

REPORT ON EXPEDITION / PROJECT

Expedition/Project Title: Taxonomic description of newly discovered cynipid gall communities at Mount Emei, Sichuan, China

Travel Dates: 16/05/2023 – 30/05/2023

Location: Mount Emei, Sichuan Province, China

Group Members: **Davis project members:**

Dr Frazer Sinclair

Dr Jack Hearn

Koorosh McCormack

Other UoE members

Professor Graham Stone

Dr Chang-Ti Tang

Dr Miles Zhang

Alex Reiss

Aims:

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1. To formally identify and describe parasitoid species associated with the pest Chestnut gallwasp, *Dryocosmus kuriphilus*, and so provide the first quantitative description of the natural enemy assemblage of this pest in its native range.
 2. To establish for the first time the host range of the biological control agent *Torymus sinensis*, by collecting and rearing other early summer cynipid galls that develop alongside *Dryocosmus kuriphilus*, and identifying those species that are also attacked by *T. sinensis*.
 3. To reduce the current taxonomic impediment to formal species identification and description in Emei gallwasp communities by providing expert training to EBG staff and setting up a minimal but adequate imaging equipment.
 4. To train EBG staff in the methods associated with preparation of specimens for DNA barcoding
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Photography consent form attached: (please refer to your award letter) Yes

No

Taxonomic description of newly discovered cynipid gall communities at Mount Emei, Sichuan, China



Report for Davis Expedition Fund, 7th August, 2023

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Cover image: Expedition team at Emeishan Botanic Gardens. From left: Alex Reiss, Jack Hearn, Chang-Ti Tang, Miles Zhang, Graham Stone©, Koorosh McCormack, Frazer Sinclair, ZhiQiang Fang.

Contents

Summary	3
Background and Rationale	4
Oak gall biology	4
Recent fieldwork has revealed many new gall-associated species in Fagaceae-rich regions of Southeast Asia.	4
The pest Chestnut gall wasp <i>Dryocosmus kuriphilus</i> and its biocontrol agent <i>Torymus sinensis</i>	5
The taxonomic impediment to work on gall wasp communities in China	5
Study site and itinerary	5
Objectives and achievements	6
Objective 1: Taxonomic description of native parasitoids of <i>Dryocosmus kuriphilus</i>	6
Objective 2: Establishing the native host range of the biocontrol agent <i>T. sinensis</i>	7
Objective 3. Reducing the taxonomic impediment.....	7
Objective 4: Training project collaborators in preparation of specimens for DNA barcoding.....	9
Conclusions	10
Acknowledgements.....	10
References	11
Appendix 1: Framework of objectives, actions, and outputs.	12
Appendix 2. Expenses	13

Summary

Cynipid oak galls contain intricate multi-trophic communities that are a useful model system for studying community ecology and evolution. East Asia is home to diverse communities of oak gall wasps, many of which are undescribed, but which also include the native populations of important species such as the global pest chestnut gallwasp *Dryocosmus kuriphilus* and its parasitoid biocontrol agent *Torymus sinensis*. Important questions – such as the vulnerability of *D. kuriphilus* to other parasitoids, and the alternative hosts of *T. sinensis* in its native range – are impeded by the lack of a formal taxonomic framework for many insect taxa, which prevents species level investigation and the comparison of past and present records. To tackle this taxonomic impediment our project set out to provide equipment, training, and laboratory and fieldwork support to project partners in China, to facilitate the formal description of new species from cynipid gall communities. A high magnification insect imaging rig was established at Emeishan Botanical Gardens and several new candidate parasitoid species reared from *D. kuriphilus* were photographed. Fieldwork was timed to coincide with the maturation of previously under-sampled spring generation galls that are likely alternate hosts for *T. sinensis*. A non-destructive method of DNA extraction was implemented to facilitate verification of candidate morphospecies with mitochondrial and nuclear DNA sequences. A collaboration was established with taxonomic experts from Sichuan University who have access to a regional entomological collection. Work to verify, photograph, and formally describe new species is ongoing.

Background and Rationale

Oak gall biology

Oak gallwasps (Hymenoptera: Cynipidae: Cynipini) are an intriguing group of insects that induce complex gall structures on trees of the Fagaceae family. An adult female gallwasp lays her eggs into host meristem tissues, and substance produced by the egg and young larva hijack development of surrounding plant tissues, resulting in a species-specific gall structure (Stone *et al.*, 2002). The larva consumes nutritive tissues within a specialised chamber, before pupating within the gall and tunnelling its way out as an adult. Most species have two generations each year, with a sexual generation developing during spring and early summer, and an asexual generation developing during late summer and autumn (Stone *et al.*, 2002). Most gall wasps do not seriously damage their plant hosts, but exceptions include the chestnut gall wasp *Dryocosmus kuriphilus*, which is native to China and is a major global pest of chestnuts (Fagaceae: *Castanea*).

Once formed, oak galls are often colonised by closely related inquiline gall wasps - close relatives of the gall inducers that are unable to induce a gall 'from scratch', but whose larvae develop within the tissues of existing galls. The galls are also attacked by species-rich communities of hymenopteran parasitoids - primarily from the chalcid and braconid families - whose larvae feed on the larvae or pupae of the gall-formers, inquilines, or other parasitoids. Oak galls therefore house multi-trophic communities, with host trees as primary producers, gall wasps and inquiline herbivores as primary consumers, and parasitoids and hyperparasitoids as secondary and tertiary consumers.

The intricate interactions and closed nature of oak gall communities make them a useful model system for studies of trait evolution and species interactions. Of particular interest is the impact of gall traits (gall structures, host plant associations, and developmental phenology) on the composition of the parasitoid assemblages that attack them (Stone & Cook, 1998; Stone & Schönrogge, 2003; Bailey *et al.*, 2009). Analysis of such community-level patterns is dependent on being able to identify the gall-inducers and their natural enemies. Furthermore, while a Linnean taxonomic framework exists for European cynipid communities, no such framework exists for other parts of the world.

Recent fieldwork has revealed many new gall-associated species in Fagaceae-rich regions of Southeast Asia.

There are around 1000 formally described gall wasp species associated with Fagaceae worldwide, with around 680 species in the Nearctic, 150 species in the Western Palaearctic, but only around 100 species from Asia. This distribution contrasts markedly with global patterns in the species richness of Fagaceae, which is highest in Southeast Asia, particularly southern China, Thailand, Myanmar, Laos and Vietnam. In addition to the familiar oaks (*Quercus*) and chestnuts (*Castanea*), Southeast Asia includes a high species richness of the purely Asian oak section *Cyclobalanopsis* and the Asian genera *Castanopsis* and *Lithocarpus*. The longstanding suspicion is that the low recorded species richness of gall wasps in Southeast Asia is an artefact of lack of study. This has been borne out by our work in SW China over the last 6 years. In 2017, we established a collaborative research project with the Sichuan Academy of Natural Resources Science based at Emeishan Botanical Gardens in western Sichuan Province. Emeishan is an isolated mountain outlier of the Tibetan Plateau further to the west, with a Fagaceae species richness (34 species) as high as the total for the entire Western Palaearctic. Field collections on previous visits (primarily in autumn) has resulted in the sampling of over 39000 gall wasp galls from a total of 28 Fagaceae species. Rearing of these galls has yielded over 200 different cynipid gall types and 62 associated parasitoid types. DNA barcoding of individual specimens supports the inference that most of the distinct morphotypes sequenced so far are also separate species, the vast majority of which are undescribed and new to science.

The pest Chestnut gall wasp *Dryocosmus kuriphilus* and its biocontrol agent *Torymus sinensis*.

The chestnut gall wasp *D. kuriphilus* is a globally important pest of chestnuts, *Castanea*. Native to southern China, since the 1940s it has been accidentally introduced to Japan, Korea, Nepal, USA, & Europe (ca. 2000) including the UK (ca. 2015), where it infests native chestnut species. Gallwasp attack reduces chestnut fruit production and can kill chestnut trees. This damage drove the search for an effective biological control agent. Japanese scientists identified a Chinese parasitoid, *Torymus sinensis*, as a candidate and this wasp has since been released throughout the introduced range of *D. kuriphilus*. While *T. sinensis* has controlled the pest successfully in many regions, there is growing evidence that, outside China, it can also attack non-target gall wasps as well as *D. kuriphilus*. Prior to our work, there was no information on which other native Chinese Gallwasp are attacked by *T. sinensis* - information that would show the ecological breadth of alternative hosts for this parasitoid and help inform risk assessments for its release outside China. There was also very little information on which other parasitoids attack *D. kuriphilus* at high levels, and so which might be alternative effective biological control agents. Our project sought to address both of these knowledge gaps by (i) sampling other early summer galls that develop alongside *D. kuriphilus* and identifying those that are also attacked by *T. sinensis* in China, and (ii) carrying out a quantitative analysis of the full natural enemy assemblage associated with *D. kuriphilus*.

The taxonomic impediment to work on gall wasp communities in China

Formal taxonomic identification and description of new species is an essential component of making biodiversity visible to science. However, while there is a strong tradition of Linnean morphological taxonomy for vascular plants in China (e.g., The Flora of China project), the same is much less true for most insect groups. As a result, major recent studies of the rich insect biodiversity of SW China have largely been based on DNA metabarcoding approaches (Wang *et al.*, 2019), which in many cases have used bulk samples of insects and so cannot match sequences to individual specimens. Most of the taxa are known only as molecular taxonomic units (MOTUs), and new specimens can only be matched with these by sequencing. This creates an enormous taxonomic impediment to matching current work with previous studies based only on morphology, and to carrying out further work where sequencing facilities are either too expensive or not available.

What is needed is a reciprocal illumination approach in which specimens are divided into morphotaxa, whose species status is validated using mitochondrial and nuclear DNA sequence data. Candidate species confirmed in this way can then be formally described using morphological characters, allowing subsequent non-molecular identification.

Because export of specimens from China is currently very difficult or impossible, formal identification of specimens and description of new species requires that necessary morphological data and images are generated in China. Lack of necessary expertise and facilities in our partner institute in Emei has meant that to date necessary data can only be generated during our brief visits. This imposes a severe bottleneck on progress, which has been underlined by covid-related interruptions to the team's ability to visit Emeishan. **It is clear that the best way to accelerate scientific advance in this system is to remove this taxonomic impediment through training of local scientists in the knowledge and techniques required alongside the installation of an independent specimen imaging system.**

Study site and itinerary

The project expedition took place from 15/05/2023 to 30/05/2023, to correspond with the maturation of sexual generation galls in late Spring*. Fieldwork (led by Jack Hearn) and microscope imaging (led by Koorosh McCormack) took place throughout this period in and around the Emeishan Botanic Gardens (see Figure 1). Laboratory training and DNA extraction (led by Frazer Sinclair) took place at

the Sichuan Provincial Academy of Natural Resources Science in Chengdu during the second week of the expedition.

* Note: The expedition was originally scheduled for April/May 2022 but was delayed due to the Covid-19 pandemic and associated mandatory quarantine for visitors to China. The mandatory quarantine requirement was only dropped in January 2023.

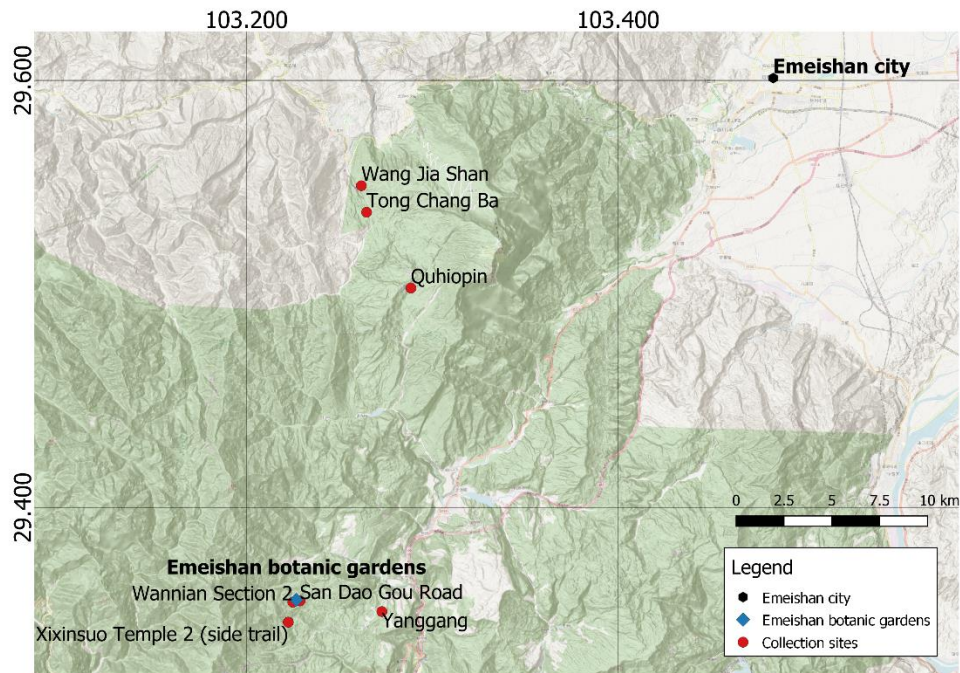


Figure 1. Map of field collection sites at Mount Emei in western Sichuan Province, China.

Objectives and achievements

Here we set out the project objectives and describe the actions completed during the expedition, together with any ongoing activities. A framework for objectives, actions, and outputs is detailed in Appendix 1.

Objective 1: Taxonomic description of native parasitoids of *Dryocosmus kuriphilus*

Sampling of *D. kuriphilus* in previous expeditions to Mount Emei had resulted in 1306 parasitoid specimens. Prior to the expedition, project collaborator Dr. ZhiQiang Fang provided dissection microscope images of all specimens, and these were used to assign them into 24 morphotypes (i.e., candidate species) using a morphological key developed at the University of Edinburgh. During the expedition, specimens from 4 parasitoid morphotypes were photographed using the project's new imaging rig and photo stacking software. We prioritised parasitoids from the genera *Bootanomyia* (x2, Chalcidoidea: Megastigmidae, see Plate 1) and *Torymus* (x2, Chalcidoidea: Torymidae), as these are the most well documented groups within China, and the only for which local specialist knowledge and collections are available (see also objective 3). Project collaborator Dr. ZhiQiang Fang will photograph further parasitoid morphotypes from *D. kuriphilus*, in conjunction with the validation of morphotypes through DNA barcoding (see also objective 4).



Plate 1. Lateral habitus images of two candidate *Bootanomyia* species (Chalcidoidea: Megastigmidae) reared from galls of *Dryocosmus kuriphilus*. (photo© Miles Zhang).

Objective 2: Establishing the native host range of the biocontrol agent *T. sinensis*.

Torymus sinensis is known to be active from late spring through to late autumn, and a parasitoid morphotype believed to be *T. sinensis* has been recorded from both sexual generation (developing during spring) and asexual generation (developing during autumn) oak galls at Mount Emei. Previous gall collections have primarily focused on asexual generation oak galls, and it is likely that the sexual generation host range of *T. sinensis* is currently underestimated. During the expedition we collected galls at 8 sites on Mount Emei ranging in elevation from 530 to 2083 meters (Figure 1). Collections followed an established protocol, whereby for each sampled tree we: (i) removed 10 x standard 1m-long branches (corresponding to 4 year's growth) using 6m long handled pruners; (ii) searched all foliage in the field and returned collected galls to the botanic gardens offices; (iii) sorted collected galls into morphotypes and photographed exemplars of each; (iv) placed galls into ventilated containers for rearing. A total of 1769 sexual generation galls were collected comprising 85 distinct morphotypes, 31 of which had not previously been recorded (Plate 2). The galls shall be monitored for at least one year by Dr ZhiQiang Fang, and any emerging insects shall be photographed and preserved in ethanol. Any parasitoids belonging to the *T. sinensis* morphotype shall be DNA barcoded to confirm their identity (see also Objective 4). Fieldwork was conducted together with two technicians from Emeishan Botanic Gardens (Mr Bangzhong Qi and Mr Feng), who received training in field sampling, gall identification, and data processing.

Objective 3. Reducing the taxonomic impediment

The components for an imaging rig were sourced in the UK, transported to China, and installed at the Emeishan Botanic Gardens. Project collaborator Dr ZhiQiang Fang and his assistant (Mrs Yingcui Zhou) were trained in specimen dissection, staging, imaging, and the use of image stacking software (see plate 3). They participated in the imaging of 4 parasitoid morphotypes during the expedition and shall continue to photograph further morphotypes in conjunction with species validation through DNA barcoding (see also Objectives 1 & 4).

In addition to high quality images, the formal description of new insect species requires expert knowledge of the taxa under consideration, access to collections of closely related species from the region, and access to an entomological collection for the curation of type specimens. To facilitate this, the project team met with Professor Kangshan Mao* and members of his research group at Sichuan University, who recently published a description of a new species from the Hymenopteran family Megastigmidae (Chen *et al.*, 2023). This family includes several known parasitoids of oak galls in the Western Palearctic from the genus *Bootanomyia*, and several new candidate *Bootanomyia* species previously reared from *D. kuriphilus* at Mount Emei (see also Objective 1). It is expected that

collaboration with Professor Mao and his lab will expediate the formal description of new oak gall parasitoid species, and voucher specimens shall be deposited in the entomology collection at Chengdu Natural History Museum.

* Note: The initial project proposal (prepared in 2021) suggested a collaboration with Dr Hui Xiao from The Chinese Academy of Sciences in Beijing. Unfortunately, Dr Xiao was unavailable to participate in the rescheduled project, but the collaboration with Professor Mao was an opportune alternative and provides access to a more conveniently located entomological collection.



Plate 2. (Left) Expedition team searching for galls at Wang Jia Shan (from left: Graham Stone©, Frazer Sinclair, Alex Reiss, & Jack Hearn). (Top right) A cynipid gall on *Lithocarpus cleistocarpus*, dissected to reveal internal chamber (Chang-Ti Tang©). (Bottom right) A cynipid gall on *Castanopsis carlesii* var *spinulosa* (Chang-Ti Tang©).



Plate 3. Imaging rig being demonstrated by team member Koorosh McKormack (photo © Graham Stone).

Objective 4: Training project collaborators in preparation of specimens for DNA barcoding

In our 'reciprocal illumination' approach to insect taxonomy, specimens are divided into morphotaxa whose species status is validated using mitochondrial and nuclear DNA sequence data. Candidate species confirmed in this way can then be formally described using morphological characters, allowing subsequent non-molecular identification.

In order to extract DNA while at the same time preserving insect specimens as morphological vouchers, previous work in the Western Palearctic has followed a protocol where a single leg is dissected under a microscope and then incubated in a Chelex™ and proteinase-K solution. Prior to the expedition this protocol was trialed on specimens from Mount Emei by project collaborator Hangyu Wu – a technician at the Sichuan Provincial Academy of Natural Resources Science, Chengdu. The success rate was poor (~20%) – as determined by Sanger sequencing of a mitochondrial DNA barcode from the extract – likely due to the very small size of many specimens and the high humidity at the insect rearing facility that may have degraded DNA prior to preservation. Subsequently, we identified an alternative method of DNA extraction using QuickExtract™ solution, where the entire specimen is gently lysed to release DNA into solution without damaging the external structures of the specimen. The method was trialed during the expedition, together with Miss Hangyu Wu, and proved to have a much greater success rate (~90%). Miss Wu has since completed extractions for ~1400 insect specimens, and these are currently being sequenced for a combination of mitochondrial and nuclear loci.

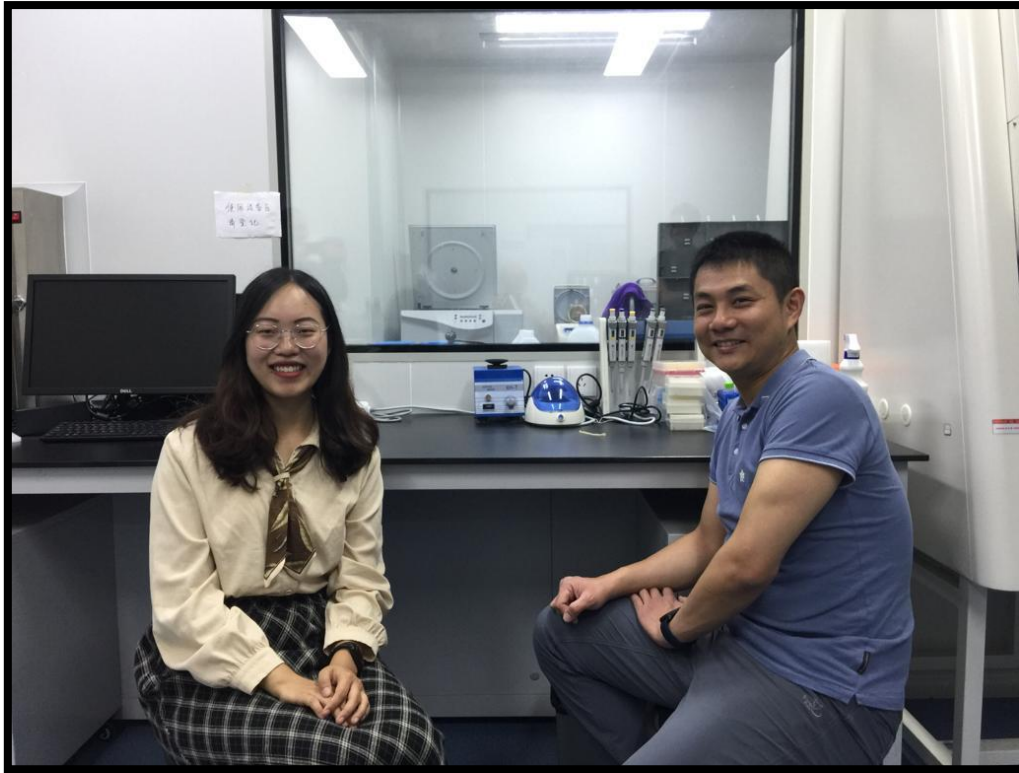


Plate 4. Project collaborator Miss Hangyu Wu and expedition team member Chang-Ti Tang in the DNA extraction laboratory at Sichuan Provincial Academy of Natural Resources Science, Chengdu (photo© Graham Stone).

Conclusions

Despite delays and logistical challenges imposed by the Covid-19 pandemic, the project expedition was successful in delivering key equipment and training to project partners in China, extending field sampling of spring generation galls, and widening collaborations with regional taxonomic experts. Through ongoing remote support and collaboration, we expect to publish numerous formal descriptions of new species from cynipid gall communities in China. This will in turn resolve the taxonomic impediment and facilitate ecological analyses of the native parasitoid assemblages associated with the global pest Chestnut gall wasp *Dryocosmus kuriphilus*, and the native host range of its biological control agent *Torymus sinensis*.

Acknowledgements

The project team are grateful for the generous support of the Davis Expedition Fund. Project partner Dr ZhiQiang Fang was instrumental in arranging permits and logistics, and his enthusiasm for oak galls is an inspiration. Many thanks also for the involvement of project collaborators at Emeishan Botanical Gardens and the Sichuan Provincial Academy of Natural Resources Science – especially Mrs Yingcui Zhou, Miss Hangyu Wu, Dr Ying Zhu, Mr Bangzhong Qi, & Mr Feng. Thanks also to Professor Graham Stone, Chang-Ti Tang, Miles Zhang, and Alexander Reiss for joining the expedition with their own funding and lending their invaluable expertise.

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Appendix 1: Framework of objectives, actions, and outputs.

Objective	Completed actions	Ongoing actions	Expected outputs
1. Taxonomic description of native parasitoids of <i>Dryocosmus kuriphilus</i>	1.1. Taxonomic description quality images for 2 parasitoid morphotypes (family Megastigmidae)	1.3. Verification of parasitoid morphotypes with DNA barcoding (linked with 4.1)	1.a. Publication of new parasitoid species descriptions.
	1.2. Allocation of 1306 Dk parasitoids into 24 morphotypes	1.4. Taxonomic description quality images for further verified parasitoid morphotypes (linked with 3.3)	1.b. Publication of the first formal taxonomic description of the parasitoid assemblage of <i>D. kuriphilus</i> in its native China.
2. Establishing the native host range of the biocontrol agent <i>Torymus sinensis</i>	2.1. Field sampling of early summer gall morphotypes.	2.3. Rearing of galls and preservation of emerging parasitoid specimens	2.a. Publication detailing the native host range of <i>T. sinensis</i>
	2.2. Training of two technicians in field collection methods and associated data management protocols.	2.4. Photographic / DNA barcode identification of <i>T. sinensis</i>	
3. Reducing the taxonomic impediment	3.1. Installation of a functional imaging rig at Emeishan Botanic Gardens	3.4. Taxonomic description quality images for further Gallwasp and parasitoid species	3.a. Enhanced in-country capacity for advances in Cynipid gall community taxonomy
	3.2. Training of project collaborators (x2) in specimen dissection, staging, and microscope imaging		
	3.3. Collaboration established with taxonomic experts at Sichuan University	3.5. Deposit of voucher specimens within the entomological collection of Sichuan University	
4. Training project collaborators in preparation of specimens for DNA barcoding	4.1. Training of one laboratory technician in non-destructive DNA extraction protocol.	4.3. DNA barcoding of gall wasp and parasitoid specimens	Linked to outputs 1.a, 1.b, 2.a, & 3.a
	4.2. DNA extractions completed for ~1400 parasitoid and gall wasp specimens		

Appendix 2. Expenses

The total project expenditure was £8761, consisting of £7500 from the Davis Expedition Fund, and personal contributions of £1261 shared between the three project members. This total conforms with the original proposed budget total of £9267.50, which included a 10% contingency line. A summary of expenditure is provided in Table A2.1, and an itemised record of all expenditure, with receipts, is available on request from Frazer Sinclair (fsincla2@exseed.ed.ac.uk).

Table A2.1. Summary of expedition expenses by type.

Expense Type	£
Imaging equipment (inc. photography rig & image stacking software)	1614.75
Field-kit (inc. notebooks, tools, field clothing)	846.48
UK T&S (including hotels, domestic flights, and rail travel)	688.86
International travel (including visa fees and international flights x3)	3071.14
China T&S (including food, accommodation, and vehicle hire)	1173.73
China lab reagents (i.e. QuickExtract™ solution x150ml)	1366.08
Grand Total	8761.04