Kaken 2016 application for 2017-2020 (Category B)
PJ Matthews, National Museum of Ethnology, Japan.
26th October 2016 (approved). Form S-1-9

Mapping Genetic Diversity in Taro (*C. esculenta*) to Test Domestication Models

Purpose of research (Outline)
Taro, *Colocasia esculenta* (L.) Schott, is a root crop and leaf vegetable found in tropical to temperate regions of Africa, Asia, Oceania, and the Americas. **Throughout lowland Southeast Asia, wild populations of *C. esculenta* grow along roadsides, ditches and canals, and on the banks of ponds, streams, and rivers** (Fig. 1, right). These rarely studied wild populations are widely used as free sources of food for local people and fodder for local pigs. **They actively invade the open habitats created by human activities, spreading vegetatively and by seed, and may represent the most abundant wild vegetable source in Southeast Asia.** To test old and new domestication models (Fig. 2), we will (i) map the distribution of wild populations of *C. esculenta*, (ii) record their morphological diversity (iii) collect leaf tissue samples for DNA analysis, and (iv) compare the wild populations with cultivated *C. esculenta* and wild *Colocasia* species using DNA tests for past hybridization. To conduct the research, convene related meetings, and prepare publications, we will establish an international "Wild Taro Working Group" composed of local counterparts and others with relevant expertise and interests.

Figure 1 In N. Vietnam, *C. menglaensis* Yin, Li, & Xu (left), and *C. lihengiae* Long & Liu (centre) carry the same chloroplast genome as wild *C. esculenta* in a natural lowland swamp (right) but have species-specific, nuclear genome ITS sequences (Matthews, Ahmed & Nguyen, 2016 "Sympatry of *Colocasia esculenta* (taro) and its wild relatives in Southeast Asia" Paper presented at 8th World Archaeology Congress, Kyoto, 28. Aug.-2. Sept. 2016).

(1) Scientific background
Before the global exchange of crops in recent centuries, *C. esculenta* was the most widely cultivated starch crop in the world (Matthews 2006). In early discussions of the domestication of *C. esculenta,* wild populations were known to exist (Model I, Fig. 2) but none had been mapped or studied. In surveys conducted since 1982, PI has recorded many wild populations (Matthews 2014) (e.g. Fig. 1 right). **Wild populations of *C. esculenta* are widespread**, and around 20 wild *Colocasia* species are known in Southeast Asia (Matthews & Nguyen 2014). Two wild species have traits similar to those of cultivated *C. esculenta*: (i) *C. lihengiae* with edible leaves

(indicating low acridity) (Fig. 1), and (ii) *C. oresbia* Hay 1996 with large corms (edibility unknown).

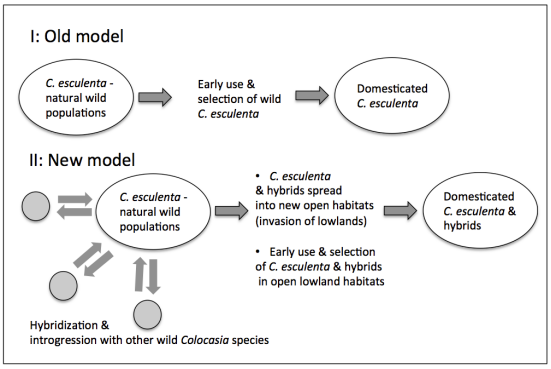

Figure 2 Domestication models:
I. Old: linear process, one species (without hybridization).
II. New: hybridization & introgression among multiple species, then spread to lowlands, use, selection, and domestication (= 'floodplain weed theory' of Smith, 1995, applied to *C. esculenta*).

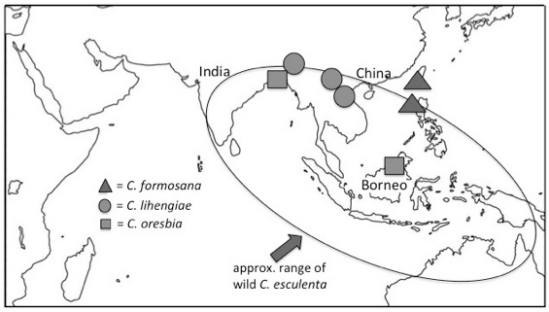

Figure 3 Wild *C. esculenta* is common in lowlands from SE Asia to Papua New Guinea and Australia. Wild *Colocasia* species of special interest are: *C. formosana* (triangles), *C. lihengiae* (circles), and *C. oresbia* (squares)

Hybridization has been important in the domestication of many crops (Smartt & Simmonds 1995), and may have stimulated the evolution of invasiveness in many plants (Ellstrand & Schierenback 2000). Hybridization may have contributed to the invasive character of wild *C. esculenta*, as well as introducing useful traits into wild populations utilized as sources of food and fodder. In open lowland habitats, *C. esculenta* and close hybrids may have been used, selected, and taken into cultivation (Model II, Fig. 2).

We previously studied chloroplast and nuclear DNA sequences in *C. esculenta* and other *Colocasia* species (Ahmed, Matthews et al. 2013). **In northern Vietnam, we found evidence for introgression from wild *C. esculenta* into wild populations of *C. lihengiae* and *C. menglaensis*** (Fig. 1). Hybridization (and then introgression) may have occurred in other regions where *Colocasia* species are sympatric. *C. formosana* Hayata in Taiwan and the Philippines (with high acridity), and *C. oresbia* in Borneo, Malaysia (with large corms) have not been tested. **The vegetative shoots of *C. esculenta* often float downstream from wild and cultivated populations upstream, so lowland populations of wild *C. esculenta* may be repositories of high genetic diversity, including hybrids.** Lowland wild populations are easily found and sampled, but have been largely ignored in previous studies focused on highly variable nuclear loci suitable for cultivar identification (e.g. Chair et al 2016), and not designed to identify species or detect hybrids. The CGIAR Research Program on "Roots, Tubers, and Bananas" recognises *C. esculenta* as "not sufficiently researched" (CGIAR, 2016: www.rtb.cgiar.org).

(2) To be elucidated in the research period

Our aim is test wild populations of *C. esculenta* - in lowland deltas and wetlands in China, Vietnam, Thailand, Myanmar, India, and Malaysia (Fig. 4) - for genetic evidence of past hybridization with other *Colocasia* species. Chloroplast and nuclear DNA sequences will be analysed using leaf tissue samples collected during the surveys. Samples of cultivated *C. esculenta,* and other *Colocasia* species, will be obtained from living collections at agricultural research centers and botanical gardens.

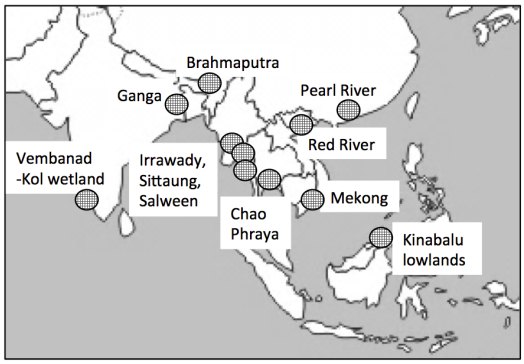

Figure 4 Target areas (lowland floodplains) for mapping & sampling wild *C. esculenta* and possible hybrids.

(3) Academic originality and characteristics

Originality: (i) A new hypothesis is proposed, namely that domesticated *C. esculenta* arose from invasive wild populations of *C. esculenta* and related hybrids, in open lowland habitats (Model II, Fig. 2). (ii) To test this hypothesis, newly developed tests will be used to detect hybrids between *Colocasia* species. (iii) The study of wild breeding populations of *C. esculenta* in lowland floodplains is new (cultivated *C. esculenta* and wild *Colocasia* species in mountainous areas have received attention from agriculturalists and taxonomists respectively).

Characteristics: (i) The survey of wild *C. esculenta* on a wide geographical scale (Fig. 4) is needed to compare lowland populations of potentially high genetic diversity, within the region where other *Colocasia* species are located (ii) Our recently developed DNA tests for 3 chloroplast and 2 nuclear loci make testing for hybrids affordable for large sample sets, using commercial diagnostic laboratories**.** The results will clarify taxonomic and evolutionary relationships among *Colocasia* species, and the domestication process in *C. esculenta*. (iii) Wild *Colocasia* species are threatened by forest clearance and agricultural expansion in SE Asia; this research will inform future conservation, development, and utilisation of *C. esculenta* and its wild relatives. (iv) Through previous experience, PI is well positioned to lead international collaboration by the proposed "Wild Taro Working Group"; all resulting papers will be co-authored in accordance to international protocols on academic benefit sharing. (v) *C. esculenta* is deeply embedded in the traditional dietary cultures of Japan (*washoku*); samples from Japan will be compared with *C. esculenta* and other *Colocasia* species in other countries.

REFERENCES: Please see list on Page 7

<u>Research Plan and Method (Outline)</u>

Year 1. Field survey and sampling in China & Vietnam. Begin genetic analysis of field samples, including samples already collected in Japan, Taiwan & Philippines. Present paper at the *XIX International Botanical Congress, Shenzen, July 23-29, 2017*. Convene "1st Wild Taro Working Group Meeting" alongside the Congress. Prepare field reports for *Annual Report on Exploration and Introduction of Plant Genetic Resources (AREIPGR)* (National Agriculture and Food Research Organization, Japan)

Year 2. Field survey and sampling in India, Myanmar & Thailand. Continue genetic analysis. Convene "2nd Wild Taro Working Group" meeting in Myanmar. Prepare field reports for *AREIPGR*.

Year 3. Field survey and sampling in India & Malaysia. Continue genetic analyses. Convene "3rd Wild Taro Working Group Meeting" in India. Prepare field reports for *AREIPGR*; and paper for the journal *Genetic Resources and Crop Evolution* (field data & early results).

Year 4. Supplementary fieldwork focused on wild *Colocasia* species identified as possible crop progenitors in Years 1-3, and laboratory analysis of the samples obtained. Collate all data (Years 1-4). Prepare three papers based on the collated data, for *Genetic Resources and Crop Evolution, PLoSOne*, and *PNAS*.

Basic protocols

Field surveys: We will map the distribution of wild *C. esculenta* and other *Colocasia* species in lowland deltas & wetlands across SE Asia (Fig. 4), collect leaf tissue samples and voucher specimens, and data on distribution, flowering, vegetative morphology, vernacular names, and uses. Special attention will be given to evidence of breeding: flowering, fruiting, seed dispersal, and seedling growth. Field notes and sample data will be linked to GPS location data, photography, and video recordings.

The field data will allow (i) assessment of the economic value of wild populations, (ii) description of sampled populations and *Colocasia* species, and (iii) assessment of opportunities for genetic exchange between *Colocasia* species. Fieldwork will be conducted together with local researchers and their students in each country visited. The PI will visit all areas and liaise with local counterparts. Collaborators from Japan will join fieldwork in their areas of special interest: Iida in Malaysia; Ikeya in Vietnam; Masuno in Thailand.

Field sample collection: Leaf samples and whole-plant voucher specimens will be collected in duplicate. Duplicates will be kept by research counterparts in each country, for submission to institutions that maintain national or regional reference collections for plants. Agreements for sample collection, exchange, analysis, and data sharing will be made following academic and legal conventions.

DNA testing. Hybridization and introgression can be detected in plants by simultaneous analysis of loci in the chloroplast and nuclear genomes. We will use PCR primers previously developed for *Colocasia* spp. to analyse: (i) three chloroplast loci (*trnY*-IGS-*trnE*, rbcl, *rpl22-rps19-rpl2*), and (ii) two nuclear loci (*phyC*, ITS) (Ahmed 2013; Ahmed, Matthews et al. 2013). For effective use of time and budget, analyses

will be carried out using commercial DNA sequencing services. Sequencing in just one direction (forward or reverse) will allow us to analyse a large number of field samples at a reasonable cost. Analyses will employ standard protocols for sequence editing, alignment and analysis (Geneious Pro, Mesquite, SplitsTree, JModelTest, PhyML, FigTree, and TreeDyn software packages).

Schedule (outline)

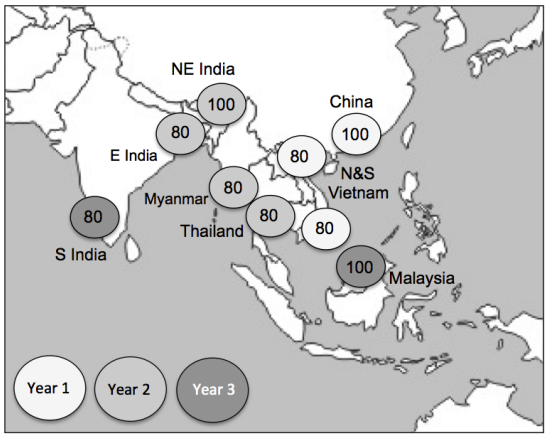

Figure 5 Target sample numbers for wild & cultivated *C. esculenta*, & wild *Colocasia* spp., project years 1-3.

Total n = 860, including already available & Yr 4 supplementary samples. To test all samples at 5 loci will require analysis of 4,300 short sequences.

Year 1 (2017): **(1)** Present paper at the *XIX International Botanical Congress, Shenzen, July 23-29, 2017* (Working title: "Chloroplast and Nuclear Genome Diversity in Japanese Diploid and Triploid Taros: Is There Evidence for Hybrid Origins?"). Convene "1st Wild Taro Working Group Meeting" alongside the Congress. Congress travel: PI, 9 days. **(2)** Survey and collection in China: Guangdong Prov. & Guanxi Prov. (vic. Pearl River delta)(3 persons, 2 weeks; Matthews, Chun-lin LONG & 1 other local counterpart). **(3)** Collect samples of *C. esculenta* from national collection, Plant Resource Center, Hanoi, with assistance of PRC staff NGUYEN Kien (1 person, 1 week; Matthews). **(4)** Survey and collection in vic. Hanoi, N. Vietnam (vic. Red River delta) (3 persons, 2 weeks; Matthews, NGUYEN Dzu & 1 other local counterpart). **(5)** Survey and collection in An Giang Province, S. Vietnam (vic. Mekong delta) (3 persons, 2 weeks; Matthews, NGUYEN Dzu & 1 other local counterpart). **(6)** DNA analysis, including samples already collected in Japan (*C. esculenta*), Taiwan & Philippines (*C. formosana, C. esculenta*) (n=300). **(7)** Prepare China & Vietnam field reports for *AREIPGR* (x2).

Year 2 (2018) **(1)** Survey and collection in Assam NE India (Brahmaputra floodplain) (3 persons, 2 weeks; Matthews, MEDHI & 1 local counterpart). **(2)** Survey and collection in upper Ganga delta, India (3 persons, 2 weeks; Matthews, 2 local counterparts). **(3)** Survey and collection vic. Irrawaddy and Sittaung deltas, and lower Salween, Myanmar (3 persons, 3 weeks, 18 person-weeks; Matthews, Kyaw Win NAING & 2 others). **(4)** Convene "2nd Wild Taro Working Group Meeting" at Yezin Agricultural University, Nay Pyi Taw, Myanmar (1 day). **(5)** Survey and collection in vic. Bueng Boraphet wetland, Chao Phraya delta, Thailand (3 persons, 2 weeks; Matthews, Masuno & 1 local counterpart). **(6)** DNA analysis (n=340). **(7)** Prepare Myanmar & Thailand field reports for *AREIPGR* (x2). Prepare first paper on

field data and DNA test results for *Genetic Resources and Crop Evolution* (results for Years 1-2).

Year 3 (2019) **(1)** Survey and collection of *C. esculenta* and *C. oresbia* in lowlands of Mt Kinabalu, Sabah, Malaysia (3 persons, 4 weeks; Matthews, IKEYA, & 1 local counterpart). **(2)** Survey and collection in Vembanad-Kol Wetland, Kerala, southern India (3 persons, 2 weeks; Matthews & 2 local counterparts from Central Tuber Crops Research Institute (CTCRI). **(3)** Convene "3rd Wild Taro Working Group Meeting" at Central Tuber Crops Research Institute (CTCRI), Trivandrum, India (incl. travel to CTCRI for 4 participants from NE India, 3 days travel and stay). **(4)** DNA analysis (n=180). **(5)** Prepare Malaysia & India (years 2&3) field reports for *AREIPGR* (x2).

Year 4 (2020) **(1)** Supplementary fieldwork in an upland area of SE Asia to target wild *C. esculenta* populations or *Colocasia* spp. identified as possible progenitors for domesticated *C. esculenta* in Years 1-3 (3 persons; 4 weeks; Matthews & 2 local counterparts). **(2)** DNA analysis (n=40). **(3)** Collate all field and laboratory data on distribution, ecology, and genetics of *C. esculenta* and other *Colocasia* species. **(4)** Prepare three papers based on completed project results, for *Genetic Resources and Crop Evolution* (further field data & early test results), *PLoSOne* (on outcome of lowland survey strategy & regional comparisons of genetic diversity in wild *C. esculenta*), and *PNAS* (on evolution of *Colocasia* species, the role of hybridization in *C. esculenta*, and the "floodplain weed theory" of domestication for this crop, cf. Model II, Fig. 2). (5) Transmit records of the "Wild Taro Working Group" to the International Network for Edible Aroids (INEA), and establish the Group as a permanent INEA branch.

Collaborating researchers

Other countries

Dr E. M. AGOO, Dept of Biology, De La Salle University-Taft, Philippines. Area advisor for SE Asia & DNA archives.

Dr Ibrar AHMED, Quaid-i-Azam University, Pakistan. Area advisor; DNA methods & analysis.

Dr Robin ALLABY, Warwick University, United Kingdom. Advisor for population genetics & analysis.

Dr. James GEORGE, ARS Director (act) & Project Coordinator AICRPTC
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Area advisor/counterpart for research co-ordination.

Dr Danny HUNTER, Global Project Coordinator of the global GEF/UNEP/FAO Biodiversity for Food and Nutrition Project, Bioversity International, Italy (former leader of Taro Genetic Resources: Conservation and Utilisation (TaroGen) Project, SPC). Advisor for research dissemination.

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Dr NGUYEN Van Du, Ethnobotany Department, Institute for Ecology and Biological Resources (IEBR), Viet Nam. Area & taxonomy advisor & local counterpart for fieldwork.

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Cooperative Investigator (*Buntansha*)
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Cooperating researchers (*Renkei-kenkyusha*)
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Dr T. MASUNO, Sokendai, Hayama. Field research on uses of wild taro & human ecology, Thailand.

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