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Out of Africa: Origin of the *Erythrina* Gall Wasp *Quadrastichus erythrinae* (Hymenoptera: Chalcidoidea: Eulophidae)

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ABSTRACT

Quadrastichus erythrinae Kim induces galls on coral trees (*Erythrina* spp.), which became an aggressive invasive pest since year 2000. Its African origin was hypothesized but remains supported only by indirect and limited evidence. In this study, molecular phylogeny and DNA haplotype were used to determine the origin of the erythrina gall wasp *Q. erythrinae* based on *cytochrome oxidase I* (*COI*) and *internal transcribed spacer 2 (ITS2)* regions of taxa from Mauritius, Tanzania, Singapore, Taiwan, Thailand, India, Indonesia, Philippines, China, Japan, Guam, and Hawaii. A total of 90 *COI* and 94 *ITS2* sequences were generated from five *Quadrastichus* species, including *Q. erythrinae* and four African congeners. Three *COI* haplotypes and one *ITS2* haplotype were detected from 66 taxa of *Q. erythrinae*. Taxa from most localities were observed to have an identical *COI* haplotype (haplotype a); the outliers with other haplotype compositions were the taxa from Indonesia (haplotype a and c) and Tanzania (haplotype n). The moelecualr phylogeny suggested that the Tanzanian taxa of *Q. erythrinae* is more primitive than the other taxa. This finding supported the hypothesis that *Q. erythrinae*, which is an invasive pest in many countries and regions, has an African origin.

Key words: Africa, coral tree, gall forming eulophid, invasive pest, Pacific region

Introduction

Erythrina gall wasp *Quadrastichus erythrinae* Kim is a fierce invasive pest found on coral trees (*Erythrina* spp.) worldwide. The pest induces galls on the newly growth sections of hosts and, consequently, causes severe damage. A heavy infestation of *Q. erythrinae* causes the curling of leaves and shoots and, eventually, the death of a plant (Kim *et al*., 2004; Yang *et al*., 2004). Outbreaks of this pest species have occurred in Pacific and Asian countries since 2000, and it has already spread to southern USA and Latin America (Kim *et al*., 2004; Yang *et al*., 2004; Huang *et al*., 2005; Faizal *et al*., 2006; Gerlach and Madl, 2007; Uechi *et al*., 2007; Howard *et al*., 2008; Kanai *et al*., 2008; Wu *et al*., 2008; Yao and Yin, 2009; Jacob and Devasahayam, 2010; Lit *et al*., 2010; Das and Talukdar, 2011; Narayana and Dhanya, 2014;

Palacios-Torres *et al*., 2017; Medianero and Zachrisson, 2019). Control measures for *Q. erythrinae*, particularly those involving chemical control (Xu *et al*., 2008; Wang *et al*., 2011) and biological control (Kaufman *et al*., 2020), have been successfully applied to suppressed this pest population.

Invasive route and origin are essential information for understanding the history and pathway of a biological invasion; they also provide baseline knowledge for establishing a predictive model for other pests with similar ecological features. Several studies have attempted to identify the invasive route and origin of *Q. erythrinae*. As *Q. erythrinae* feeds on species of *Erythrina* with various susceptibilities; therefore, Messing *et al*. (2009) proposed the use of host species with less pest infestation to infer the origin of *Q. erythrinae*. The basis for this proposal is that long term insect-host interactions may result in the establishment of a balance as an evolutionary consequence; therefore, the host species becomes less affected by a pest species, and this applies particularly to the pest's native ranges. Thus, Central Africa was inferred to be the origin of *Q. erythrinae*. In addition, although several other *Erythrina*-associated *Quadrastichus* species were identified in South Africa (Prinsloo and Kelly, 2009), *Q. erythrinae* was the only species known to infesting *Erythrina* hosts in the outbreak regions. Because the origin center of a taxon often exhibits more diversity compared with other regions, the aforementioned finding may imply that Africa is the origin of *Erythrina*-associated *Quadrastichus* species, including *Q. erythrinae*.

Two studies have conducted preliminary investigations of the invasive route of *Q. erythrinae* using genetic information. Tung *et al*. (2008) examined three genes (*cytochrome oxidase I* [*COI*], *internal transcribed spacer 2* [*ITS2*], and *12s rDNA*) and taxa from Asia (Taiwan and Singapore) and Africa (Mauritius). Rubinoff *et al*. (2010) analyzed two genes (*COI* and *elongation factor-1 alpha* [*ef-1α*]) and taxa from the Asia-Pacific region (Hawaii, Guam, American Samoa, Japan, Singapore, Taiwan, and China). However, the *Q. erythrinae* examined in these two studies lack of genetic variation; this finding suggests that the *Q. erythrinae* is genetically homogenous but not aid in clarifying its origin.

Quadrastichus erythrinae is speculated to originally from Africa (Messing *et al*., 2009; Prinsloo and Kelly, 2009), but evidence supporting the "Out of Africa" hypothesis is lacking. In particular, molecular information on African taxa remains scarce. In the present study, *Q. erythrinae* samples from Africa and congener of the species were examined to clarify their divergence and the origin of *Q. erythrinae*.

Materials and Methods

Specimen collection

Of the 96 *Quadrastichus* specimens (Table 1) obtained for DNA polymerase chain reaction (PCR) tests, 67 were *Q. erythrinae* specimens from Thailand, India, Indonesia, Philippines, China (Shenzhen, Hong Kong, and Hainan), Japan, Hawaii, Guam, and Tanzania. Four additional *Quadrastichus* species with 29 samples were obtained from Kenya (*Quadrastichus* sp. 1) and South Africa (*Quadrastichus* sp. 2-4). In addition, the other 30 *Q. erythrinae* sequences from Taiwan, Singapore, and Mauritius were obtained from a previous study conducted by Tung *et al*. (2008).

DNA extraction, polymerase chain reaction, and sequencing

Genomic DNA was obtained from a single wasp using a Purgene DNA Isolation kit (Gentra Systems, MN, USA) per the extraction protocol of the manufacturer. Precipitated DNA was resuspended in 30 μLof dH_20 and used to amplify the partial mitochondrial *COI* and nuclear region *ITS2*; this was achieved by conducting PCR. Each PCR was performed with a final volume of 25 μL with 0.8 μL of 10 μM dNTP, 1.5 μL of 25 mM $MgCl_2$, 0.5 μL of each 10 μM of primer, 2.5 μL of 10× *Taq* buffer, and 0.1 μL of Amersham *Taq* (Amersham Biosciences, Buckinghamshire, UK). Touchdown PCR conditions were as follows: an initial denaturation was performed at 95°C (2 min), followed by 10 cycles consisting of denaturation at 94°C (30 s), annealing at 60°C (30 s) with a 0.5°C temperature reduction for each succeeding cycle, and extension at 72°C (1 min); thereafter, 30 cycles of denaturation at 94°C (30 s),

Table 1. Specimen information of *Quadrastichus* species examined in the present study

Year	Location	Longitude	Latitude	Hostplants (sampled number)	Haplotype (Number of sequences)	
					ITS2	*COI*
Quadrastichus erythrinae						
2005	Shenzhen, China.	22.55208	114.086727	*E. variegata*(2) *E. variegata var. orientalis*(4)	A(6)	a(6)
2005	Lantau Is., Hong Kong.	22.337072	114.138925	*E. variegata*(2) *E. variegata var. orientalis*(3)	A(5)	a(5)
2005	Hainan Is., China.	18.28569	109.461853	*E. variegata*(5)	A(5)	a(5)
2007	Bangkok, Thailand.	13.723523	100.535395	*E. variegata var. orientalis*(2)	A(1)	a(2)
2005	Kerala, India.	10.422412	76.128595	*E. variegata*(4) *E. stricta*(1)	A(5)	a(4)
2008	Sumbawa Is., Indonesia.	−8.436402	117.303992	*E. variegata*(5)	A(5)	a(2), c(3)
2007	Manila, Philippines.	14.97294167	120.89815	*E. variegata*(1)	A(1)	a(1)
2007	Talavera, Philippines.	15.29933333	120.9242472	*E. variegata*(4)	A(4)	a(4)
2007	Mabuni, Okinawa.	26.09111111	127.7249444	*E. variegata*(1) *E. crista-galli*(1)	A(2)	a(2)
2007	Ie Is., Okinawa.	26.71338889	127.8070278	*E. variegata*(2)	A(2)	a(2)
2007	Shuri, Okinawa.	26.203348	127.672098	*E. variegata*(1)	A(1)	a(1)
2007	Onnoyama park, Okinawa.	26.203348	127.672098	*E. variegata*(1)	A(1)	a(1)
2007	Nago, Okinawa.	26.59252778	127.9715833	*E. variegata*(1)	A(1)	a(1)
2005-2008	Big Is., Hawaii.	20.073811	−155.845985	*E. variegata*(5) *E. sandwicensis*(4)	A(9)	a(9)
2005	Oahu Is., Hawaii.	21.390643	−158.150537	*E. variegata*(11)	A(11)	a(11)
2006	Mangilao, Guam.	13.467192	144.746068	*E. variegata*(6)	A(6)	a(6)
2007	Morogoro, Tanzania.	−5.181046	38.408375	*E. abyssinica*(1)	A(1)	n(1)
***Quadrastichus* sp. 1**						
2006	Lolgorien Kenya.	−1.274359	36.813106	*E. abyssinica*(2)	C(1), E(1)	h(2)
***Quadrastichus* sp. 2**						
2007	Bummer, S. Africa.	−26.066652	28.157959	*E. zeyheri*(2)	D(2)	k(1)
***Quadrastichus* sp. 3**						
2008	Univ. Kwa-Zulu Natal, S. Africa.	−29.6132	30.3926	*E. latissima*(5)	F(5)	q(1), m(1)
***Quadrastichus* sp. 4**						
2008	Durban, S. Africa.	−29.837647	31.027837	*E. abyssinica*(2)	B(1)	b(2)
2008	I.C.C. Durban, S. Africa.	−29.852648	31.028824	*E. latissima*(5) *E. princeps*(5)	B(10)	b(9)
2008	Ushaka marine world, Durban, S. Africa.	−29.865638	31.044638	*E. latissima*(4) *E. caffra*(4)	B(8)	d(1), e(2), f(1), g(2), o(1), p(1)

annealing at 50°C (30 s), and extension at 72°C (1.5 min) were performed, followed by a final extension at 72°C (7 min). Varying annealing temperatures and primer pairs were used to improve PCR quality as per the protocol used by Tung *et al*. (2008). The primers used in this study are listed in Table 2. The PCR products were run on 1.0% agarose gels in 1× Tris-borate-EDTA

(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, Taipei, Taiwan) on an ABI3730 DNA Analyzer (Applied Biosystems, Taipei, Taiwan). Two strands were sequenced, and the sequences were checked and assembled into contiguous arrays using Sequencher 4.7 (GeneCode, Boston, USA).

Genetic analysis and phylogeny

The population variation was described using the following general statistics: the number of unique haplotypes, variable nucleotide number, measured genetic diversity that included nucleotide diversity (π), average number of nucleotide differences per site between two randomly chosen sequences, and haplotype diversity (h) of two randomly chosen sequences from a sample that differed from other samples. These statistical analyses were performed using DNASP 6.0 (Rozas *et al*., 2017). In addition, haplotype reconstruction with PHASE algorithms was performed for heterozygous sequences (Stephens *et al*., 2001; Stephens and Donnelly, 2003).

To determine the relationship among the *Quadrastichus* taxa, both mitochondrial *COI* and nuclear *ITS2* sequences were used to infer the phylogenies by performing Bayesian inference with MrBayes 3.2 (Ronquist *et al*., 2012). TrN+G (Tamura-Nei + gamma distribution) and K80 models were determined using jModelTest 2.1.10 (Darriba *et al*., 2012) for *COI* and *ITS2* regions. *Quadrastichus mendeli* Kim & La Salle were selected as outgroup species according to Nugnes et al. (2016), and DNA sequences (*COI*: KU133520, ITS2: KU133506) were downloaded from the National Center for Biotechnology Information. We ran four chains (three heated and one cold); and each chain initially began from a random tree and ran for 10^6 generations. The log-likelihood scores were sampled every 1000 generations and plotted against generation time to determine when they became stationary. The first 25% of the steps were discarded as burn-in. The topology and supported values (posterior probability) were checked using FigTree v1.4.2. (Rambaut, 2014).

To understand the diversification processes of five *Quadrastichus* species, the haplotype networks of *COI* and *ITS2* regions were analyzed using TCS v1.21 with 1,000 steps in fix connection limitation and the indel was treated as missing data (Clement *et al*., 2000).

Results and Discussion

Haplotypes of *Quadrastichus* species

Ninety sequences of *COI* regions (764 bp) and 94 sequences of *ITS2* (508 bp) were successfully generated from 96 *Quadrastichus* specimens. Among five *Quadrastichus* species, 14 *COI* and six *ITS2* haplotypes were detected (Fig.1c, 1d; Table 1). Three *COI* haplotypes (type a, c, and n) were detected among 66 *Q. erythrinae* taxa. Haplotype a was detected for all sampling localities; the exceptions were Tanzania and Indonesia, whose taxa only presented haplotype n and haplotypes a and c, respectively. In addition, the 30 *COI* sequences of *Q. erythrinae* from Taiwan, Mauritius, and Singapore, which were obtained from a study by Tung *et al*. (2008), were identical to haplotype a. Among the four outgroups (*Quadrastichus* sp. 1-4), many haplotype(s) were detected (Table 1). For *ITS2,* one *ITS2* haplotype (haplotype A) was detected among 66 *Q. erythrinae* taxa. Akin to *COI*, multiple numbers of haplotypes were detected among the other four *Quadrastichus* species (Table 1). In addition, the 30 *ITS2* sequences of *Q. erythrinae* from Taiwan, Mauritius, and Singapore, which were obtained from a study by Tung *et al*. (2008), were identical to haplotype A. *Quadrastichus erythrinae* generally exhibited low genetic diversity (*COI*: h: 0-0.006, π: 0-0.00079; *ITS2*: h:0, π:0; Table 3) and monotype (*COI* haplotype a and *ITS2* haplotype A) in the invasive regions. Genetic variation is a key basis for inferring the dispersal pathway of a target organism such as a global invasive pest. A geographical population with a high level of genetic diversity is generally considered a source population, whereas those with relatively low genetic diversity tend to be introduced populations (Zepeda-Paulo *et al*., 2010; Ascunce *et al*., 2011; Perdereau *et al*., 2013; Ryan *et al*., 2019). Monomorphic genotypes of *Q. erythrinae*

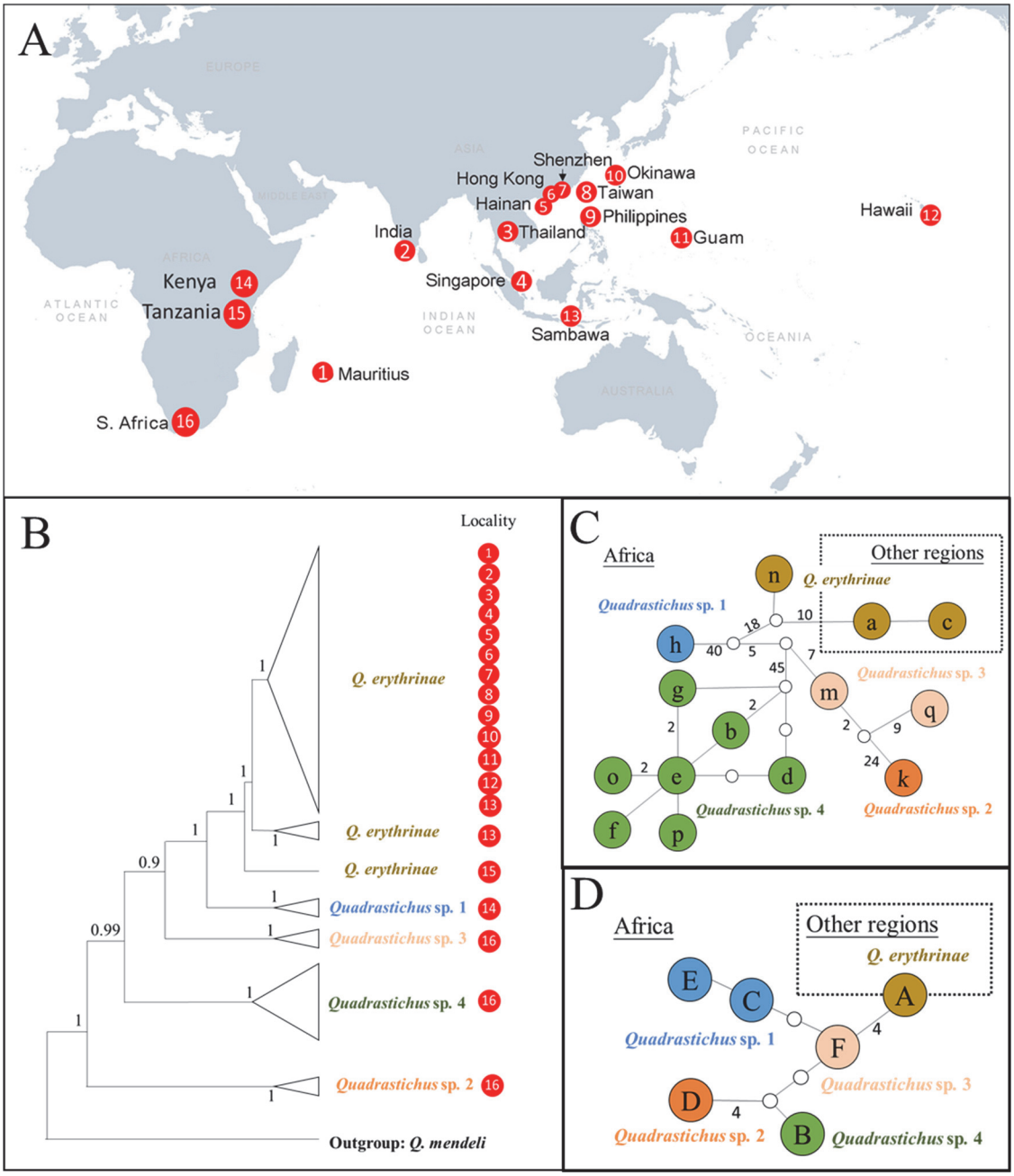

Fig. 1. Sampling localities, phylogeny, and haplotype networks: (A) Map showing each material-collection locality as a red numbered circle; (B) Cladogram of *Quadrastichus* species based on *COI* and *ITS2* regions; (C) *COI*-based haplotype network; (D) *ITS2*-based haplotype network (D) Locality of each taxon (i.e., red numbered circle) in (B) is indicated by its number, which corresponds to the number found in (A). Colors and letters in both (C) and (D) represent distinct species and haplotypes, respectively. Supported values in (C) shown beside nodes are Posterior Probabilities. The number beside a connected line in (D) indicates distance between haplotypes; an unnumbered lines indicates a distance of 1. "Other regions" indicates areas outside Africa.

have been reported in the Asia-Pacific region (Tung *et al*., 2008; Rubinoff *et al*., 2010). In the present study, we included more taxa from Africa and the Asia-Pacific region to establish a sampling regime with a broader geographical scale relative to the two aforementioned studies. The present study is the first to detect heterogenetic *COI* haplotypes (a, c, n) of *Q. erythrinae* from Tanzania and Indonesia (Table 1); by contrast, a monomorphic genotype (haplotype a) was detected among the taxa from other localities.

"Out of Africa" hypothesis based on phylogeny

The present study clarifies phylogenetic

Table 2. *COI* and *ITS2* primers used in the present study

Name	Sequence (5'→3')
CO1-2183F[a]	CAACATTTATTTTGATTTTT
Gallcox1-F1	GATTTTTTGGTCACCCAGAAGT
Gallcox1-F2	GAACWGGDTGAACHGTHTAYCC
Gallcox1-F3	GGAGATCCAATTTTATATCA
Gallcox1-F4	CAACATTTATTTTGATTTTTTGG
Gallcox1-F5	GGTACTGGTACTGGATGAACAG
Gallcox1-R1	ATGAATGATAAGCTGGAGGAT
Gallcox1-R2	CCATTTATTGAAGCAAGCCATCT
Gallcox1-R3	TGACATAAYTGATCCYATAGATGA
Gallcox1-R4	GGAATTTCATATAATGAATGA
Gallcox1-R5	GAAAAAATTACTAATCGTTGGCT
ITS2-28sBld[b]	TTCTTTTCCTCCSCTTAYTRATATGCTTAA
ITS2-5p8sFc[b]	TGAACATCGACATTTYGAACGCACAT

[a] Primer listed in Simon *et al*. (1994); [b] Primers listed in Campbell *et al*. (1993).

relationship among the five *Quadrastichus* species (Fig. 1b). *Quadrastichus* sp. 2 is the basal clade followed by *Quadrastichus* sp. 4 and sp. 3. *Quadrastichus* sp. 1 and *Q. erythrinae* are sister groups and situated at termi nal. At the intraspecific level of *Q. erythrinae*, the taxon from Tanzania is the sister group to the taxa from other non-African areas. Messing *et al*. (2009) indicated that *Q. erythrinae* may have originated from Central Africa rather than South Africa because the infestation level of the species is lower in the native hosts from Central Africa. This serves as indirect evidence for inferring the source of *Q. erythrinae* from the macroevolutionary perspective. However, pest infestation levels can be influenced by the ecological performance or physiological responses of plants in varying situations, and these relationships can be complex. Microevolutionary factors are also involved, including interactions among the insect pest, host plants, natural enemies and the environment (Kolesik, 2000; Vidart *et al*., 2013; Veenstra *et al*., 2014; Kaufman *et al*., 2020). Despite the complexity of the topic, the finding that the Tanzanian taxon is situated at the basal position of the *Q. erythrinae* lineage in the topology (Fig. 1b) suggests that *Q. erythrinae* may have originated from Tanzania or its neighboring regions; in other words, the finding supports the "Out of Africa" hypothesis. However, more *Q. erythrinae* samples from multiple African regions (particularly South and Central Africa) are required to pinpoint the origin of *Q. erythrinae* to a finer degree than what the present study did.

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Table 3. Genetic diversity of *COI/ITS2* genes of *Quadrastichus* species

Population	N	Number of variations	Haplotype number	Haplotype diversity (h)	Nucleotide diversity (π)
Q. erythrinae					
Singapore.	7/7	0/0	1/1	0/0	0/0
Mauritius.	4/4	0/0	1/1	0/0	0/0
Taiwan.	19/19	0/0	1/1	0/0	0/0
Shenzhen, China.	6/6	0/0	1/1	0/0	0/0
Hong Kong, China	5/5/	0/0	1/1	0/0	0/0
Hainan Is., China.	5/5/	0/0	1/1	0/0	0/0
Bangkok, Thailand.	2/1	0/0	1/1	-/-	-/-
Kerala, India.	4/5	0/0	1/1	0/0	0/0
Sumbawa Is., Indonesia.	5/5	1/0	2/1	0.600/0	0.00079/0
Manila, Philippines.	1/1	-/-	1/1	-/-	-/-
Talavera, Philippines.	4/4	0/0	1/1	0/0	0/0
Mabuni, Okinawa.	2/2	0/0	1/1	0/0	0/0
Ie Is., Okinawa.	2/2	0/0	1/1	0/0	0/0
Shuri, Okinawa.	1/1	-/-	1/1	-/-	-/-
Onnoyama park, Okinawa.	1/1	-/-	1/1	-/-	-/-
Nago, Okinawa.	1/1	-/-	1/1	-/-	-/-
Big Is., Hawaii.	9/9	0/0	1/1	0/0	0/0
Oahu Is., Hawaii.	11/11	0/0	1/1	0/0	0/0
Mangilao, Guam.	6/6	0/0	1/1	0/0	0/0
Morogoro, Tanzania.	1/1	-/-	1/1	-/-	-/-
***Quadrastichus* sp. 1**					
Masi Mara, Kenya.	2/2	0/1	1/2	-/-	-/-
***Quadrastichus* sp. 2**					
Bummer, S. Africa.	1/2	-/0	1/1	-/-	-/-
***Quadrastichus* sp. 3**					
Univ. Kwa-Zulu Natal, S. Africa.	2*/5	24/0	3/1	-/0	-/0
***Quadrastichus* sp. 4**					
Durban, S. Africa.	2/1	0/-	1/1	-/-	-/-
I.C.C. Durban, S. Africa.	9/10	0/0	1/1	0/0	0/0
Ushaka marine world, Durban, S. Africa.	8/8	9/0	6/1	0.929/0	0.00341/0

"-" indicates that no data are provided or that number of analyzed sequence(s) is less than 3; "*" indicate heterozygous sequence.

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遠離非洲：刺桐釉小蜂（膜翅目：小蜂總科：釉小蜂科）的起源

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摘　要

刺桐釉小蜂 *Quadrastichus erythrinae* Kim 為 2000 年間開始入侵多國並嚴重危害刺桐 (*Erythrina* spp.) 的造癭昆蟲，咸認起源於非洲，但迄今多為間接推測或片段證據。本研究以模里西斯、坦尚尼亞、新加坡、台灣、泰國、印度、印尼、菲律賓、中國、日本、關島及夏威夷之刺桐釉小蜂與其他四種來自肯亞和南非的同屬釉小蜂 (*Quadrastichus* spp.) 標本，分析粒線體細胞色素 c 氧化酶亞基 I 基因 (cytochrome c oxidase I, *COI*) 及核醣體 DNA 第二區間 (*ITS2*) 片段，配合分子親緣關係與 DNA 基因型來確認刺桐釉小蜂之起源。由刺桐釉小蜂及四種非洲近緣種共計取得 90 條 *COI* 及 94 條 *ITS2* 序列。於刺桐釉小蜂兩基因各 66 條序列中，發現三種 *COI* 基因型及一種 *ITS2* 基因型，多數樣點個體之序列 *COI* 基因型為 haplotype a，僅印尼 (基因型 a 及 c) 與坦尚尼亞 (基因型 n) 組成不同。以分子特徵建構的親緣關係指出，坦尚尼亞的刺桐釉小蜂較其他地區為古老，顯示入侵的源頭為非洲。

關鍵詞：非洲、刺桐、造癭釉小蜂、入侵物種、太平洋地區