

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

Project outline for years 2017-2020 (JSPS Kaken Category B, approved)

PJ Matthews. Short Version, 26th October 2016 (not to be cited). Form S-1-9

### Purpose of research

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.

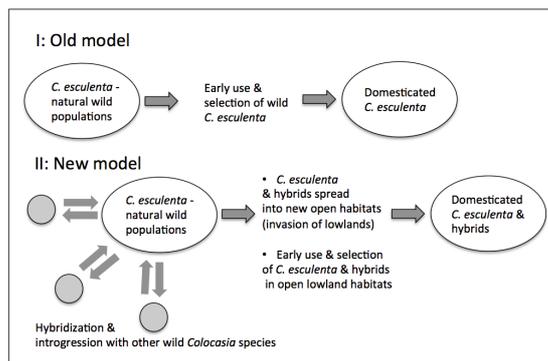


**Figure 1** In N. Vietnam, *C. menglaensis* Yin, Li, & Xu (left), and *C. lihengiae* Long & Liu (centre) carry the same chloroplast genome as wild *C. esculenta* in a natural lowland swamp (right) but have species-specific, nuclear genome ITS sequences (Matthews, Ahmed & Nguyen, 2016 “Sympatry of *Colocasia 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 acidity) (Fig. 1), and (ii) *C. oresbia* Hay 1996 with large corms

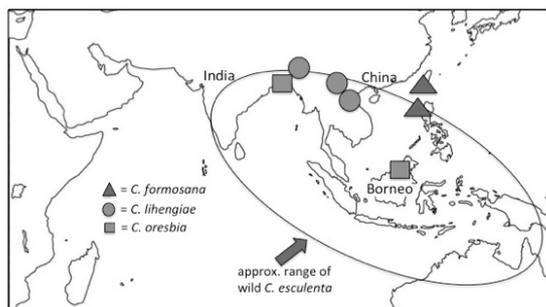
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**Figure 2** Domestication models:

**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*).



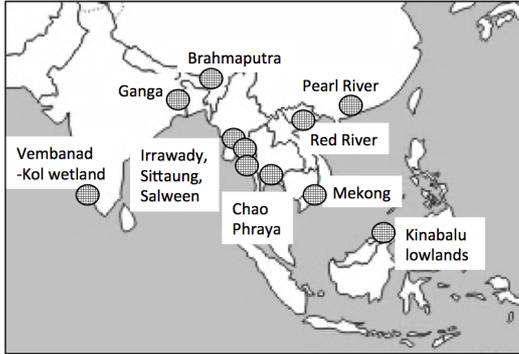
**Figure 3** Wild *C. esculenta* is common in lowlands from SE Asia to Papua New Guinea and Australia. Wild *Colocasia* species of special interest are: *C. formosana* (triangles), *C. lihengiae* (circles), and *C. oresbia* (squares)

**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 acidity), 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](http://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

Research Plan and Method (Outline)

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, Shenzhen, 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.

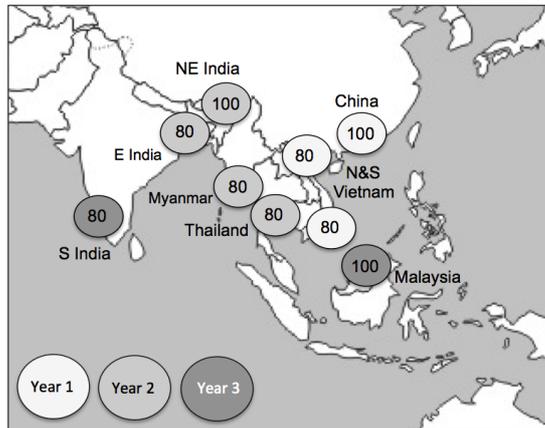
The field data will allow (i) assessment of the economic value of wild populations, (ii) description of sampled populations and *Colocasia* species, and (iii) assessment of opportunities for genetic exchange between *Colocasia* species. Fieldwork will be conducted together with local researchers and 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, Shenzhen, 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 ICAR-Central Tuber Crops Research Institute, Trivandrum, Kerala, India. 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.

Dr Vincent LEBOT, scientific coordinator of International Network for Edible Aroids ([www.EdibleAroids.org](http://www.EdibleAroids.org)), CIRAD, PMB 946, Port-Vila, Vanuatu. Advisor for collections & methods.

Dr Peter LOCKHART, Institute of Fundamental Sciences, Massey University, New Zealand. Advisor for methods & analysis.

Dr Chunlin LONG, College of Life and Environmental Sciences, Minzu University of China. Area advisor, aroid taxonomy expert; local counterpart for fieldwork.

Dr Melanie MEDECILLO, Director, University Research Office, De La Salle University-Dasmariñas, Philippines. Area advisor (SE Asia), aroid taxonomy expert.

Dr Dilip MEDHI, Emeritus Professor. Dept of Anthropology, Gauhati University, Assam, India. Area advisor; local counterpart for fieldwork.

Mr NGUYEN Kien, Plant Resources Center, Vietnam. Local counterpart for national taro collection.

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

Dr Tin THUT, Permanent Secretary of the Ministry of Agriculture Livestock and Irrigation, Myanmar. Area advisor.

### *Japan*

#### Principle investigator

Dr Peter J. MATTHEWS, National Museum of Ethnology, Osaka (time 80%)

#### Cooperative Investigator (*Buntansha*)

Dr Kazunobu IKEYA, National Museum of Ethnology, Osaka (time 5%). Field research on uses of wild taro & human ecology, Vietnam.

#### Cooperating researchers (*Renkei-kenkyusha*)

Dr Taku IIDA, National Museum of Ethnology, Osaka. Field research on cultivated taro in Austronesian speaking communities, Malaysia.

Dr T. MASUNO, Sokendai, Hayama. Field research on uses of wild taro & human ecology, Thailand.

### **References**

Ahmed 2013 Evolutionary Dynamics in Taro. PhD thesis, Massey University, New Zealand. [supervised by Matthews].

- Ahmed, Matthews, et al. 2012. Mutational dynamics of aroid chloroplast genomes. *Genome Biology and Evolution* 4:1316–1323.
- Ahmed, Matthews, et al. 2013 Identification of chloroplast genome loci suitable for high-resolution phylogeographic studies of *C. esculenta* and closely related taxa. *Molecular Ecology Resources* 13: 929-937.
- Chair et al. (2016) Genetic diversification and dispersal of taro (*Colocasia esculenta* (L.) Schott). *PLoS ONE* 11 (6) e0157712.
- Ellstrand, NC & Schierenbeck, K 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? *PNAS* 97:7043-7050.
- Hunt H, Moots H & Matthews PJ 2013. Genetic data confirms field evidence for natural breeding in a wild taro population in northern Queensland, Australia. *Genetic Resources & Crop Evolution* 60,1695-1707.
- Ikeya K 2014a. Biodiversity, Native Domestic Animals, and Livelihood in Monsoon Asia: Pig Pastoralism in the Bengal Delta of Bangladesh. K. Okamoto and Y. Ishikawa (eds.), *Traditional Wisdom and Modern Knowledge for the Earth's Future*, International Perspectives in Geography 1, 51-77, Springer Japan
- Ikeya K 2014b. 「世界の畜産飼養の起源—ブタ遊牧からの視点—」 池内了編 『「はじまり」を探る』 105-126 頁、東京:東京大学出版会
- Masuno T, Matthews PJ, et al. 2012. Fodder sources and backyard pig husbandry in BaVi, Hanoi province, northern Vietnam. Proc. of the 1st International Conference on Animal Nutrition and Environment. A.-N. 2012. Khon Kaen: Khon Kaen University Press: 657 - 660.
- Matthews PJ 1997. Field guide for wild-type taro, *Colocasia esculenta* (L.) Schott. *Plant Genetic Resources Newsletter* No. 110: 41-48.
- Matthews PJ 2004. Genetic diversity in taro, and the preservation of culinary knowledge. *Ethnobotany Research & Applications*, 2:55-71.
- Matthews, PJ 2006. Written Records of Taro in the Eastern Mediterranean, in F Ertug (ed) *Ethnobotany: At the Junction of the Continents and the Disciplines*. Istanbul, Yayinlari, pp. 419-426.
- Matthews PJ et al. 2012. Ethnobotany and Ecology of Wild Taro (*Colocasia esculenta*) in the Philippines: Implications for Domestication and Dispersal. In: *Irrigated Taro (Colocasia esculenta) in the Indo-Pacific: Biological, Social and Historical Perspectives*. pp. 307-340.
- Matthews PJ 2014. *On the Trail of Taro: An Exploration of Natural and Cultural History*. Osaka, National Museum of Ethnology.
- Matthews, PJ & DV Nguyen 2014. Origins and Development of Taro, in C Smith (ed)

*Encyclopedia of Global Archaeology*. Berlin, Springer, pp. 419-426.

Matthews PJ, Nguyen DV, Tandang D, Agoo EM and Madulid DA 2015. Taxonomy and ethnobotany of *Colocasia esculenta* and *C. formosana* (Araceae): implications for evolution, natural range, and domestication of taro. *Aroideana Supplement* **38E**(1): 153-176.

Matthews PJ, Lockhart PJ, & Ahmed I 2015. Phylogeography, ethnobotany, and linguistics: Issues arising from research on the natural and cultural history of taro *Colocasia esculenta* (L) Schott. *Man in India* **95**(4) in press.

Nguyen DV, Matthews PJ, et al. (2016) *Colocasia yunnanensis* (Araceae), a new record for the flora of Vietnam. *The Journal of Japanese Botany* 91: 223-229 (publication expected in October 2016).

Smarrt & Simmonds (1995) *Evolution of Crop Plants*. Harlow: Longman Scientific and Technical.

Smith, BD (1995) Seed Plant Domestication in Eastern North America. In Price & Gebauer (eds) *Last Hunters, First Farmers*. Santa Fe, School of American Research, pp. 193-213.