# A report of Davis Expedition Fund--Expedition to Okinawa

## Abstract

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*Titanotrichum oldhamii* (Gesneriaceae) is a rare endemic plant from wetlands in the mountains of Taiwan, South-Eastern China and the Okinawa Islands (Japan). This plant was first discovered in 1864 by Richard Oldham, the last collector of the Royal Botanic Gardens Kew, during his trip in Formosa (Taiwan).

This enigmatic species is notable not only for its scarce status, but also for its spectacular morphology. Although it produces inflorescences of showy flowers (which may allow outbreeding, although seed-set is low), it also produces inflorescences where the primordia produce numerous embryo-like bulbils in place of flowers.

This field trip allowed me to investigate its farest distribution on these remote Southern Okinawa Islands, especially when most of its populations in Taiwan and China have been surveyed. It was observed that this species encoutered a severely imbreeding pressure which is due to the lack of effective pollinators. Yet, when it was cultivated in green house, it is easy to get seeds from hand pollination. Since lowland forests in these area have been greatly disturbed by human activities. Its direct pollinators may have been extinct. Therefore, in order to survive in highly diverse niches under subtropical natural forests, *Titanotrichum oldhamii* has an alternative breeding strategy which replaces generative seeds with incredible bulbils.

A pollination experiment previously conducted in the wild and molecular data collected from this trip both indicated the population genetic diversity of *Titanotrichum oldhamii* is quite limited. It can be very prone to the selection pressure simply because clonal inbreeding may lead to reduce the genetic variation. This result is hope to provide adequate suggestion for local government on species conservation.

From the living materials collected from this journey, the ontogenetic difference between flowers and homologus bulbils has been distinguished. It is my sincere wish that the fruitful result of this field trip can eventually contribute to academic researches thus encouraging subsequent collaboration between Britain and other countries.

# Field trip report of investigating *Titanotrichum oldhamii* in Okinawa islands.

**Discovery of Titanotrichum oldhamii and its significance on southern Okinawa flora**

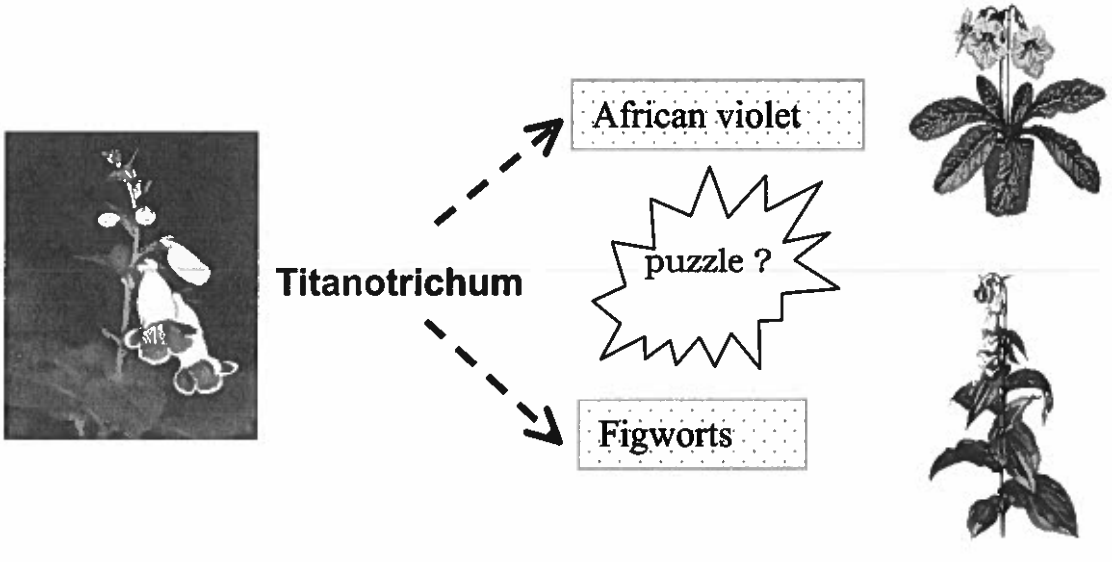
This plant was first discovered in 1864 by Richard Oldham, the last collector of the Royal Botanic Gardens Kew, during his trip in Formosa (Taiwan). This young botanist shortly afterward died of fever disease in China. Therefore, when Hemsley in 1890 described it as a new Scrophulariaceae species, he chose the name Rehmannia oldhamii as a memorial to Mr. Oldham.

It is now reduced to scattered locations in Taiwan, the Fujien province of China and the Okinawa Islands of Japan, according to the red list of local flora. After I had surveyed its populations in China and Taiwan, this trip offered me a great chance to investigate its farest distribution on these remote Okinawa islands. In addition, its distribution pattern may strikely serves as an example of the glacial/interglacial cycle effect on Okinawa archipelago flora (Fig.2). Many plants and even some reptiles species distributed in this area showed the similar pattern (endemism in Taiwan, subtropical SE China and Southern Okinawa). This journey also gave me a precious opportunity to briefly examine this hypothesis, by comparing the local flora of Southern Okinawa with the emphasis on its relation to Taiwan and China.

**The mysterious plant**

This enigmatic species is notable not only for its scarce status, but also for its spectacular morphology. With its unique bulbils, seed morphology, scaly rhizome and racemose inflorescence, botanists still do not yet know its phylogeny and where to place it in their classifications. Although tubular flowers in long tapering spikes suggest a resemblance between itself and Foxglove (*Digitalis*), its scaly rhizomes and habit suggest its affinity with African violet family.

Recent chemotaxonomic and molecular phylogeny data even anomaly included it in New World Gesneriaceae. From these intriguing conclusions, *Titanotrichum oldhamii* will be the first Gesner species in Old World disjunctively related to New World ones.



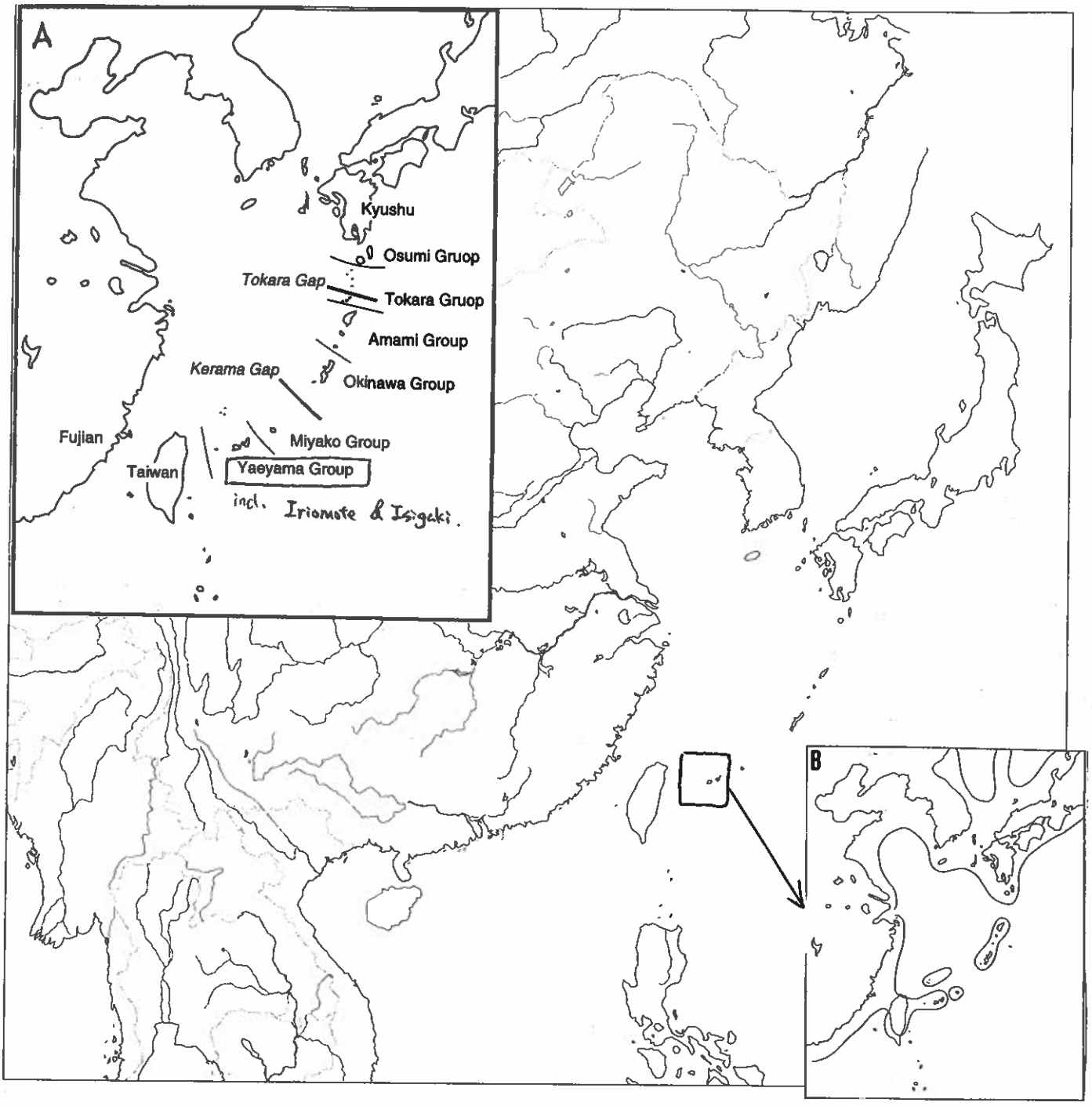



Fig. 2 Map of East Asia, Square indicates research area of this report. (Iriomote, left; Ishigaki, right)

2A Map of East asia showing the location of the okinawa islands (Ryukyu Archipelago); map is after Ota (1999)

2B Inferred land configulations of the ryukyu and adjacent regions during the middle Pleistocene. (Yaeyama group, i.e. Iriomote & Ishigaki was connected to Taiwan during that time.); map is after Ota (1999)

From this trip I collected specimen and living plants together with DNA samples back to Edinburgh. The centre for Plant Science within the Institute of Cell and Molecular biology (ICMB) and Royal Botanic Garden Edinburgh (RBGE) have had a long history involving plant diversity research in Old World Gesneriaceae species. Take this advantage, it is convenient to conduct comprehensive researches on this plant. These materials I collected may therefore provide crucial suggestions to reveal its morphological and molecular phylogeny between Gesneriaceae and Scrophulariaceae.

**Unusual breeding strategies for this endangered species---making clones and mating**

Plants have evolved a variety of adaptive breeding strategies subject to selection. *Titanotrichum oldhamii* has three methods of reproduction: bulbils, branching rhizomes and seeds.

The plants produce many showy flowers each summer, which indicate it is still eager to attract pollinators. However, seeds are hardly ever set, both in the wild and cultivation. Instead, most populations seem to be clonally maintained by propagation from bulbils. From my observation in this trip, this plant is apparently less competitive to other companion species because of its habitats requirement is strict. It only grows in deep forest on partial shaded slope with constant water dripping. It always stand on limestone soil perhaps some essential nutritions are needed. No specific pollinators in the wild visit the plants regularly. This severely inhibits its pollination process. Therefore, in order to survive in highly diverse niches under subtropical natural forest, *Titanotrichum oldhamii* has an alternative breeding strategy which replaces generative seeds with incredible bulbils (Fig.3).

This can be seen from the result of a pollination experiment previously conducted in Taiwan (Table1). The fruit and seed set from crossings within populations is apparently less than crossings among distant populations. This indicates it is generally an outcrossing species. Moreover, seed set is extremely low in those individuals which are naturally pollinated.
It can be said that the genetic diversity within population of *Titanotrichum oldhamii* is quite restricted. And insufficient pollen flow among individuals is probably due to the lack of effective pollinators. In other words, they can be very prone to the selection pressure simply because clonal inbreeding may lead to reduce the genetic variation.

Fig. 3 Bulbiferous inflorescence is presented on top of the right hand side. 4, 5 show how bulbils clustering. →

To support this conclusion firmly, the study of population genetic variation based on molecular methods is proceeding in RBGE molecular lab. By comparing the genetic polymorphism individually within and among populations, we can then estimate the genetic diversity of *Titanotrichum oldhamii* to provide adequate information on species conservation.

Table 1. Pollination results of Titanotrichum oldhamii

| | fruits/flowers (Fruit set) | Seeds per capsule | Germination rate | Individuals |
|---|---|---|---|---|
| **Crossing among distant popul.** | 20/25(80%) | 323.75±154.57 | 0.92±0.05 | n=10 |
| **Crossing among adjacent popul.** | 17/30(56%) | 253.67±134.30 | 0.80±0.21 | n=25 |
| **Crossing within popul.** | 19/35(54%) | 93.29±60.06 | 0.55±0.27 | n=25 |
| **Natural Crossing/Control** | 35/385(9%) | 30.06±42.78 | 0.16±0.21 | n=95 |
| **Selfing** | 4/25(16%) | 4.00±2.83 | 0 | n=25 |

**Unusual inflorescence development ----- The ontogenetic change from flower to bulbils**

Rather than a typical raceme inflorescence, *Titanotrichum oldhamii* has bulbils and flowers arise in the same spike, which is never reported among the flowering plants around the world. These tiny bulbils are generated from floral meristems, along with ordinary flowers in the inflorescence. Otherwise, completely bulbiferous inflorescence can evolve simultaneously especially in the end of the flowering season or in one to two years old young individuals. As the living materials collected from field trips grew well in RBGE green house, I have been able to closely examine the development of bulbils.

To distinguish the ontogenetic differences between bulbil, flower and leaf (bract) formation along an inflorescence shoot, developmental stages of all the primordia were examined by SEM (Scanning Electronic Microscopy). It is found that the floral meristems of *Titanotrichum oldhamii* can convert into the primordia of bulbils or leaves immediately after their two lateral bracteoles (prophylls) divide (Fig. 4). After two bracteoles (prophylls) developed, three subsidiary meristems arise from the axile of the primary meristem between bract and bracteoles. Then all the meristems divide repeatedly to generate the numerous bulbil primordia. Thus, each floral meristem might give rise eventually to hundreds of bulbils.

**Diary of the field trip**

**Day 1, September 3**

I arrived at the Okinawa airport on morning of September 3 and soon transferred to Japanese southern most Main Island (Ishigaki). From this island, I took an express boat to reach an islet (Iriomote) on its west. Iriomote is a national reserved area which remains the only tropical forest in Japan. By assistance of Dr. Tokushiro Takaso in Tropical Biosphere Research Centre from the University of Ryukyu, I therefore could

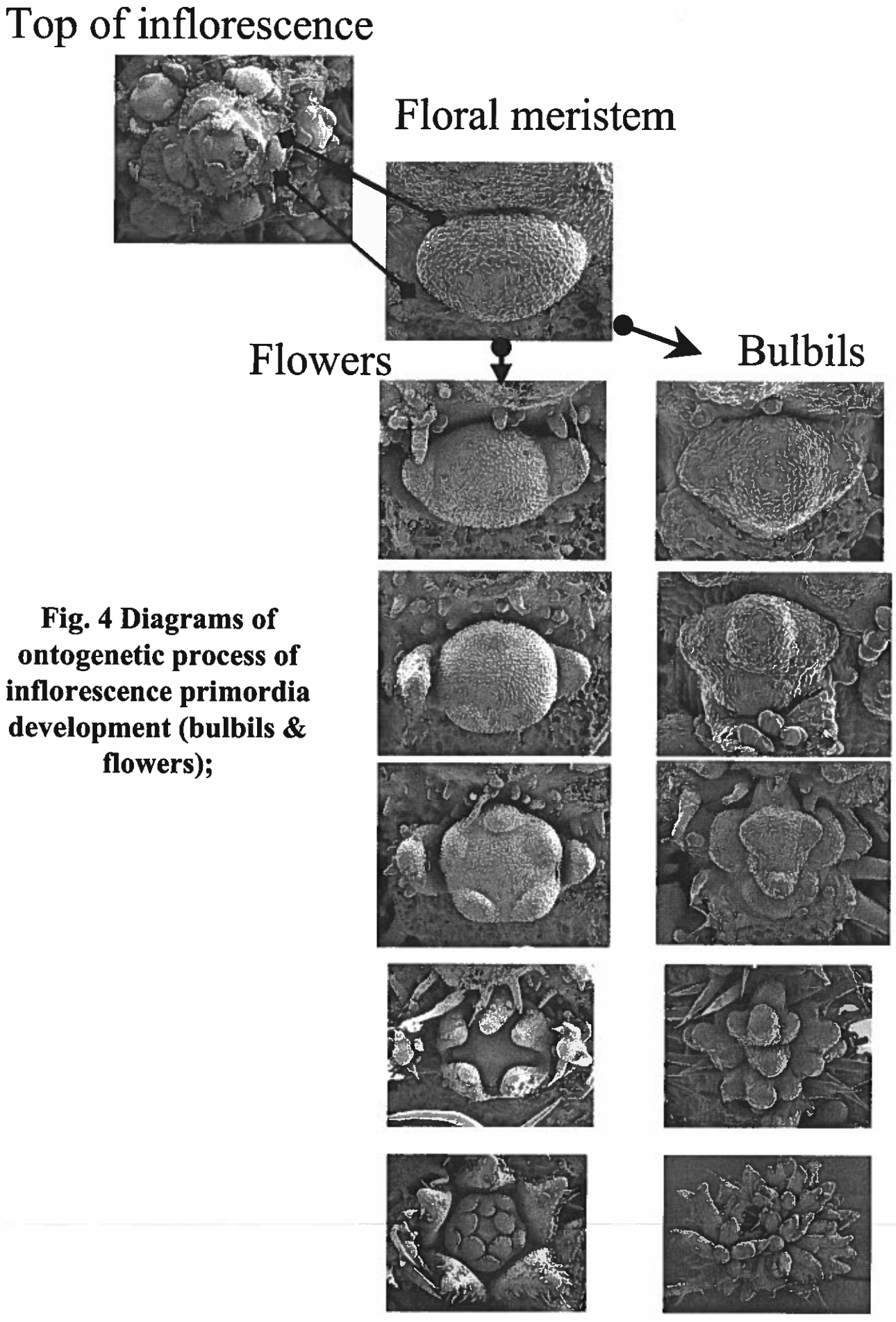



**Fig. 4 Diagrams of ontogenetic process of inflorescence primordia development (bulbils & flowers);**

less than 400 square kilometres. Inhabitants live near seashore thus most of the forests remain naturally in the central mountain area. According to the location where the literature listed and a botanist's former footnote, Dr. Takaso and I got a rough idea where *Titanotrichum oldhamii* should be. As shown on the map, we had to swim across the mangrove from seashore, then continue upstream after climbing two steep waterfalls. Followed by that is walking on slippery rocks at least one hour for searching this species along the creek. We were not sure whether we could find it out accordingly or not. However, we both realised this would be a very tough mission.

**Day 2, September 4**

This morning we woke up in a tropical full sun. With wellingtons and helmet, we attempted to keep our journey soothingly and safely. In spite of that, our feet were still bound by mud in mangrove. The skin of our feet soon went torn as we walked and swam in water for a long time.
It took us five hours to reach the top of the creek. Yet, we could not find any *Titanotrichum oldhamii* since it was so scarce. After we gave up to backtrack downstream, we crossed another tributary creek which was different from we did. Fortunately we found a tiny population on a steep cliff where flowing water was dripping. Less than thirty individuals survived there. Apparently they were clonally propagated from one individual on the top. Eventually I was able to collect few living materials and fix some leaves sample for DNA.
Dr. Takaso was appointed a committee member of the university entrance exam abruptly. He left Iriomote that evening.

**Day 3, September 5**

Without the help from Dr. Takaso, I managed to go for a general collection in another side of the island. The forests are dominated by Moraceae and Lauraceae species. Quite a few lowland species have their relations to species in Southern Taiwan and even Philippines. In contrast, most of the mountain species have their relations to species in Northern Taiwan. This mixed flora not only implied the land bridge connection between Southern Ryukyu archipelago and Taiwan, but also postulated species migration of tropical elements from Southern Taiwan or Northern Philippines. To strengthen this hypothesis, I decided to go to the herbarium of Ryukyu University for evidence.

**Day 4, September 6**

Finishing the collection trip in Iriomote, I returned to Okinawa Main Island where the University is. Professor Hidetoshi Ota, a zoologist specialised on reptiles, gave me his precious opinion on the formation of local fauna. This led me to correlate it with local flora. Dr. Masatsugu Yokota, the herbarium curator, allowed me to stay in herbarium after hours especially a typhoon attacked this island in the following two days. Great thanks are extended to all.

**Day 7, September 9**
I left Okinawa with specimen I collected.

## Conclusion and acknowledgement

Since only few biodiversity research in species abundant area of Subtropical Asia nowadays, this study explored the new field of adding molecular techniques to systematic biology and conservation biology studies. The result can offer adequate suggestions to local government in shaping a conservation policy for *Titanotrichum oldhamii* thus providing an example for subsequent collaborative conservation research between British and other countries. It is also my sincere hope that this field trip can extend our understanding on the biology of endangered species.

These results will be published in appropriate journals with full acknowledgement of the kind financial support from the Davis Expedition Fund.

## Appendix: Photographs in this field trip

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|---|---|---|---|
| 1 | 2 | 3 | 4 |
| 5 | 6 | 7 | 8 |
| 9 | 10 | 11 | 12 |
| 13 | 14 | 15 | 16 |
| 17 | 18 | 19 | |

Photo 1,2 Coastline of the Iriomote 7 Ishigaki island
Photo 3 From moutain to oversee the coast
Photo 4,5 Mangrove forest along the coast
Photo 6 The erect root of *Ficus*, which is common in mangrove
Photo 7 A bat pollinated species
Photo 8,9 Diverse habitate for subtropical forest
Photo10-14 *Titanotrichum oldhamii,* 10, habit; 11, inflorescence; 12, bulbilous inflorescence; 14, habit; 15, rhizome
Photo 15 Waterfall and riverbank
Photo 16 An orchid
Photo 17 A Gesner species, *Cyrtandra yaeyama*
Photo 18 A carnivous plant
Photo 19 A *Rhododendron* species