service Director in response to a written petition. (50 CFR 13.21(c)) Have you or any of the owners of the business, if applying as a business, been convicted, or entered a plea of guilty or nolo contendere, forfeited collateral, or are currently under charges for any violations of the laws mentioned above?

    ☐ Yes ☑ No If you answered "Yes" provide: a) the individual's name, b) date of charge, c) charge(s), d) location of incident, e) court, and f) action taken for each violation.

Landwirtschaftskammer Niedersachsen

Landwirtschaftskammer Niedersachsen • Wunstorfer Landstr. 9 • 30453 Hannover

Pflanzenschutzamt
Fachbereich 3.7
Wunstorfer Landstraße 9
30453 Hannover
Telefon: 0511 4005-0
Telefax: 0511 4005-2215

Internet: www.lwk-niedersachsen.de

Bankverbindung
Landessparkasse zu Oldenburg
BLZ 280 501 00 | Kto 000-199 4599

To whom it may concern

Ihr Zeichen	Unser Zeichen	Ansprechpartner	In	Durchwahl	E-Mail	Datum
	SG 3.7.2	Brunhild Köhler		-2201	Brunhild.Koehler@LWK-Niedersachsen.de	05.04.2016

Information to the responsible Plant Protection Office about the import of seeds / dried plants into an EU-Member State:
Seeds from: *Melicope*, *Platydesma und Zanthoxylum* (Rutaceae), *Myrsine* (Primulaceae) and various dried plant samples

Importer:
Dr. Marc Appelhans, Curator of the Herbarium
University of Goettingen
Albrecht-von-Haller Institute for Plant Sciences Department of Systematic Botany
Untere Karspuele 2, 37073 Goettingen, Germany

This is to certify that the import of the **above mentioned seed species and of dried plants in general** into the Europian Union from third countries is allowed without phytosanitary measures or restrictions.

Kind regards

Brunhild Köhler
Plant Health
Import, Export, EU-Single Market

Deutscher Pflanzengesundheitsdienst • Pflanzengesundheitszentrale • Amtliche • NI 01

Project Description – Project Proposals

Principal investigator:
Dr. Marc S. Appelhans

Georg-August-Universität Göttingen, Albrecht-von-Haller-Institute for Plant Sciences, Department of Systematics, Biodiversity and Evolution of Plants, Untere Karspüle 2, 37073 Göttingen

Phone: ++49 (0) 551 3922220
Email: marc.appelhans@biologie.uni-goettingen.de

Title: Biogeography and evolution of the largest adaptive radiation of woody plants (*Melicope*, Rutaceae) on the Hawaiian Islands.

Project Description

1 State of the art and preliminary work

The proposed study aims at deciphering the evolutionary history, phylogeography and putative hybridization events of Hawaiian *Melicope* (*Citrus*- or Rue-family; Rutaceae) as an example of a recent and species-rich radiation of flowering plants.

The Hawaiian Islands are a perfect "natural laboratory" to study recent and species-rich radiations. With approximately 5 MA (millions of years) Kaua'i and Ni'ihau are the oldest of the current main islands and the archipelago ranks among the most isolated landmasses on the planet (Price & Elliott-Fisk, 2004). Due to their recent age and their isolated geography, only relatively few organismal lineages successfully colonized the archipelago and some of them underwent adaptive radiations that lead to great numbers of species (Funk & Wagner, 1995; Price & Wagner, 2004; Givnish *et al.*, 2009). There are often vast morphological differences between those species, which is usually contrasted by very low genetic variation (Baldwin & Sanderson, 1998; Cronk *et al.*, 2005; Knope *et al.*, 2012). The *Melicope* lineage is a very good example for this phenomenon as it shows a wide array of morphological variation (e.g., growth form, connation of carpels, leaf shape, indumentum), but low genetic variation (Wagner *et al.*, 1990; Harbaugh *et al.*, 2009; Appelhans *et al.*, 2014a, b). *Melicope* constitutes one of the most species-rich plant lineages on the Hawaiian archipelago and, with a total number of 52 species, it accounts for the largest lineage of woody plants (Wagner *et al.*, 1990). The bird-dispersed genus *Melicope* also occurs on other Pacific Islands, Australasia, Malesia, S Asia, Madagascar and the Mascarene Islands. Altogether, with about 230 species, *Melicope* is the largest genus of Rutaceae (Hartley, 2001). In the course of a postdoctoral project at the Smithsonian Institution (Washington DC, USA), the applicant studied the phylogeny and biogeography of *Melicope* and related genera. Six nuclear and plastid markers were sequenced using Sanger sequencing. The phylogenetic trees inferred from this dataset were highly supported and well resolved for most branches, but the Hawaiian clade showed relatively poor resolution (Fig. 1). Similar results are reported for other Hawaiian lineages (Baldwin & Sanderson, 1998; Cronk *et al.*, 2005; Knope *et al.*, 2012). Until the advent of Next Generation Sequencing (NGS) techniques, studies on recent and species-rich radiations were often hampered by the low quantity of DNA sequence information, which did not deliver enough variable sites to untangle the evolutionary histories of these rapid radiations (Maddison & Knowles, 2006; Rubin *et al.*, 2012; Eaton & Ree, 2013). Phylogenetic and biogeographic studies mostly do not require the sequencing of a complete and annotated genome or transcriptome. Instead, a sufficiently large number of single nucleotide polymorphisms (SNP's), which carry useful phylogenetic information, is needed. Restriction site associated DNA sequencing (RADseq; Baird *et al.*, 2008) is a very cost-effective NGS method to recover large amounts of SNP data across the genome. Because not the whole genome is sequenced using this method, several samples can be multiplexed and sequenced together on a single Illumina lane, which makes the method very time- and cost-effective (Baird *et al.*, 2008; Eaton & Ree, 2013). It is well suited for non-model taxa for which no

fully sequenced close relative is present, because it does not involve the assembly of whole genomes. So far, RADseq has mainly been used for population genetic studies (e.g., Narum *et al.*, 2013), but has recently shown its utility in resolving phylogenetic relationships at and below species-level and even postglacial radiations as well as the radiation of cichlid fishes in Lake Victoria – "the fastest known vertebrate species radiation" – have been untangled using this method (Emerson *et al.*, 2010; Rubin *et al.*, 2012; Cariou *et al.*, 2013; Eaton & Ree, 2013; Wagner *et al.*, 2013; Cruaud *et al.*, 2014; Hipp *et al.*, 2014; Ebel *et al.*, 2015; Herrera & Shank, 2015). At the time of submitting this proposal, no RADseq study based on Hawaiian plants has been published, so that the proposed study will serve as a case study in Hawaiian biogeography.

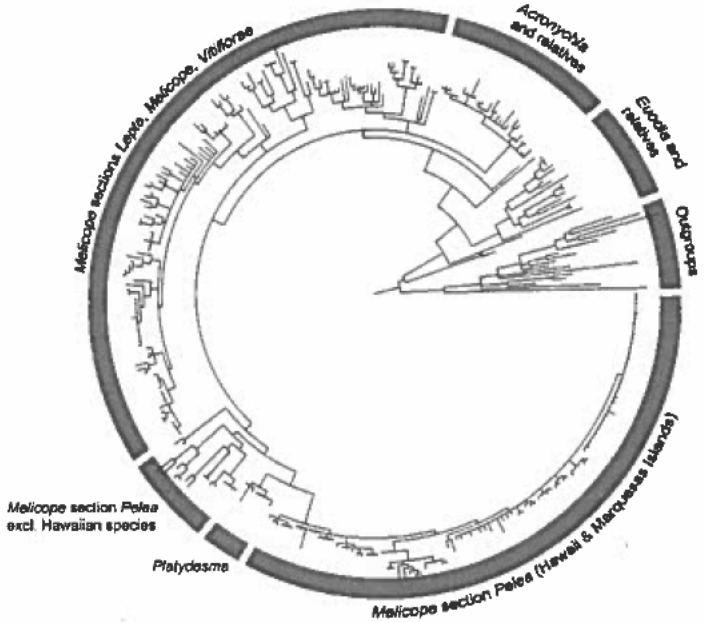

Fig.1: Bayesian consensus tree of *Melicope* and related genera based on Sanger sequencing of six chloroplast and nuclear markers. Most branches of the tree are well resolved and supported, except for the clade that contains the Hawaiian radiation (=*Melicope* section *Pelea* (Hawaii & Marquesas Islands) (adapted from Appelhans *et al.*, 2014b).

In the course of preparing this project proposal, we tested the suitability of the RADseq method to untangle phylogenetic relationships among Hawaiian *Melicope* species. In total 23 samples – 12 Hawaiian and 11 non-Hawaiian *Melicope* – were sequenced on one Illumina HiSeq 2500 using *Pst*I as restriction enzyme. All Hawaiian material used in our previous publications (Appelhans *et al.*, 2014a, b) did not deliver the sufficient DNA quantity needed for RADseq, and our collaboration partner Kenneth Wood (National Tropical Botanical Garden, Kalaheo, Hawaii/USA) sent us newly collected silica-material. Non-Hawaiian samples were taken from cultivated plants or used from recently collected material. This demonstrates the need for recently collected material for RADseq. Library preparation and sequencing were carried out by Eurofins MWG and we analyzed the DNA sequence reads with the program pyRAD (see "Lab work and data analyses" under 2.3 for details). A total of 78,614 RAD loci that included 75,233 phylogenetically informative sites were detected, and used for phylogenetic reconstruction using MrBayes 3.2.5 (Ronquist *et al.*, 2012). The resulting consensus tree showed very high resolution and support (Fig. 2). All except one node in the tree were highly supported (>0.95 posterior probability, pp). The only node that was not supported (0.77pp) was part of the backbone phylogeny suggesting that the RADseq method might not be suited to resolve deeper nodes due to problems with homology assessment of RAD loci. All nodes within the Hawaiian clade were strongly supported and the two species (*M. barbigera*, *M. degeneri*) of which more than one sample was sequenced were monophyletic (Fig. 2).

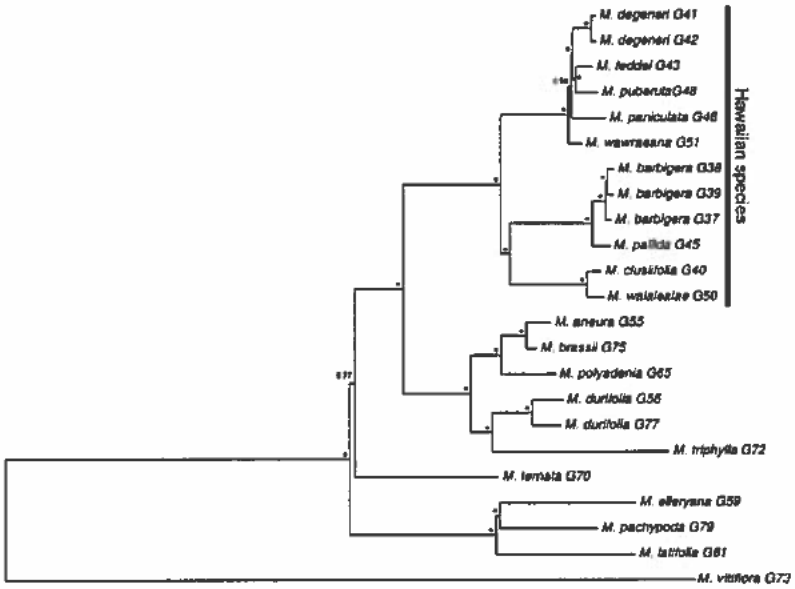

Fig.2: Preliminary RADseq results. Bayesian consensus tree of 23 *Melicope* specimens including 12 specimens from the Hawaiian Islands based on 75,233bp. The phylogenetic tree is completely resolved and all but one basal node are statistically supported (≥0.95pp). Nodes with maximum support (1.00pp) are marked with an asterisk (*). *M.* = *Melicope*. (Appelhans, unpublished results).

A resolved phylogeny of the large radiation of Hawaiian *Melicope* is the starting point of a series of analyses to answer biogeographic and evolutionary questions. The location of the Hawaiian Islands on a moving volcanic hotspot has created an archipelago in which islands are arranged in sequence with geological age (Price & Elliott-Fisk, 2004). Our preliminary molecular dating results (BEAST software; Drummond & Rambaut, 2007) inferred from our Sanger sequencing dataset point to a relatively old age of the Hawaiian *Melicope* lineage (Appelhans *et al.*, in preparation). We assume that the lineage was an early colonizer of the archipelago at a time when Nihoa and Necker (now small, rocky islands without forests) were established, but the oldest current main islands Kaua'i and Ni'ihau were still submerged. With each newly emerging island, *Melicope* could have colonized the archipelago in a stepwise manner (rule of progression; Funk & Wagner, 1995). The islands Maui, Lanai, Moloka'i and Kaho'olawe were merged into one island (Maui Nui) until Moloka'i started to separate at around 0.6 MA ago and the remaining islands were probably connected until the Last Glacial Maximum during periods with low sea level (Price & Elliot-Fisk, 2004). Because of alternating periods of low and high sea levels, islands were separated by the ocean repeatedly, and a vicariance pattern might be expected for species endemic to these islands (Funk & Wagner, 1995).

A common problem for plants colonizing new and distant areas is the small size of the founder population reflecting a low genetic diversity. Inbreeding depression, loss of genetic variation and accumulation of deleterious mutations are connected to higher extinction rates on island systems (Frankham, 1998). Polyploid taxa are thought to be better suited to colonize new areas than diploid taxa due to their higher genetic diversity (Seehausen, 2004; te Beest *et al.*, 2012). This pattern is very obvious in the Hawaiian flora, which has the highest incidence of polyploidy worldwide (Carr, 1998). However, not much is known about hybridization and ploidy levels in *Melicope*. Based on intermediate morphological characters, the New Zealand endemic *Melicope mantellii* is often considered to be a hybrid of the other two species (*M. simplex* & *M. ternata*) from New Zealand (Hartley, 2001). However, genetic studies verifying this hypothesis are still lacking. Hybridization in *Acronychia*, a very close relative of *Melicope* (Fig. 1; Holzmeyer *et al.*, 2015), has been observed (Rossetto, 2005). Chromosome numbers have been counted for only 21 out of about 230 *Melicope* species including nine Hawaiian species (Hartley, 2001; Kiehn, 2005, unpublished). With the data at hand it seems likely that the genus is diploid with a basic chromosome number of x=18 (2*n*=36).

Interestingly, one of the measured Hawaiian species shows a tetraploid pattern, which suggests that at least some of the Hawaiian species are polyploid. Up to seven species of *Melicope* are reported to grow in sympatry on the Hawaiian Islands (Wagner *et al.*, 1990), which suggests that there is a potential for hybridization. The indication that several of the measured Hawaiian *Melicope* species are diploid suggests that the earliest colonizing populations might have been diploid and that allopolyploid hybridization occurred later on during the radiation of the genus. A contrasting pattern of hybridization has been observed in other Hawaiian lineages, in which the hybridization events usually preceded the colonization events (Carr, 1998; Barrier *et al.*, 1999; Lindqvist & Albert, 2002; Lindqvist *et al.*, 2003; Soltis *et al.*, 2009; Baldwin & Wagner, 2010; Marcussen *et al.*, 2012; Roy *et al.*, 2013).
In the course of this project, we will determine the hybrid origin of a taxon by comparing the RADseq data (Partitioned D statistics test; see 2.3) with our results from flow cytometry and chromosomal counts. Prof. Dr. Michael Kiehn (University of Vienna) is a specialist on both Hawaiian plants and chromosome counting and will be involved in the chromosomal counting. He has already provided six unpublished chromosomal counts for Hawaiian *Melicope* and will be involved in measuring more species from plant material that will be collected during fieldwork and then cultivated in the Göttingen Botanical Garden. We tested if silica gel dried plant material of *Melicope* is suited for flow cytometry by measuring fresh material and one month old silica dried material obtained from the same *Melicope triphylla* plant cultivated at Göttingen Botanical Garden. Our measurements are highly congruent and ascertain that the species is diploid with a C-value of about 0.90pg. We also tested older (2007-2010) silica-dried material of Hawaiian species and these samples did not deliver clear results, thus we confirm the findings by Suda & Trávníček (2006), who do not recommend using material older than 20 months. Our measurements will be based on the silica gel material and plants grown from seeds collected in the course of this study.

1.1 Project-related publications

Holzmeyer L, Duretto M, Crayn D, Hörandl E, Heslewood M, Jayanthan J, **Appelhans MS. 2015**. Phylogeny of *Acronychia* (Rutaceae) and first insights into its historical biogeography and evolution of fruit characters. *PLoS ONE* **10:** e0136296.

Appelhans MS, Wen J, Wagner WL. **2014**. A molecular phylogeny of *Acronychia*, *Euodia*, *Melicope* and relatives (Rutaceae) reveals polyphyletic genera and key innovations for species richness. *Molecular Phylogenetics and Evolution* **79:** 54–68.

Appelhans MS, Wen J, Wood KR, Allan GJ, Zimmer EA, Wagner WL. **2014.** Molecular phylogenetic analysis of Hawaiian Rutaceae (*Melicope*, *Platydesma* and *Zanthoxylum*) and their different colonisation patterns. *Botanical Journal of the Linnean Society* **174:** 425–448.

Appelhans MS, Wagner WL, Wood KR. **2014**. *Melicope balgooyi* Appelhans,W.L.Wagner & K.R.Wood, a new species and new record in *Melicope* section *Melicope* (Rutaceae) for the Austral Islands. *PhytoKeys* **39:** 77–86.

Appelhans MS, Janssens SB, Smets E, Razafimandimbison SG, Keßler PJA. **2012.** Age and historical biogeography of the pantropically distributed Spathelioideae (Rutaceae, Sapindales). *Journal of Biogeography* **39:** 1235–1250.

Appelhans MS, Van Heuven BJ, Lens F, Baas P. **2012.** Phylogenetic and ecological signals in the wood of the Spathelioideae (Rutaceae). *IAWA Journal* 33: 337-353.

Appelhans MS, Smets E, Razafimandimbison SG, Haevermans T, van Marle EJ, Rabarison H, Couloux A, Randrianarivelojosia M, Keßler PJA. **2011.** Phylogeny, evolutionary trends, and classification of the *Spathelia* / *Ptaeroxylon* clade: morphological and molecular insights. *Annals of Botany* **107:** 1259–1277.

Razafimandimbison SG, **Appelhans MS**, Rabarison H, Haevermans T, Rakotondrafara A, Rakotonandrasana SR, Ratsimbason M, Labat J-N, Keßler PJA, Smets E, Cruaud C, Couloux A, Randrianarivelojosia M. **2010**. Implications of a molecular phylogenetic study of the Malagasy genus *Cedrelopsis* and its relatives (Ptaeroxylaceae). *Molecular Phylogenetics and Evolution* 57: 258-265.

Appelhans MS, Smets E, Baas P, Keßler PJA. **2010.** *Cneorum* (Rutaceae) in Cuba? The solution to a 150 year old mystery. *Taxon* 59: 1126-1134.

Appelhans M, Weber HC, Imhof S. **2008.** Rutaceae sampled from Germany, Malta, and Mallorca (Spain) are associated with AMF clustering with *Glomus hoi* Berch & Trappe. *Mycorrhiza* 18: 263-268.

2 Objectives and work programme

2.1 Anticipated total duration of the project

The project is designed for a total duration of three years. See time schedule in 2.3.

2.2 Objectives

Within the proposed project we will study the evolutionary history of Hawaiian *Melicope* as an example of a recent and species-rich plant radiation. The main bottleneck until now has been the lack of phylogenetic resolution (Appelhans *et al.*, 2014a), which hindered the understanding of mechanisms of speciation and colonization. RADseq is a promising approach to better resolve the phylogeny and our preliminary study has proven that it is perfectly suited for Hawaiian *Melicope* (Fig. 2). RADseq opens the door to more integrative studies on species radiations on the Hawaiian Islands. The proposed project involves RADseq of 120 specimens of the genus *Melicope* with subsequent phylogenetic reconstruction, biogeographical analyses and hybridization/introgression studies. The latter will also involve flow cytometry and chromosome counting. Our results will deliver new and important insights into island biogeography, hybridization and the question of how to tackle lineages with low genetic variation. The project will be among the first plant radiations studied with RADseq and the very first for the Hawaiian Islands. The exploration of RADseq data for molecular dating and diversification analyses is at the very beginning (Cavender-Bares *et al.*, 2015), so that our study will be among the very first case studies using RADseq in biogeographical analyses.

Our particular research questions and hypotheses are as follows:

1. RADseq method & Phylogenetic reconstruction
Previous molecular phylogenetic studies on Hawaiian *Melicope* (Harbaugh *et al.*, 2009; Appelhans *et al.*, 2014a, b) were successful in resolving the major clades and showed that the Hawaiian Islands were not a dead-end for dispersal. However, a resolution at species-level was not possible using Sanger sequencing. Our preliminary RADseq dataset containing 12 Hawaiian and 11 non-Hawaiian *Melicope* specimens delivered 75,233 SNPs and the resulting phylogenetic tree was completely resolved and statistically supported for the Hawaiian taxa (Fig. 2). This means a nearly 100-fold increase in variable characters as compared to the six-marker dataset used by Appelhans et al. (2014a; 768 variable characters of which 430 were parsimony-informative).
A resolved phylogeny is the first goal of the proposed study and questions related to the monophyly of sections and species will be answered. The resolved phylogeny will also be the basis for the following questions. The proposed study will be the first NGS project about a major adaptive radiation on the Hawaiian Islands and it will serve as a case study for future studies on Hawaiian taxa and studies in other geologically young areas or young species groups in which Sanger Sequencing does not provide sufficient data quantity.

2. (Hawaiian) Biogeography
When and where did the ancestor of Hawaiian *Melicope* arise, when was the archipelago colonized and how did *Melicope* spread over the Hawaiian Islands?
Our hypotheses based on preliminary results (Appelhans *et al.*, in preparation) are that the ancestors of Hawaiian *Melicope* stem form New Guinea and/or nearby islands and that the Hawaiian lineage is slightly older than the current islands, suggesting that older islands of the Hawaiian-Emperor seamount chain such as Nihoa and Necker were colonized first. From there,

Kaua'i and the younger islands were colonized. Judging from the high number of species and the fact that all four sections of Hawaiian *Melicope* are present on Kaua'i (Wagner *et al.*, 1990), an initial radiation on this island seems likely. With the emergence of the younger islands, new areas became available for colonization and our hypothesis is that they were colonized in a stepwise manner mainly from the older to the younger islands.

Our existing Sanger sequencing data (Appelhans *et al.*, 2014a) does not allow many biogeographical conclusions, but the habitat preferences of Hawaiian *Melicope* shows an interesting pattern. Most Hawaiian *Melicope* species (79%) are single-island endemics and many of these species are restricted to wet and bog forest habitats. The few multi-island species (species that occur on more than one Hawaiian Island) of *Melicope* are found also in dry and mesic forest. Hawaiian lobeliads (Campanulaceae) show a similar pattern (Givnish *et al.*, 2009). Givnish *et al.* (2009) argued that forest-interior bird species, which disperse the diaspores, often baulk at habitat barriers. The same could be true for the wet-forest species of the bird-dispersed *Melicope*. Dispersal by very stationary birds would mean very restricted dispersal capabilities, so that dry and mesic ancestors might account for most inter-island dispersal with repeated dispersal into wet-forest habitats. Figure 3 pictures this scenario and shows the placement of wet- and dry-habitat species in a phylogenetic tree. The rare occasions in which a wet-forest species disperses to an adjacent island would immediately lead to a genetic barrier with a potential of subsequent speciation. In this case, the phylogenetic trees would show wet-forest species (or species groups) from adjacent islands as sister groups.

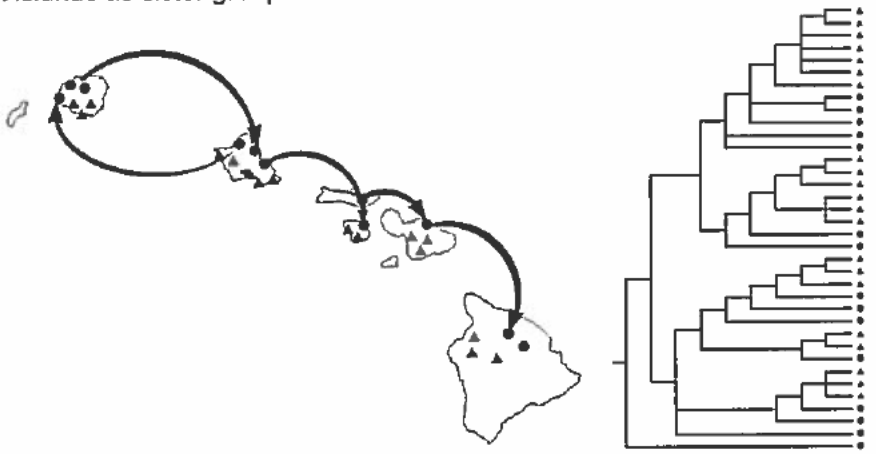

Fig.3: A possible colonization scenario of Hawaiian *Melicope*. Taxa that are adapted to dry or mesic habitats (black dots) account for the inter-island colonization (black arrows; mainly from older to younger islands) while wet-forest taxa (gray triangles) descended from dry or mesic taxa and hardly cross the barrier to another island. In a phylogenetic tree, dry or mesic taxa would mainly represent early diverging lineages that are sister to clusters of wet-forest taxa.

The major modes of speciation of Hawaiian *Melicope* might be geographic isolation (relatively rare inter-island colonizations) and ecological isolation (adaptation to different habitats [e.g. moist forest habitats]). An additional factor, especially in areas where several *Melicope* species co-occur, could be hybridization, a topic that will be discussed in the following paragraph.

3. Hybridization & Species-richness

Do several Hawaiian *Melicope* species have a hybrid origin? Did this hybridization happen before or after the colonization of the archipelago? Did the hybridization event(s) have an influence on the net diversification rates (hybridization as driver of adaptive radiation)? Are homoploid hybrids more common than allopolyploid hybrids?

The available information on chromosome numbers and ploidy levels in Hawaiian *Melicope* (Hartley, 2001; Kiehn, 2005, unpublished), suggests that the ancestor of Hawaiian *Melicope* was diploid and that polyploidization occurred at least once on the Hawaiian Islands. The tetraploid species *M. wawraeana* belongs to the most species-rich clade of Hawaiian *Melicope*, which

includes about 30 species (Appelhans *et al.*, 2014a). The species has been confused with *M. oahuensis* and *M. peduncularis* and its fruits sometimes approach the features of both species (Wagner *et al.*, 1990). We will test the hypothesis that *M. oahuensis* and *M. peduncularis* are the potential parent species of *M. wawraeana*. In addition to the tetraploid *M. wawraeana*, chromosome counts for three more species of the species-rich clade are known (Kiehn, 2005, unpublished). The three species are diploid ($2n$=36) and our hypothesis is that the ancestor of the clade was diploid and that allopolyploid hybridization occurred at least once in this clade.
Allopolyploidy is the result of a meiotic mismatch that might be expected when two less closely related species hybridize (Seehausen, 2004; Buggs *et al.*, 2009; Paun *et al.*, 2009). The Hawaiian radiation of *Melicope* is relatively young and genetic divergence is probably low. One might therefore not expect meiotic mismatches during a putative hybridization event in this case, and hybrid species would have the same chromosome number as the parent species. Our hypothesis is that homoploid hybrids might exist in Hawaiian *Melicope* and that they are more common than polyploid hybrids. Analysis of the RADseq data will directly enable us to differentiate between homoploid hybrids and their parent species, and also between allopolyploid and autopolyploid species (see paragraph about the Partitioned D-statistics test in 2.3). Up to seven species of *Melicope* occur sympatrically on the Hawaiian Islands (Wagner *et al.*, 1990) so that an important requirement for hybridization exists. Another important requirement for hybrid speciation is genetic isolation between the hybrid and its parental taxa. In allopolyploids this is directly achieved through reproductive isolation caused by the different chromosome numbers of gametes. Seehausen (2004) argued that some hybrid genotypes might be better adapted in extreme habitats and novel niches compared to their parent taxa, while at the same time showing lower fitness under parental ecological conditions. Transgressive segregation in F2 and later hybrid generations is the putative mechanism behind this type of hybrid speciation (Rieseberg & Willis, 2007). In this way, hybrids would be ecologically isolated from the parents and might develop into new species. Seehausen (2004) hypothesized that hybridization might be a driver of adaptive radiation in unstable or new environments. *Melicope* probably arrived very early in the history of the Hawaiian Islands (Appelhans *et al.*, in preparation). Molecular dating of most other Hawaiian plant lineages suggests a younger age than the oldest of the current main islands (Lindqvist & Albert, 2002; Clark *et al.*, 2009; Havran *et al.*, 2009; Keeley & Funk, 2011; Knope *et al.*, 2012; Sebastian *et al.*, 2012) so that one might expect *Melicope* to be among the first colonizers of the then underutilized and developing ecological niches on the single islands. Many Hawaiian plant lineages are paleopolyploids (Carr, 1998; Barrier *et al*, 1999; Lindqvist & Albert, 2002; Lindqvist *et al.*, 2003; Soltis *et al.*, 2009; Baldwin & Wagner, 2010; Marcussen *et al.*, 2012; Roy *et al.*, 2013) and *Melicope* is among the few Hawaiian lineages that show different ploidy levels. *Melicope* therefore is a perfect model taxon to test Seehausen's (2004) theories with modern NGS methods.

2.3 Work programme incl. proposed research methods

Study taxa
Melicope is an ideal model taxon to study island biogeography. Of the total +/- 230 species in this genus, 52 are endemic to the Hawaiian Islands making *Melicope* the largest Hawaiian radiation of woody plants (Hartley, 2001; Wagner *et al.*, 1990). The species grow at a number of different habitats including bog forests, wet forests, mesic forests and dry forests. About 79% of the species, especially those adapted to very humid areas (wet forests and bogs), are single island endemics. The patchy information on chromosome numbers suggests that at least some Hawaiian species are polyploid (Hartley, 2001; Kiehn, 2005, unpublished). Wagner *et al.* (1990) highlight that as many as seven species of *Melicope* occur sympatrically in some areas.

Fieldwork on the Hawaiian Islands
The samples for this project have to be collected in the wild. RADseq requires high quality and quantity of DNA and four weeks of fieldwork are therefore planned in the first year of the project. Due to the high proportion of single-island endemics, collecting on all larger islands (Kaua'i, O'ahu, Maui, Moloka'i and Hawai'i) is necessary in order to obtain a sufficiently high taxon sampling. The

fieldwork will be carried out by the principal investigator and the PhD student. Kenneth Wood (National Tropical Botanical Garden [NTBG], Kalaheo, Kaua'i) has agreed to assist during the fieldwork and Warren Wagner (Washington DC) will help to assemble a list of locations that will be visited. Several collection and export permits are needed (separate permit for each National Park). We will apply for these permits prior to the start of the project together with our counterparts from NTBG (Kenneth Wood, David Lorence) as well as Warren Wagner and Michael Kiehn, who are both affiliated to NTBG (Wagner: McBryde Chair of Hawaiian Plant Studies; Kiehn: Research Associate).
During the fieldwork, about 120 specimens of most Hawaiian *Melicope* species will be collected. Some species are regarded as (possibly) extinct, so that our taxon sampling will not reach 100% of the described species. Our goal is to sample most species with two or three specimens and to include up to five samples for species that either occur on several islands (e.g., *M. clusiifolia*, *M. hawaiiensis*, *M. peduncularis*, *M. volcanica*), that are very variable (e.g., *M. clusiifolia*, *M. hawaiiensis*, *M. knudsenii*) or for which the delimitation towards other species is not clear (e.g., *M. elliptica* species complex, *M. kavaiensis* species complex, *M. oahuensis*/*M. anisata*, *M. wawraeana*).
Seeds, needed for germination and chromosome counting on root tips, will be collected for cultivation at Göttingen Botanical Garden. Leaf material for RADseq and flow cytometry will be collected in silica gel. Three duplicates of herbarium vouchers will be collected and one set of specimens will be sent to Göttingen University, Washington DC (Smithsonian Institution), and NTBG (Kalaheo, Kaua'i/ Hawaii) each, so that the involved cooperation partners will have access to the collections and can help with the identification of specimens. We will digitize the Göttingen specimens and make the scans and metadata available through the webpage of the Göttingen herbarium, so that researchers worldwide have the possibility to review and verify the species identifications.

Lab work and data analyses

Lab work will be carried out by the PhD student and a technical assistant (Jennifer Krüger).

Sequencing will be performed on 120 samples (40 species, 3 individuals per species) collected during our field trip as well as five non-Hawaiian *Melicope* species as outgroups. Outgroup-samples have been sequenced in the course of generating preliminary data and sequencing can be repeated if necessary from *Melicope* species cultivated in the Botanical Gardens in Göttingen (*Melicope elleryana*, *M. triphylla*, *M. ternata*), Leiden (Netherlands; *M. denhamii*) and NTBG (*M. latifolia*).
Total DNA will be extracted using the DNeasy Plant Mini kit (Qiagen, Hilden, Germany). We will do two extractions per sample in order to assure that the high DNA quantity needed for RADseq (~5µg) is provided. DNA quantification will be measured using a Qubit 3.0 fluorometer (Life Technologies, Darmstadt, Germany). The actual library preparation and sequencing will be outsourced to Eurofins MWG Operon (Ebersberg, Germany; http://www. eurofinsgenomics.eu/), which is – to our knowledge – the only sequencing company in Germany that offers RADseq service. DNA will be digested with the 6-base cutter restriction enzyme *Pst*I, which has a large number of cutting sites in the genomes of both animals and plants (Davey & Blaxter, 2011; Eaton & Ree, 2013; Wagner *et al.*, 2013) and yielded more than 75,000 RAD loci in our preliminary study. A barcoded P1 adapter (Primer site & adapter that binds to Illumina flow cell & barcode for individual samples so that several samples can be multiplexed) will be ligated to the ends of the digested DNA. The barcoded DNA samples will be sheared by sonication in order to generate fragments of a length of several hundred basepairs (bp). For size selection, the multiplexed samples will be run on an electrophoresis gel and fragments of approximately 300-500bp will be isolated by gel extraction. We expect tetraploid and hybrid species in Hawaiian *Melicope*, for which high coverage (at least 50 fold) is essential to identify heterozygous SNPs (Hirsch & Buell, 2013). Multiplexing of 23 samples on one lane (23 samples plus one internal control) will be appropriate for this coverage (see cost estimation by Eurofins MWG GmbH). A P2 adapter, which carries the reverse primer sequence, will finally be ligated to the fragments, which will then be ready for single-end

sequencing using an Illumina HiSeq 2500 system (Etter *et al.*, 2011; read length 100bp; 150-180 million reads per lane).
A free software pipeline (*pyRAD*; http://pyrad.googlecode.com) for processing Illumina FASTQ sequence files into a multi-sequence alignment has been developed by Dr. Deren Eaton (Eaton & Ree, 2013; Eaton, 2014), who will be involved in our project as a collaborator. *pyRAD* will be used to de-multiplex the Illumina sequence data, trimming barcodes, restriction sites and adapters as well as removing low quality sequence reads. Sequences of each de-multiplexed sample are clustered by similarity using USEARCH (Edgar, 2010) and clusters with low coverage will be deleted. The remaining clusters will be used for SNP calling and to calculate consensus sequences for each sample using the MUSCLE algorithm (Edgar, 2004). Multi-sequence alignments will be generated from the consensus sequences using MUSCLE and used for further analysis.
In addition to *pyRAD*, which we used for our preliminary data analysis, we will explore other analysis software such as stacks (Catchen *et al.*, 2011), AftrRAD (Sovic *et al.*, 2015) and the CLC genomics workbench (Aarhus, Denmark). RADseq datasets often contain a larger amount of missing data because some RAD loci will not be sequenced for all samples (most likely due to mutations in the cutting sites). The effect of missing data has been explored by creating different data matrices that consisted of as many RAD loci as possible and allowing RAD loci to be missing in several samples, versus including only RAD loci that were available for most samples (trade-off: number of included RAD loci vs. amount of missing data for each RAD locus) (Eaton & Ree, 2013; Wagner *et al.*, 2013; Chattopadhyay *et al.*, 2014). We will assemble and analyze different datasets accordingly to find a balance between missing data and number of loci included. A known problem with RADseq is the orthology assessment of RAD loci (Rubin *et al.*, 2012; Leaché *et al.*, 2015). Low orthology of RAD loci is correlated with mutations in the cutting sites of restriction enzymes and are thus more problematic with higher genetic variation and old clade ages (>50MA; Rubin *et al.*, 2012). In a study (Cavender-Bares *et al.*, 2015) focusing on a group of oak species that were slightly older than the estimates for Hawaiian *Melicope* (Appelhans *et al.*, in preparation), homology assessment proved not to be problematic. This, together with the results of our preliminary study, indicates that homology assessment will most probably not be a critical point for the planned study.
Phylogenetic analyses of the preliminary RADseq dataset were done using MrBayes 3.2.4 (Ronquist *et al.*, 2012). However, the final datasets will contain much more data, so that phylogenetic analyses will be carried out using mainly fast maximum likelihood (ML) algorithms implemented in programs such as RAxML (Stamatakis, 2006) and GARLI (Zwickl, 2006). We are working together with the *Gesellschaft für wissenschaftliche Datenverarbeitung mbH Göttingen* (GWDG) who are maintaining high-performance computer clusters on which we will run the analyses. We will further explore the feasibility of Bayesian Inference using MrBayes (Ronquist *et al.*, 2012), Phycas (Lewis *et al.*, 2015) and BUCKy (Larget *et al.*, 2010) using partial datasets (e.g. using only variable sites). Hybridization networks as implemented in SplitsTree 4.0 (Huson & Bryant 2006) will be used to study reticulate evolution and to reconstruct hybrid-parent relationships (Pirie *et al.*, 2009; Pellino *et al.*, 2013).

We will explore the use of RADseq for molecular dating and will also carry out molecular dating analyses based on the already existing Sanger sequencing data (Appelhans *et al.*, 2014b) for comparison. No fossils of Hawaiian *Melicope* are known, but a root age of the lineage can be taken from our biogeographical work on the whole genus (Appelhans *et al.*, in preparation). In a second approach we will use the age of islands as calibration points for dating. Molecular dating analyses will be performed using the BEAST package (Drummond & Rambaut, 2007). Additionally, we will perform diversification analyses using BAMM (Rabosky, 2014), MEDUSA (Alfaro *et al.*, 2009) and BayesRate (Silvestro *et al.*, 2011). A particular interesting feature of the BAMM package is its ability to account for missing taxa and the non-random distribution of missing taxa in the phylogeny. Net diversification rates between different subclades will be compared and a specific hypothesis that will be tested is that hybridization events with subsequent speciation might have caused diversification rate changes.

In order to trace back inter-island colonization patterns, we will perform ancestral area analyses (AAR) using the programs BioGeoBEARS (Matzke, 2013) and Lagrange (Ree *et al.*, 2005; Ree & Smith, 2008). An advantage of both programs in comparison with other AAR methods is the

possibility to exclude certain areas during certain geological epochs and to allow the dispersal probabilities between two areas to change through time. In our case, the age/emergence of each Hawaiian island (and its availability for colonization) can be incorporated into the analyses. BioGeoBEARS also features a model that accounts for founder-event speciation ("jump-dispersal"), which is an important model in islands systems and which has not been implemented in other AAR programs so far. In addition, ecological parameters such as habitat preferences and altitudinal ranges will be plotted on the phylogenetic trees using Mesquite (Maddison & Maddison, 2011) in order to shed further light on the colonization and occupation of ecological niches of species.

Screening for hybridization and polyploidy will involve three aspects. To test for polyploid taxa, we will carry out chromosome counts based on root tips from germinated seeds and we will determine genome sizes and ploidy levels using flow cytometry. Flow cytometry will be done using silica gel dried leaves and seedlings on a Partec CyFlow Space flow cytometer (Münster, Germany) with the DAPI (4', 6-diamidino-2-phenylindole) fluorochrome applying a standard protocol (Hojsgaard *et al.*, 2012). The differentiation between auto- and allopolyploid taxa and also the differentiation between parent species and homoploid hybrids will be done using the RADseq data. Hybrid taxa are characterized by high levels of heterozygosity because alleles were inherited by parents that belong to different species. The *pyRAD* package contains the Partitioned D-statistics test, which detects introgression and the directionality of gene flow, based on the occurrence of heterozygosity in the RADseq data (Eaton & Ree, 2013). Based on five-taxon subsets of a defined tree topology (from the phylogenetic analyses), the test monitors alleles (SNPs) that are incongruent with the tree topology. There are six possibilities of how an incongruency can be distributed in a five-taxon tree (Fig. 4, right side). If one incongruency pattern is dominant across all alleles, the incongruence is asymmetric. While a symmetric incongruence would be a signal of stochastic lineage sorting, asymmetric incongruence points to introgression.

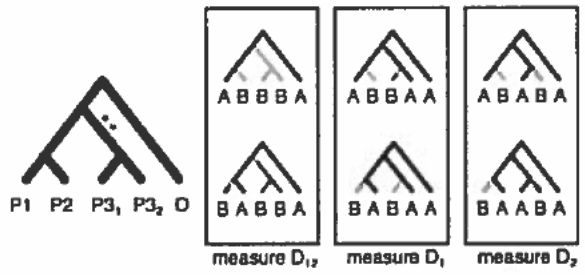

Fig.4: Principle of the Partitioned D statistics test. A derived character (symbol B) is shared by $P3_1$ and/or $P3_2$ with one of the two taxa P1 and P2 (patterns: ABBBA, BABBA, ABBAA, BABAA, ABABA, BAABA). The distribution of that particular character is incongruent with the fixed tree topology. If the overall frequency of one of the patterns across the genome (=across the RAD loci, as they represent a random subset of the whole genome) is higher than the frequency of the other patterns, the incongruence is asymmetric which is a clear indicator of introgression. In case ABBBA is the dominant pattern, there would have been introgression between P2 and the ancestor of $P3_1$ and $P3_2$. Figure adapted from Eaton & Ree (2013).

During the proposed study, we will perform the Partitioned D statistics test based on a series of five-taxon subsets. We will focus on taxa for which chromosomal counts and flow cytometric measurements show differing ploidy levels, taxa growing in sympatry (Wagner *et al.*, 1990), and species-complexes that are morphologically difficult to untangle or show intermediate characters (Wagner at al., 1990; i.e. *M. elliptica* species complex, *M. kavaiensis* species complex, *M. oahuensis*/*M. anisata*). The results of the hybridization studies will be compared with the phylogenetic analyses, the BEAST analyses, and the diversification rate analyses in order to evaluate the time of origin(s), the number of hybridization events and the effect of hybridization on net diversification.

Time schedule

	First year				Second year				Third year			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
Fieldwork Hawaii												
Preparation	X											
Collection of plant material		X										
Lab work & analyses												
RADseq (DNA extraction and QC; further steps done by company)		X	X	X								
Seed germination		X	X	X								
Flow cytometry				X	X							
Chromosome counting				X								
Data analysis					X	X	X	X	X	X		
Writing manuscripts							X			X	X	X
Conferences							X				X	

The application for collecting permits will be handled prior to the start of the project and will be done in cooperation with Warren Wagner (Smithsonian Institution, Washington DC/USA) and Kenneth Wood (National Tropical Botanical Gardens; Kalaheo, Hawaii/USA).

2.4 Data handling

The sequence data will be stored on servers of Göttingen University and published sequence data will be uploaded to EMBL/Genbank. Microscopic images and flow cytometric histograms will be saved and stored in Göttingen. Silica-dried leaf samples and DNA extracts will be kept at -80°C at Göttingen University. Herbarium specimens (3 duplicates of each collection) will be stored in the herbaria of Göttingen University (GOET), the Smithsonian Institution (US) and NTBG (PTBG). In order to provide direct access to the collected herbarium vouchers for researchers worldwide, we will digitize (scanning and databasing) the GOET specimens and make them available through our webpage. Living plants from collected seeds will be kept for further cultivation at the Botanical Garden of Göttingen University.

2.7 Information on scientific and financial involvement of international cooperation partners

See 5.4.1 for a list of cooperation partners and their scientific involvement in the project. The two main cooperation partners will be Dr. Warren Wagner and Dr. Jun Wen (both Smithsonian Institution, Washington DC, USA). Dr. Wagner has worked on the Hawaiian flora and biogeography for many years. He is an expert on Hawaiian *Melicope* and he will be participating in many parts of the project, e.g. assembling a list of locations that will be visited during the fieldwork, identifying species, discussing the results and writing manuscripts. Dr. Wen is an expert in the fields of phylogenetics and biogeography. She leads ongoing projects using several NGS methods including among others RADseq. Dr. Wen has been involved in the study design (e.g., choice of restriction enzyme, number of multiplexing) and she will participate in data analysis, discussing results and writing manuscripts.
Dr. Deren Eaton (Yale University) is a specialist in the field of Next Generation Sequencing and he developed and maintains the software pipeline *pyRAD*. He will be involved in data analysis. Prof. Dr. Michael Kiehn from the University of Vienna is an expert on chromosome counting and he has extensive knowledge about the Hawaiian flora. He will be involved especially in chromosome counting and discussing hybridization and polyploidization. Kenneth Wood is a research botanist at NTBG and has excellent knowledge on the Hawaiian flora and *Melicope*. He will be involved in the application for collection permits, the field trip and the identification of specimens. We are also in

contact with other researchers from Hawai'i (Dr. Marian Chau [University of Hawai'i at Manoa], Dr. Shelley James [Bishop Museum, Honolulu] Dr. David Lorence [NTBG], Hank Oppenheimer [Maui Nui Plant Extinction Prevention Program, Maui], Lara Reynolds [Department of Land & Natural Resources, Pearl City/O'ahu]) and these contacts will be helpful during the fieldwork.
No financial involvement of the international cooperation partners is intended.

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4 Requested modules/funds

4.1 Basic Module

Total amount (staff, consumable, travel, publication costs) requested: 166,985.50€

4.1.1 Funding for Staff

Salary for a PhD student (N.N., 65% of TV-L, E13) is requested for three years.
Quantity: 0,65
Sum per year: 39,390.00€ (according to DFG Personnel Rates for 2015 [65% of 60,600.00€])
Total: 118,170.00€

Student assistant (N.N.) for the digitization of herbarium specimens: 50h in the first year of the project. The hourly rate from Göttingen University for a student assistant with a BSc degree is 14.91€ (including taxes and employer´s share of social security contributions).
Total: 745.50€

4.1.2 Direct Project Costs

In addition to the funding for staff, we are requesting funds for lab work, travel (fieldwork, conferences) and publication costs.

4.1.2.1 Equipment up to €10,000, Software and Consumables

Total amount requested: 27,900.00€

Consumables	
Description	**Amount**
Fieldwork Hawaii	
Permits, sending costs, silica gel, bags, herbarium sheets, paper tags	500.00€
Cultivation of plants	
Seed germination (pots, substrates, labels)	250.00€
RADseq	
DNA extraction (DNeasy Plant Mini Kit, 120 samples, double extraction; 682.20€ plus VAT)	811.82€
DNA quality and quantity check (240 samples) using the Qubit 3.0 Fluorometer (Qubit dsDNA HS Assay Kit, 250.64€ plus VAT)	298.26€
Spot quality assessment/quality control at the sequencing company (Eurofins MWG) (220€ plus VAT)*	261.80€
DNA normalization (Eurofins MWG) (1000€ plus VAT)*	1,190.00€
RADseq library preparation (Eurofins MWG) (120 samples à 90€ plus VAT)*	12,852.00€
Sequencing costs (Eurofins MWG; single-end Illumina HiSeq) (120 samples multiplexed on five channels à 1920€ plus VAT)*	11,424.00€
Data management fee (Eurofins MWG) (200€ plus VAT)*	238.00€

Flow cytometry	
Flow cytometric measurement (120 samples à 0.30€) and glassware	74.12€
Total	27,900.00€

*See the attached price offer by Eurofins MWG GmbH.

4.1.2.2 Travel Expenses

Total amount requested: 17,920.00€

Fieldwork to collect plant material is needed in the first year of the project.

We request support for participation in international conferences for the applicant (principal investigator) and the PhD student in the second and third year of the project (1500€ per person per conference visit). We are envisaging the "XIX International Botanical Congress" in Shenzen (China) in 2017 and the North American "Botany 2018" meeting (organized among others by ASPT [American Society of Plant Taxonomists]; venue not announced yet).

Travel expenses First year	
Description	**Amount**
Fieldwork in Hawaii	
2x airfare Frankfurt - Honolulu	3.000€ (2x 1.500€)
2x airfare between Hawaiian Islands	1.400€ (2x 700€)
Car rental and gasoline (24 days x 80€)	1.920€
Accommodation for Principal Investigator and PhD student (28 days x 60€ per person)	3.360€
Per diem (28 days x 40€ per person)	2.240€
Total	11.920€

Travel expenses Second year	
Description	**Amount**
Conference	
2x travel, accommodation and conference fee (Principal Investigator and PhD student)	3000€ (2x 1500€)
Total	3000€

Travel expenses Third year	
Description	**Amount**
Conference	
2x travel, accommodation and conference fee (Principal Investigator and PhD student)	3000€ (2x 1500€)
Total	3000€

4.1.2.6 Project-related publication expenses

750€ are requested per year (2250€ in total). We will publish our results in ISI ranked, peer-reviewed journals within the field of plant systematics, biogeography and hybridization (e.g., Journal of Biogeography, Molecular Phylogenetics and Evolution, Annals of Botany, Systematic Biology, Evolution).

5 Project requirements

5.1 Employment status information

Appelhans, Marc S., Dr; Scientific staff and curator of the university herbarium (until 3/2019), University of Göttingen

5.2 First-time proposal data

Appelhans, Marc S., Dr. (see 5.1)

5.3 Composition of the project group

Scientific group members

Appelhans, Marc S., Dr.; see above (5.1); *Principal investigator*

Hörandl, Elvira, Univ. Prof., Dr.; Professor for Systematics, Biodiversity and Evolution of Plants (permanent position), University of Göttingen; *Polyploidy & Hybridization, supervisor of PhD student*

Hojsgaard, Diego, PhD; Assistant position in the Department of Systematics, Biodiversity and Evolution of Plants (until 12/2019), University of Göttingen; *Polyploidy, Chromosome counting & Flow cytometry techniques*

Non-scientific group members

Krüger, Jennifer; Lab technician in the Department of Systematics, Biodiversity and Evolution of Plants (permanent 100%), University of Göttingen; *Assisting molecular lab work and preparation of samples for Flow Cytometry*

Friedrichs, Sylvia, Schmidt Sabine; Gardeners in the Department of Systematics, Biodiversity and Evolution of Plants (both permanent 50%), University of Göttingen; *Cultivation of collected seeds form field trip to Hawaii in greenhouses of the Botanical Garden*

Freundt, Heike; Secretary in the Department of Systematics, Biodiversity and Evolution of Plants (permanent 50%), University of Göttingen; *Help with administration of the project*

Liessmann, Gabriele; Herbarium assistant in the Department of Systematics, Biodiversity and Evolution of Plants (permanent 50%), University of Göttingen; *Preparing and mounting of herbarium specimens*

5.4 Cooperation with other researchers

5.4.1 Researchers with whom you have agreed to cooperate on this project

Wagner, Warren L., PhD; Research Scientist, Curator & Chair of Botany, Smithsonian Institution, Washington DC/ USA & McBryde Chair of Hawaiian Plant Studies, National Tropical Botanical Garden, Kalaheo, Hawaii/USA; *Hawaiian biogeography, Hawaiian flora, Melicope*

Wen, Jun, PhD; Research Scientist & Curator, Smithsonian Institution, Washington DC/ USA; *Phylogeny & Biogeography, NGS methods*

Eaton, Deren, PhD; Postdoc, Yale University, New Haven, Connecticut/USA; *Bioinformatics (especially pyRAD package), NGS methods, Introgression and hybridization analyses*

Kiehn, Michael, Prof. Dr.; Director of the Botanical Garden of the University of Vienna/ Austria & Research Associate National Tropical Botanical Garden, Kalaheo, Hawaii/USA; *Chromosome counts, Hawaiian flora*

Wood, Kenneth, MSc; Research Biologist, National Tropical Botanical Garden, Kalaheo, Hawaii/USA; *Field trip to Hawaii, Collection permits, Plant Identification*

Bohrer, Rainer, Dr.; Staff member at the *Gesellschaft fur wissenschaftliche Datenverarbeitung mbH Göttingen* (GWDG); *Bioinformatics software, Computational resources*

5.4.2 Researchers with whom you have collaborated scientifically within the past three years

Marc Appelhans:
Steven **Janssens** (University of Leuven, Belgium), Sylvain **Razafimandimbison** (Bergianska Institute Stockholm, Sweden), Erik **Smets**, Paul **Keßler**, Pieter **Baas**, Frederic **Lens** (all Leiden University, Netherlands), **Nina Stock**, Matthias **Niedrig** (Robert Koch-Institute Berlin, Germany), Laurence **Dorr****, Vicki **Funk****, Warren **Wagner**, Jun **Wen**, Elizabeth **Zimmer** (all Smithsonian Institution, Washington DC/USA), Kenneth **Wood** (National Tropical Botanical Garden, Kalaheo, Hawaii/USA), Gerard **Allan** (Northern Arizona University, Flagstaff, Arizona/USA), Timothy **Sharbel***, Marco **Pellino*** (both Leipniz Institute of Plant Genetics and Crop Plant Research Gatersleben, Germany), Darren **Crayn** (Australian Tropical Herbarium, Cairns, Australia), Marco **Duretto** (Royal Botanic Garden Sydney, Australia), Elvira **Hörandl**, Laura **Holzmeyer**, Bastian **Steudel**** (Göttingen University), Stefanie **Ickert-Bond**** (University of Alaska, Fairbanks, USA)
**Manuscripts in preparation*, ***Manuscripts have been submitted.*
Joint publications with all other persons have been published.

5.5 Scientific equipment

The department of Systematics, Biodiversity and Evolution of Plants is equipped with a modern molecular laboratory that will be used to extract DNA and prepare the samples for the outsourced RADseq. A Qubit 3.0 Fluorometer, needed for the quantification of DNA extracts, is also present in our laboratories. A flow cytometer (Partec Flow Cytometer CyFlow Space with High-speed Autoloader) used to measure genome sizes and ploidy levels as well as microscopic equipment for chromosome counting are available in the department.

In terms of computer facilities, we work together with the *Gesellschaft für wissenschaftliche Datenverarbeitung mbH Göttingen* (GWDG), who provide the computing capacity (computer cluster) needed for the computationally elaborate bioinformatic analyses. The needed software packages mentioned in the methods section are available. Commercial software packages such as

the CLC genomics workbench and Geneious, which might be needed in addition to the *pyRAD* package, are available in the department. In addition to computer clusters at the GWDG, a high-performance computer with 16 cores (2x8) and 64GB-RAM is available in the department.

All equipment needed for the digitization of the herbarium specimens ("HerbScan" [a large-format scanner especially designed for herbarium specimens], PC, barcodes) is available in our department. The Göttingen herbarium is currently migrating its databases to the DiversityWorkbench system (http://diversityworkbench.net) and the digitization will be done using this system.

Cogliano, Mary <mary_cogliano@fws.gov>

RE: Status of processing request for Permit No. "96221B""

1 message

Dang, Charmian C <charmian.c.dang@hawaii.gov> Thu, Aug 18, 2016 at 9:16 PM
To: "Cogliano, Mary" <mary_cogliano@fws.gov>, "Appelhans, Marc" <marc.appelhans@biologie.uni-goettingen.de>
Cc: "Wagner, Warren" <WAGNERW@si.edu>

Hi Mary,

Attached is a draft copy of the Hawaii T&E plant permit for Marc. Once Dr. Wagner and Marc receive an approved permit from your office I can proceed on my side so that Marc will be covered on both the state and federal level. Please feel free in contacting me if you have any questions.

Aloha,

charmie

From: Cogliano, Mary [mailto:mary_cogliano@fws.gov]
Sent: Thursday, August 18, 2016 2:50 AM
To: Appelhans, Marc
Cc: Dang, Charmian C; Wagner, Warren
Subject: Re: Status of processing request for Permit No. "96221B""

Dear Mr. Appelhans,

Thank you for the clarification concerning *Platydesma cornuta*.

The continued processing of your application is pending our receipt of copies of the required collection permits.

Best regards,

Mary Cogliano

On Thu, Aug 18, 2016 at 3:49 AM, Appelhans, Marc <marc.appelhans@biologie.uni-goettingen.de> wrote:

Dear Dr. Cogliano, Dear Charmian,

(Charmian: see email below)

Thank you for informing us about the status of our export permit request.

In addition to the export permit from USFWS we have applied for a permit to collect threatened and endangered species and permits to collect in forest reserves on Hawaii (Big Island), O'ahu, Maui & Moloka'i and Kaua'i. Our main contact regarding these permits is Charmian Dang from the Hawaiian Division of Forestry and Wildlife.

I am sending this email to the two of you so that you can evaluate if all requirements for these permits are fulfilled.

Concerning the question about Platydesma cornuta: We are planning to collect one specimen for each variety. The herbarium specimens will stay in Hawaii (Bishop Museum) and we would like to export a single leaf each for the two collections.

Best wishes,

Marc Appelhans

Dr. Marc Appelhans

Curator of the Herbarium

University of Goettingen

Albrecht-von-Haller Institute for Plant Sciences

Department of Systematic Botany

Untere Karspuele 2

37073 Goettingen

Germany

Email: Marc.Appelhans@biologie.uni-goettingen.de

Phone: ++49 (0)551 3922220

Fax: ++49 (0)551 3922329

Research Associate

National Museum of Natural History

Smithsonian Institution

PO Box 37012

Washington, DC 20013-7012

From: Cogliano, Mary [mary_cogliano@fws.gov]
Sent: Wednesday, August 17, 2016 9:39 PM
To: Wagner, Warren
Cc: Appelhans, Marc
Subject: Re: Status of processing request for Permit No. "96221B""

Dear Mr. Wagner,

I'm contacting you to follow up on your permit application for export of plant specimens collected from Hawaii. First, I want to let you know that since the plants on your application are not CITES-listed, you only need an export permit for the Federally Threatened and Endangered Species. Concerning collection of specimens of these species that are either Threatened or Endangered under the U.S. Endangered Species Act, please provide me with copies of all the required collection permits. Your permit application indicated that you have applied for these collection permits.

In addition, concerning *Platydesma cornuta*, please clarify whether you intend to export var. cornuta or var. decurrens or both.

Please be aware that if the requested information is not received by this office within 45 days, your application will be abandoned and administratively closed. Once a file is closed, you will need to submit a new application and all required fees for the Service to consider your proposed activity.

Thank you,

Mary Cogliano

--

Mary Cogliano, Ph.D.

Supervisory Biologist & Policy Specialist

Division of Management Authority

U.S. Fish and Wildlife Service

5275 Leesburg Pike, MS: IA

Falls Church, VA 22041-3803

Phone: (703) 358-1991

P-242 draft copy.pdf
107K

PERMIT FOR THREATENED AND ENDANGERED PLANT SPECIES
Department of Land and Natural Resources
Division of Forestry and Wildlife
1151 Punchbowl Street, Room 325
Honolulu, Hawaii 96813
(808) 587-4155, Fax (808) 587-0160

Permit No.	P-242
Date of Issue:	August 15, 2016
Expiration Date:	August 14, 2017

The Board of Land and Natural Resources hereby grants permission under the authority of Hawaii Administrative Rules §13-104 and §13-107, Hawaii Revised Statutes §195D and all other applicable laws, to the person(s) listed below.

Persons in violation of the terms and conditions of this permit and /or related or appropriate laws may be subject to criminal and or administrative penalty under Hawaii Revised Statutes 195D-8, §195D-9, §195D-27, §183-4, §183-5, § 183-18, §183-21, §183C-7, §171-6.4 §171-31.6, Hawaii Administrative Rules §13-104-3, §13-107-8, or as otherwise provided by law.

Marc Sebastian Appelhans
Goettingen University
Department of Systematic Botany
Untere Karspuele 2
37073 Goettingen Germany

Phone: ++49 5513922220
Emai: mappelh@gwdg.de

Warren Wagner
Smithsonian Institute
Email: WAGNERW@si.edu

To possess and transport for the purpose of research, the following endangered plant life:

For the Plant Extinction Prevention Program (PEPP including POP and ROI) species each island PEPP Coordinator will make the collection. A minimum of a 3 cm by 3 cm leaf sample may be collect if a whole leaf is not available.

For T&E species that are not PEPP species the DOFAW Island Botanist or DOFAW staff assigned by the Island Botanist will make the collection or will provide direct assistance in the field by accompanying the researcher during collecting activities. A minimum of a 3 cm by 3 cm leaf sample may be collect if a whole leaf is not available.

For species located on Kauai, Ken Wood, of the National Tropical Botanical Garden (NTBG), will be collecting for the Permit holder. A minimum of a 3 cm by 3 cm leaf sample may be collect if a whole leaf is not available.

NTBG and PEPP possess their own T&E plant permit to collect the material listed below and must contact the DOFAW Island Botanist prior to field work to coordinate work and communication plans to access state managed lands.

Permit holder will provide collectors the necessary supplies and paperwork for the plant material collection.

Kauai: *Melicope cruciata* (PEPP-POP), *Melicope degeneri* (E/PEPP), *Melicope haupuensis* (E/PEPP), *Melicope knudsenii* (E/PEPP), *Melicope macropus* (PEPP-POP), *Melicope nealae* (PEPP-POP), *Melicope pallida* (E/PEPP-POP), *Melicope paniculata* (E/PEPP-POP), *Melicope puberula* (Endangered), *Melicope quadrangularis* (E/PEPP), *Melicope waialealae* (PEPP-POP), *Platydesma rostrata* (E/PEPP-POP), *Zanthoxylum dipetalum* (Endangered), *Myrsine fosbergii* (E/PEPP-POP), *Myrsine knudsenii* (E/PEPP), *Myrsine linearifolia* (Endangered), *Myrsine mezii* (E/PEPP), *Myrsine petiolata* (PEPP-POP), *Myrsine punctata* (PEPP), *Zanthoxylum dipetalum* var *dipetalum* (PEPP-ROI)

Oahu: *Melicope christophersenii* (Endangered/Oahu DOFAW target), *Melicope cinerea* (PEPP- no known locations on Oahu), *Melicope hiiakae* (E/PEPP- from greenhouse), *Melicope lydgatei* (E/PEPP- from greenhouse), *Melicope makahae* (Endangered/Oahu DOFAW target), *Melicope ovata* (PEPP- no known locations on Oahu), *Melicope pallida* (E/PEPP- no known locations on Oahu), *Melicope saint-johnii* (Endangered/Oahu DOFAW target), *Melicope sandwicensis* (rare OPEP/Oahu DOFAW target), *Platydesma cornuta cornuta* (E/PEPP - from greenhouse), *Platydesma cornuta decurrens* (Endangered/Oahu DOFAW target), *Myrsine fosbergii* (Endangered/Oahu DOFAW target), *Myrsine juddii* (Endangered), *Myrsine punctata* (PEPP- from greenhouse).

Molokai: *Melicope mucronulata* (E/PEPP), *Melicope munroi* (E/PEPP), *Melicope reflexa* (E/PEPP)

Maui: *Melicope adscendens* (E/PEPP), *Melicope balloui* (Endangered), *Melicope cinerea* (PEPP), *Melicope knudsenii* (E/PEPP), *Melicope mucronulata* (E/PEPP), *Melicope ovalis* (Endangered), *Myrsine vaccinioides* (Endangered)

Hawaii Island: *Melicope zahlbrueckneri* (E/PEPP)

It is the State of Hawaii's understanding that the leaf samples of the species listed above will be acquired without exchange of funds or any type of transaction that could be construed as commerce. The leaf samples may not be transferred, sold, or given away without the authorization from the State of Hawaii Division of Forestry and Wildlife (DOFAW) Rare Plant Program. A copy of this permit and any other applicable permits, and specimen history reports must accompany the samples in transit from Hawaii to Dr. Appelhans' laboratory at Goettingen University in Germany. Upon completion of research, any reports or published results will be sent to the State of Hawaii DOFAW Office in Honolulu.

Subject to the following conditions:

I. GENERAL CONDITIONS

A. This permit authorizes the permit holder(s) to conduct described activities at location(s) noted, on State Forest Reserves, or lands that are under the control of the control of the Division of Forestry and Wildlife (DOFAW), Department of Land and Natural Resources (DLNR).
B. Activities conducted in DOFAW's Natural Area Reserves System (NARS) require a Special Use Permit. Activities conducted on other lands under the jurisdiction of DOFAW/DLNR, will require access permits.
C. The permit holder(s) must obtain approval from other landowners on lands where activities are planned, including other divisions of the DLNR, private landowners, tenants, and County, State, and Federal agencies prior to conducting activities on lands under their jurisdiction.
D. This permit is not transferable or assignable. A signed copy must be carried by permit holder(s) while engaging in activities authorized by this permit. Each permit holder is individually responsible and accountable for his or her actions under this permit.
E. This permit does not authorize activities with any other plant species except those stated. Permission to collect additional plant material must be obtained from district DOFAW offices.
F. Appropriate DOFAW district office must be notified in advance of proposed fieldwork, for a access permit, to coordinate collections, plant propagation needs, district requests, and approval of additional field personnel other than the listed associates for state reintroduction projects and/or their island cooperators.
G. Primary repositories are cooperating rare plant nurseries for live storage. Lyon micropropagation laboratory (for tissue culture) and seed storage facilities (for seed storage) are secondary depositories for these propagules.
H. This permit does not in any way make the Board of Land and Natural Resources of the State of Hawaii liable for any claims of personal injury or property damage to the permit holder(s)

or his or her party which may occur while engaged in activities permitted under this permit; further, the permit holder(s) agrees to hold the State harmless against any claims of personal injury, death or property damage resulting from the activities of the permit holder(s).

I. This permit shall become valid upon completion of the following:

1. **All persons who are actively involved in activities authorized by this permit have read this permit in its entirety and acknowledge understanding & agreement to abide by its conditions by signing this permit.**
2. **The signed permit is returned to DOFAW. Upon approval by the DOFAW Administration, a copy of the signed permit will be returned to the principal investigator.**

J. The permit holder(s) will provide copies of all publications/reports of any study resulting from the activities of this permit to DOFAW. The permit holder(s) will also provide or make available for inspection any raw data that is obtained under this permit when requested by the Division. In addition, permit holder(s) will notify DOFAW and provide product drafts of, and receive clearance prior to, final publication of any outreach or publicity projects involving interviews, films, brochures and print articles.

K. Any person violating any of the conditions stipulated under this permit will be subject to the penalty provision provided by law. Further, any infractions of this permit may be cause for revocation of this permit and/or denial of future permit requests.

L. This permit is issued for one year. This permit can be renewed at the end of this period. Please submit plans for the coming year and the need for permit renewal or extension before expiration of present permit.

II. SPECIAL CONDITIONS

A. The purpose of this permit is possession and transport of rare Hawaiian plants for a research, and project.

B. Any person holding a permit shall allow the Administrator's authorized representatives to enter the premises of the Permit Holder at any reasonable hour to inspect any plant held or to inspect, audit, or copy any permits, books, or records required to be kept by the Permit Holder.

§13-104-2 Definitions.

"Administrator" means the administrator of the division of forestry and wildlife.

"Authorized representative" means the administrator, foresters, conservation enforcement officers, and other persons authorized by the board of land and natural resources to act for the board.

The Permit Holder agrees to comply with all the terms and conditions of the applicable permit, as well as applicable laws and regulations; and consent to be subject to inspection by a duly authorized representative of the department. A refusal of inspection by a duly authorized representative of the department will be considered a violation of this permit, and thus the permit is subject to suspension or being revoked until such time the permit can be reviewed for renewal.

C. In advance of entry, permit holder(s) will notify District DOFAW of communication contacts in the field (cell phone/radio) and notify authorities of specific collection dates and times.

D. Permit holder(s) is strictly prohibited from collecting whole plants unless under specific DOFAW request.

E. The permit holder(s) will adhere to methods that are in accordance with established procedures as published by the Hawaii Rare Plant Restoration Group (HRPRG) for collection of Threatened and Endangered species. Completion of HRPRG Rare Plant Monitoring Forms is required for all collections.

F. Plant Extinction Prevention (PEP) plants will be documented by photo vouchers unless a new population is discovered; if material is limited for the new population, photographs may be the most appropriate voucher method to document the population along with DNA samples. Ideally, all PEP plants will be labeled to prevent over collection.

G. **The permit holder(s) is required to consult with island Plant Extinction Prevention (PEP) Coordinators prior to any planned work with PEP species. If deemed necessary, PEP Coordinators may require to accompany the permit holder(s) to oversee field work in and around PEP plants. The permit holder(s) is asked to alert land managers, PEP Coordinators, and landowners of any observed threats or negative changes in the health of PEP plants so immediate mitigative actions may be taken.**

H. Photos taken by Permit Holders, Sub-Permit Holders and Volunteers of rare and endangered wildlife and plants, encountered during research activities described in this endorsement, shall not be posted on social media outlets with location information, except in the broadest of terms i.e. island and mountain range. An example of an acceptable location tag for a site on O`ahu would be "Northern Ko`olau Mountains". Failure to comply will result in the revocation of this permit and the denial of further permits. If Principal Permit Holder, Sub-Permit Holders, or Volunteers encounter rare and endangered wildlife and plants while conducting research activities outlined in this endorsement, said permit holders and volunteers will not visit the given rare or endangered wildlife or plant again except if absolutely necessary for conducting the research outlined in this endorsement. Similarly, Permit Holders, Sub-Permit Holders, and Volunteers will not provide directions or location information of rare and endangered wildlife and plants to non-agency or non-permitted individuals. Failure to comply will result in the revocation of this permit and the denial of further permits.

I. For the protection of the forest areas from transmission of Rapid Ohia Death (ROD), no clothing, gear, equipment, or tools used on Hawaii Island will be permitted for use on Maui Nui, Oahu, or Kauai. Prior to conducting any work under this permit, the permittee is required to contact and receive approval of the district manager or designee. Permittee clothing, gear, equipment, or tools may be subject to inspection pursuant to compliance with this condition.

J. Yearly reports must include information on collection, propagation, transfers, outplanting and monitoring and maintenance information and will be in electronic form. The report must list where the Permit Holder got each T&E species. For collection the following information is required in the report: collection location, collector's name, species name, propagative form (cuttings, seeds, etc.), the amount obtained per species, plant identification number, date of collection, and accession number. For donations the following information is required for the report (receiving organization and their State Permit Number, T&E species name, the amount for each species, date of transfer, accession number and purpose of why the species

was donated). For outplanting the following information is required in the report: outplanting location, species name, the amount outplanted per species, plant accession number, and date of outplanting. Accurate records will be kept for individual plants and must include scientific name, source of origin, accession number, date of outplanting, plant location, phenology (vigor, flowering, fruit health, reproduction, survival status), herbivory (disease and insect information), and management and maintenance information (fertilization schedule, pesticide applications, and watering schedule). If a new population is discovered, GPS information will be supplied when possible.

The undersigned have read, understood, and hereby agree to abide by the conditions as stated above.

Principal Investigators:

_____ _____

Marc Sebastian Appelhans, Principal Investigator (Date)

Associates:

_____ _____

Claudia Patzold

APPROVED: _____ Date_____

DAVID G. SMITH, Administrator,
Hawaii Division of Forestry and Wildlife