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## Genetic differentiation of the eulophid wasp *Quadrastichus erythrinae* Kim (Hymenoptera: Eulophidae) from various *Erythrina* hosts based on mitochondrial and nuclear genes **【Research report】**

### 由粒線體與核基因探討各類寄主植物之刺桐釉小蜂的遺傳變異 **【研究報告】**

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#### Abstract

Since 2000, many *Erythrina* trees have been found initially wilting and later suffering die-back due to severe infestations by *Quadrastichus erythrinae* Kim (Hymenoptera: Eulophidae) in Mauritius, Réunion, and in Singapore. These infestations have since spread into several tropical and subtropical countries. Various degrees of damage and different symptoms have been observed in different host species which has raised the question whether the pests all belong to the same species or not. In order to measure the differentiation in the eulophid wasps from different geographical areas and various *Erythrina* host plants, 36 samples on 6 host taxa from 14 localities in Taiwan and 11 samples on 2 host species from Singapore and Mauritius were collected. Singapore and Mauritius were two of the three countries included in the original description of *Q. erythrinae*. We analyzed the mitochondrial genes of 12S ribosomal RNA and cytochrome oxidase I, and the nuclear sequence of intergenic spacer 2 to understand their genetic variation and phylogenetic relationship. Sequences of the same region in all the three genes were identical among all the samples. The homogeneity of the genetic composition suggests that there were no cryptic species in the samples. This confirms that all the wasps sampled in this study, despite their different origin of both area and host, belong to the same species. It is evident that all these pests from these countries came from the same monomorphic genotype which quickly and widely spread to other areas.

#### 摘要

刺桐釉小蜂於2000年首先被報導嚴重感染非洲東部模里西斯、留尼旺島以及亞洲新加坡的刺桐樹，並造成部份植株死亡，旋即迅速蔓延到全球熱帶與亞熱帶島嶼或陸塊邊緣地區。由於受害的寄主植物種類及病徵程度不同，這些感染的昆蟲是否為同一種，令人產生質疑。我們選擇台灣本島與其離島等14個地區的36個樣本，及該物種最早報導之發生地模里西斯與新加坡的11個樣本；應用粒線體的12S核糖體RNA (12sRNA)、細胞色素氧化酵素第一單元 (COI) 以及核DNA的核糖體第二區間 (ITS2) 等區域之DNA片段，分析來自不同地區、不同寄主植物上的刺桐釉小蜂之遺傳變異與親緣關係。結果顯示所有不同地點與不同寄主植物上的昆蟲樣本在同片段的序列皆無差異，顯然全台各地及取樣之其他國家的刺桐屬植物上的害蟲均為同種之刺桐釉小蜂，這些受害嚴重地區的刺桐釉小蜂顯然來自同一個基因型快速擴散的結果。

**Key words:** invasive insect, *Quadrastichus erythrinae*, *Erythrina*, cytochrome oxidase I, 12 rRNA, intergenic spacer 2

**關鍵詞:** 外來種昆蟲、刺桐釉小蜂、刺桐、細胞色素氧化酵素第一單元、12S核糖體RNA、核DNA的核糖體第二區間。

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# Genetic differentiation of the eulophid wasp *Quadrastichus erythrinae* Kim (Hymenoptera: Eulophidae) from various *Erythrina* hosts based on mitochondrial and nuclear genes

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## ABSTRACT

Since 2000, many *Erythrina* trees have been found initially wilting and later suffering die-back due to severe infestations by *Quadrastichus erythrinae* Kim (Hymenoptera: Eulophidae) in Mauritius, Réunion, and in Singapore. These infestations have since spread into several tropical and subtropical countries. Various degrees of damage and different symptoms have been observed in different host species which has raised the question whether the pests all belong to the same species or not. In order to measure the differentiation in the eulophid wasps from different geographical areas and various *Erythrina* host plants, 36 samples on 6 host taxa from 14 localities in Taiwan and 11 samples on 2 host species from Singapore and Mauritius were collected. Singapore and Mauritius were two of the three countries included in the original description of *Q. erythrinae*. We analyzed the mitochondrial genes of 12S ribosomal RNA and cytochrome oxidase I, and the nuclear sequence of intergenic spacer 2 to understand their genetic variation and phylogenetic relationship. Sequences of the same region in all the three genes were identical among all the samples. The homogeneity of the genetic composition suggests that there were no cryptic species in the samples. This confirms that all the wasps sampled in this study, despite their different origin of both area and host, belong to the same species. It is evident that all these pests from these countries came from the same monomorphic genotype which quickly and widely spread to other areas.

**Key words:** invasive insect, *Quadrastichus erythrinae*, *Erythrina*, cytochrome oxidase I, 12 rRNA, intergenic spacer 2

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## Introduction

Coral trees, *Erythrina* spp. (Fabaceae), are native to tropical and subtropical regions and are popular ornamental trees all over the world. Since 2000, many *Erythrina* trees have been found initially wilting and later suffering die-back due to severe infestations by *Quadrastichus erythrinae* Kim (Hymenoptera: Eulophidae) in Mauritius, Réunion, and Singapore (Kim *et al.*, 2004). It then spread widely into various countries in Asia (Gramling, 2005; Horticulture Working Group, 2003; Yang *et al.*, 2004; Huang *et al.*, 2005; Yang *et al.*, 2006; Uechi *et al.*, 2007), the Pacific Islands (Heu *et al.*, 2006; Schmaedick, 2006; Uechi *et al.*, 2007), and was recently found in Florida (Wiley and Skelley, 2006; <http://www.doacs.state.fl.us/pi/enpp/ento/gallwasp.html>). This wasp forms galls on the tender part of the plant, and unlike most galling insects which live harmoniously with their hosts, it is a ferocious pest which causes acute and severe damage. A heavy infestation causes curling of leaves and shoots, loss of plant growth and vigor, severe defoliation, and eventually the plant dies.

Among the 112 named species of *Erythrina* genus worldwide (Bruneau, 1996), field reported hosts of the *Q. erythrinae* have to date covered a wide range of *Erythrina* species, including *E. variegata*, *E. crista-galli*, *E. corallodendron*, *E. abyssinica*, *E. berteriana*, *E. fusca*, *E. indica*, *E. stricta* and *E. sandwicensis* (Yang *et al.*, 2004; Heu *et al.*, 2006; Faizal *et al.*, 2006).

In Taiwan, there are many species of *Erythrina* distributed all over the main island and the surrounding islands (Yang *et al.*, 2004). Various degrees of damage and different symptoms have been observed on different host species. We found that most cases of plant deaths occurred on *E. variegata* and its varieties. Although the differential damage might be due to different susceptibility of plant

species, the heterogeneity of the pests due to multiple invasions may play a role. This raises the question of whether the insect that infested *E. variegata* and other host species differ among each other. It is speculated that there might be cryptic species which causes variations in the level of plant damages. However, an examination of the wasp's morphology did not find a significant variation among individuals infesting different host species. Further examinations using molecular characteristics may offer a better answer to this question.

Molecular sequences provide useful information for phylogeographic studies (Avice *et al.*, 1987) and provide fast and accurate identification of morphologically indistinct species (Hebert *et al.*, 2002; Armstrong and Ball, 2005). Therefore, in order to understand the variation and the possible origin of these wasps, we used different molecular markers, i.e. COI, 12S, ITS2, covering both mitochondrial and nuclear genes to investigate the pests from different host plants in the early outbreak stage in Taiwan, Singapore and Mauritius.

## Materials and Methods

### *Samples collection*

In order to measure the geographic variation and identify the eulophid wasps from different *Erythrina* host plants, samples from various areas and different hosts were collected. These included 36 samples from 14 localities in Taiwan (Fig. 1) and 11 samples from Singapore and Mauritius (Table 1). Singapore and Mauritius were two of the three countries included in the original description of the new species of *Quadrastichus erythrinae* Kim. The samples that were collected in Taiwan were sampled from six *Erythrina* taxa, including 19 samples from *E. variegata*, 10 from *E. variegata* var. *orientalis*, 3 from *E. corallodendron*, 2 from *E. crista-galli*, 1 from *E. abyssinica*, and 1 from *E. berteriana*. Samples collected outside of Taiwan included 7



Fig. 1. Sampling localities of the *Erythrina* eulophid wasp, *Quadrastichus erythrinae* Kim from *Erythrina* host plants in Taiwan.

from *E. fusca* in Singapore and 4 from *E. variegata* in Mauritius.

**DNA extraction, polymerase chain reaction (PCR), and sequencing**

Genomic DNA was obtained from a single wasp using the Purgene DNA Isolation kit (Gentra Systems, Minnesota, USA), following the extraction protocol of the manufacturer. Precipitated DNA was resuspended in 30  $\mu$ L of dH<sub>2</sub>O and was used to amplify the target regions, including mitochondrial cytochrome oxidase I (COI) and 12S ribosomal DNA (12S rDNA), and the nuclear region of internal transcribed spacer 2 (ITS2) by PCR. Each PCR was

carried out in a final volume of 25  $\mu$ L with 0.8  $\mu$ L of 10  $\mu$ M dNTP, 1.5  $\mu$ L of 25 mM MgCl<sub>2</sub>, 0.5  $\mu$ L of each 10  $\mu$ M of primer, 2.5  $\mu$ L of 10x *Taq* buffer, and 0.1  $\mu$ L of Amersham *Taq* (Amersham Biosciences, Buckinghamshire, UK). Touchdown PCR conditions were as follows: an initial denaturation step of 95°C (2 min), then 10 cycles consisting of denaturation at 94°C (30 s), annealing at 60°C (30 s) but with 0.5°C degree down each cycle, extend at 72°C (1 min), then proceed to 30 cycles of 94°C (30 s), 50°C (30 s), extend at 72°C (1.5 min), followed by a final extension at 72°C (7 min). Different annealing temperatures and primer pairs were used to improve the

Table 1. Sampling information of *Quadrastichus erythrinae* specimens

Location	Year of collection	Host plant species of <i>Erythrina</i> (Number of wasps sampled)
Singapore	2003-2005	<i>E. fusca</i> (7)
Mauritius	2003	<i>E. indica</i> (= <i>E. variegata</i> ) * (4)
Taiwan		
Taipei	2004-2005	<i>E. variegata</i> (2), <i>E. variegata</i> var. <i>orientalis</i> (1), <i>E. corallodendron</i> (1)
Taichung	2004	<i>E. variegata</i> var. <i>orientalis</i> (1)
Chiayi	2004	<i>E. variegata</i> (2), <i>E. variegata</i> var. <i>orientalis</i> (2)
Tainan	2004	<i>E. variegata</i> (2), <i>E. variegata</i> var. <i>orientalis</i> (2)
Kaohsiung	2004	<i>E. variegata</i> (1)
Pingtung	2004	<i>E. variegata</i> (1), <i>E. crista-galli</i> (1), <i>E. corallodendron</i> (1), <i>E. abyssinica</i> (1), <i>E. berteroa</i> (1)
Ilan	2006	<i>E. variegata</i> (1)
Hualien	2006	<i>E. variegata</i> (2), <i>E. variegata</i> var. <i>orientalis</i> (1)
Taitung	2004	<i>E. variegata</i> (1), <i>E. variegata</i> var. <i>orientalis</i> (2), <i>E. corallodendron</i> (1), <i>E. crista-galli</i> (1)
Nantou	2004	<i>E. variegata</i> (1)
Orchid Is.	2004	<i>E. variegata</i> (1)
Penghu Is.	2004	<i>E. variegata</i> (1), <i>E. variegata</i> var. <i>orientalis</i> (1)
Liuchiu Is.	2004	<i>E. variegata</i> (1)
Kinmen Is.	2005-2007	<i>E. variegata</i> (2), <i>E. corallodendron</i> (1)

\* Originally recorded as *E. indica*; however, it is a synonym of *E. variegata* (GBIF, 2008).

PCR quality. The primers used in this study are listed in Table 2. The PCR products were run on 1.0% agarose gels in 1x TBE buffer to ensure correct amplification. PCR products were cleaned using a Gel/PCR DNA Fragments Extraction kit (Geneaid, Taipei, Taiwan) when only a single DNA band was visible in a gel. Sequencing reactions were conducted using a 96-well Gel/PCR Clean Up kit (Geneaid) on an ABI3730 DNA Analyzer (Applied Biosystems, Taipei, Taiwan). Both strands were sequenced, and the sequences were checked and assembled into contiguous arrays using Sequencher 4.7 (Gene Codes Corp, Boston, USA).

### Sequence Analyses

The genetic variation was described by general statistics, such as the number of unique haplotypes, variable nucleotide positions. These statistical analyses were performed using DNASP 4.50 (Rozas *et al.*,

2003).

## Results and Discussion

### Sequence variations of mitochondrial COI and 12S rDNA regions, and nuclear ITS2 rDNA region

There were a total of 74 sequences obtained, 30 from COI, 30 from ITS2 and 14 from 12S rDNA. The length of mitochondrial COI, 12S rDNA and nuclear ITS2 was 764bp, 508bp, and 298bp, respectively (Fig. 2). Within the analyzed COI region of mitochondrial genes, the average nucleotide compositions among the *Q. erythrinae* individuals were 32.2%, 44.5%, 9.8%, and 13.5% for A, T, C, and G, respectively; while those of 12S rDNA region were 41.4%, 44.9%, 8.6%, and 5.1%, respectively. Base compositions of the ITS2 region among these wasps were with average A, T, C, and G of 22.2%, 20.7%, 28.5%, and 28.5%, respectively. The A+T

Table 2. Primer sequence information for the molecular analysis of the eulophid wasp, *Quadrastichus erythrinae* Kim from *Erythrina* spp.

Name	Sequence(5'→3')
Gallcox1-F1	GATTTTTTGGTCACCCAGAAGT
Gallcox1-R1	ATGAATGATAAGCTGGAGGAT
SR-J-14199 <sup>a</sup>	TACTATGTTACGACTTAT
SR-N-14594 <sup>a</sup>	AAACTAGGATTAGATACCC
ITS2-28sBld <sup>b</sup>	TTCTTTTCCTCCSCTTAYTRATATGCTTAA
ITS2-5p8sFc <sup>b</sup>	TGAACATCGACATTTYGAACGCACAT

<sup>a</sup> Primers used are listed in Kambhampati and Smith (1995)

<sup>b</sup> Primers used are listed in Campbell *et al.* (1993)

composition of 12S rDNA was 86.3%, which is higher than COI (76.7%) or ITS2 (42.9%). The observed bias towards adenine and thymine was consistent with the mitochondrial genome of *Apis mellifera* (Crozier and Crozier, 1993). There were no variations found among sampling locations or among different host plants. Variations in these amplicons were also absent either intra/inter populations in Taiwan or between samples from Mauritius, Singapore, and Taiwan.

#### ***Episode of possible derivation of Quadrastichus erythrinae***

One hypothesis that might explain the homogeneity seen in this study is that a founder population of *Q. erythrinae* invaded islands in the Indian Ocean, where it underwent a genetic bottleneck and had the potential for rapid evolution. It might have led to a more invasive strain of this species which spread into other regions in a short time. The results of little variation found among these invasive wasps sampled from countries across 8,546 kilometers by Mercator Distance in this study provide congruent evidence to support the hypothesis.

The homogeneity of the genetic composition both from mitochondrial and nuclear amplicons suggest that there are no cryptic species in the samples, despite variations in the infectious symptoms observed. While little taxonomic knowledge

was established before the outbreak of the invasive pest *Q. erythrinae*, it was believed that the origin of the pest is Africa (Messing *et al.*, 2008). Moreover, many other species were found after the outbreak of *Q. erythrinae* and there are several new phytophagous eulophid species from *Erythrina* under description (Wright *et al.*, 2008). Samples from other countries, especially from Africa, will help to clarify the fact whether the invasive pest is a variant out of Africa which may undergone a bottleneck effect and shortly became a vicious pest in many other countries.

#### ***Differential host infestations***

Among samples from different infested plant species of *Erythrina*, the insects reveal no genetic diversity, despite the observed variations in the levels of infestation. This suggests that plant physiology may play a major role in their response to the attack by this insect. Among the infested plants, *E. variegata* and allied are the ones most seriously attacked in Taiwan, and this seems to be true in other countries as well. The most severe case is exemplified by a widely planted windbreak tree, with the common names “Tropic Coral”, “tall williwilli” or “tall erythrina”, in Hawaii. It is a special hybrid form of *E. variegata* and has an upright, tall and slender tree shape. Within a year after this pest was discovered

## A. COI

1 TTATATTTTAAATTTTACCGGGATTTGGGTTAGTATCTCATATAATTAATA  
51 ATGAAAGAATAAAAAAAGAAGTTTTTGGTAGAATAGGTATAATTTATGCA  
101 ATAATTTCTATTGGTTTATTAGGATTTATTGTTTGGAGCTCATCATATATT  
151 TACTGTAGGTATAGATGTTGATACTCGAGCTTATTTTACATCGGCAACCA  
201 TAATTATTGCTATTCCTACTGGGATTAATAATTTTATAGATGATTAGCATCA  
251 ATAAATGGTATAAAAAATTAATTTAATATTTATAATTTATGATTATTAGG  
301 TTTTATTTTTTTTATTTACTGTAGGTGGATTAACAGGAATTATTTTATCAA  
351 ATTCTTCTATTGATATTGTTTTACACGACACATATTATGTAGTAGCTCAT  
401 TTTCATTATGTATTATCTATAGGAGCTGTTTATGCTATTTTTGGCAGATT  
451 TATTTATTGATACCCATAAATACTGGTTTAAATATAAATAAATAAATGAT  
501 TAAAAATTCATTTTTATTTTTATATTTATTGGAGTTAATAACTTTTTTTT  
551 CCTCAGCATTTTTTAGGATTGAGTGGAATACCTCGACGTTATTCTGATTA  
601 TCCTGATTCATATACCTGTTGAAATATCATCTCATCTATGGGATCAGTTA  
651 TGTC AATATTTAGAACTTTATTATTTTTTTTTTTATTATTTGAGAGAGTTTT  
701 ATTAGCCAACGATTAGTAATTTTTTCTTTAAATAATAACAATTCATTGA  
751 ATGATTAATATCTA

## B. 12S rDNA

1 TTCAAAATTTCAAATTA AAAATTTTTACTATTAAATCCATTTTCATTAAA  
51 ATTTAAATATTTAAATTCATAAATTTAATTTATTGTAACCTAATTAATTCT  
101 AAAATATTAATTATATATTGATCTGAAATATAATTTTAAAATTATTA AAA  
151 AATAGTTTTTTATAAAAATTTTTTTATAACGAACATACATAAATATAAAAAT  
201 TTTAGTAGGTCTTTTTGTGGGTTATCAATTATTTAACAAGTTCCTCTAAT  
251 TAGATTA AAAATACTGCCATAATTTTTTAATTTTCATAATTTAC

## C. ITS2

1 ATTCAGCGGGTAGTCTCGCCTGCTCTGAGGTCGTCGCTATGTACACACGC  
51 ATTCGTGCGATAAACCTTCTTGTACGAACGAGCCTTTCAACTGAAATACG  
101 AACAGTCGATTTTCGTCAAGCGCAGAGGCACGCGCGCGCCCGCGTCGTAGC  
151 TCACGAGGAAGACGTCGGTCCGCTCGGCGCGCGCCGTGAGGCGCCCAAA  
201 GAGCTCCGGGACTCGACGTACGACGCACGAGAGAGACGCTTCGCTTTCGA  
251 AGTCACGCGTGCCGTACGACATTGACTTTGGTTCGTTTTCGCAACGTTTCG  
301 ACAAGTCATTGGGTGTTTATTAGAGCGACACCGAGTGCGCCGTTCGGATA  
351 CTCATTGCGAGGTTACGATGAACCGTCGCGAATGAAATCTCGAACGACGA  
401 CTCGACGAACGCTCAGGCGTACGCTCGCCAATGAGACGAGCAGTCTGGTT  
451 TTATCTAATGAACGACCCTCAGCCAGGCGTGGTCCGGGAATCGTATCCGA  
501 GGACCGCA

Fig. 2. Sequences of COI (A), 12S rDNA (B), and ITS2 (C) amplicons.

in Hawaii, severe infestations had caused the death of many tall erythrina and only few trees have survived (Heu *et al.*, 2006; Hessian *et al.*, 2008). The precious endemic williwilli, *E. sandwicensis*, of Hawaii is also threatened due to the attack of these wasps and its devastation is of great concern (Gramling, 2005). Although the characteristic of variegated flowers makes it unique among the many different *Erythrina* species, the vegetative characters of the species is similar to *E. variegata* which suggests that they might be closely related.

### **Perspectives**

The savage method of attack by *Q. erythrinae*, which eventually causes the death of its own host plants, is supposedly a maladapted characteristic of the pest. However, the insect seems to be well adapted in most areas it is currently distributed in, including Taiwan. Nevertheless, the current success of the *Erythrina* eulophid wasp may not be a guarantee for its future prosperity. As pointed out by Sakai *et al.* (2001), the characteristics required for initial colonization and the traits needed for establishment may be different in invasive organisms. The monomorphic genotype may be an accidentally well fitted type at present but may be detrimental to its long-term survival. Future investigations are necessary to monitor the populations and to measure the fitness of this pest in regard to its host adaptation.

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# 由粒線體與核基因探討各類寄主植物之刺桐釉小蜂的遺傳變異

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## 摘 要

刺桐釉小蜂於 2000 年首先被報導嚴重感染非洲東部模里西斯、留尼旺島以及亞洲新加坡的刺桐樹，並造成部份植株死亡，旋即迅速蔓延到全球熱帶與亞熱帶島嶼或陸塊邊緣地區。由於受害的寄主植物種類及病徵程度不同，這些感染的昆蟲是否為同一種，令人產生質疑。我們選擇台灣本島與其離島等 14 個地區的 36 個樣本，及該物種最早報導之發生地模里西斯與新加坡的 11 個樣本；應用粒線體的 12S 核糖體 RNA (12sRNA)，細胞色素氧化酵素第一單元 (COI) 以及核 DNA 的核糖體第二區間 (ITS2) 等區域之 DNA 片段，分析來自不同地區，不同寄主植物上的刺桐釉小蜂之遺傳變異與親緣關係。結果顯示所有不同地點與不同寄主植物上的昆蟲樣本在同片段的序列皆無差異，顯然全台各地及取樣之其他國家的刺桐屬植物上的害蟲均為同種之刺桐釉小蜂，這些受害嚴重地區的刺桐釉小蜂顯然來自同一個基因型快速擴散的結果。

**關鍵詞：**外來種昆蟲，刺桐釉小蜂，刺桐，細胞色素氧化酵素第一單元，12S 核糖體 RNA，核 DNA 的核糖體第二區間。

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