Two new Aprostocetus species (Hymenoptera: Eulophidae: Tetrastichinae), fortuitous parasitoids of invasive eulophid gall inducers (Tetrastichinae) on Eucalyptus and Erythrina

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Abstract

Two closely related new species of Aprostocetus Westwood (Hymenoptera: Eulophidae: Tetrastichinae) are described as fortuitous parasitoids of invasive gall inducers in two other genera of Tetrastichinae, Leptocybe Fisher & LaSalle and Quadrastichus Girault. Aprostocetus causalis La Salle & Wu is a parasitoid of Leptocybe invasa Fisher & La Salle on Eucalyptus spp. (Myrtaceae) in China and Thailand, and A. felix La Salle, Yang & Lin is a parasitoid of Quadrastichus erythrinae Kim on Erythrina spp. (Fabaceae) in Taiwan. Epitetrastichus nigriventris Girault, 1913 is removed from synonymy from Aprostocetus gala (Walker), and treated as the valid species A. nigriventris (Girault).

Key words: Leptocybe invasa, Quadrastichus erythrinae, Myrtaceae, Fabaceae

Introduction

Fortuitous biological control has been defined as “cases where biological control has occurred as a result of the accidental immigration and establishment (ecesis) of an exotic natural enemy or conversely ecesis of an exotic pest which is then attacked and controlled by indigenous natural enemies” (DeBach 1974: 64). DeBach (1974) pointed out that examples of fortuitous biological control often happen accidentally and go unheralded. Indeed, although this may be an extremely important phenomenon in biological pest control (DeBach 1974; La Salle 1993), there are relatively few documented cases in the literature.

DeBach (1974) pointed to several examples of fortuitous biological control of armoured scales (Hemiptera: Diaspididae) that could be attributed to parasitoids in Aphytis Howard (Hymenoptera: Aphelinidae). Bennett & Noyes (1989) reported the fortuitous biological control of spiraling whitefly, Aleurodicus dispersus Russell (Hemiptera: Aleyrodidae), in Florida by Aleurotonus vittatus (Dozier) (Hymenoptera: Eulophidae, as...

The two species of *Aprostocetus* Westwood (Hymenoptera: Eulophidae: Tetastrichinae) described in this paper represent additional examples of an indigenous parasitoid expanding its host range onto invasive pest species. Whether they will ever build up to population sizes capable of providing effective biological control for these species remains to be seen.

This paper arose out of two separate research initiatives, both involving teams of workers. One team was studying the systematics and biology of the parasitoid complex of *Leptocybe invasa* Fisher & La Salle (Eulophidae: Tetrastichinae) on *Eucalyptus* (Myrtaceae) in China and Thailand, the other was studying the systematics and biology of the parasitoid complex of *Quadrastichus erythrinae* Kim (Eulophidae: Tetrastichinae) on *Erythrina* (Fabaceae) in Taiwan. Due to the similarity of the *Aprostocetus* species involved, both in terms of relationships and biology, it seemed appropriate to combine these projects into a single paper.

## The invasive gall inducers

*Leptocybe invasa* is an Australian native that has subsequently spread to *Eucalyptus* growing areas around the world (Mendel *et al.* 2004; CABI 2007; Neser *et al.* 2007; Costa *et al.* 2008; Gaskill *et al.* 2009; Tung & La Salle 2010). Several parasitoids have been discovered in Australia, and subsequently released as biological control agents in various regions (Kim *et al.* 2008; Kelly *et al.* 2012). Indigenous species of *Megastigmus* Dalman (Hymenoptera: Torymidae) have already been recorded in Israel, Turkey, Thailand and Brazil as fortuitous parasitoids of this species (Protasov *et al.* 2008; Doğanlar & Hassan 2010; Sangtongpraw & Charernsom 2013; Doğanlar *et al.* 2013).

*Quadrastichus erythrinae* is another highly invasive species that is originally from eastern Africa. This pest has spread extremely rapidly and is now recorded in Mauritius, La Réunion, Singapore, Hawaii, Taiwan, Hong Kong, China, India, Thailand, American Samoa, Guam, Japan, Okinawa, and Florida (USA) (Kim *et al.* 2004; Yang *et al.* 2004; Heu *et al.* 2006; Gates & Delvare 2008; La Salle *et al.* 2009). It induces galls on shoots, twigs, leaves and petioles of several species of *Erythrina*, and can cause extensive damage, defoliation, or even death of trees. Several African parasitoids of this species have been identified (Gates & Delvare 2008; Prinsloo & Kelly 2009; La Salle *et al.* 2009), with some of them being used in biological control programs.

## Material and methods

Terminology follows Gibson (1997) and Graham (1987). OOL, ocellar-ocular distance; POL, post-ocellar distance; CC, costal cell; SMV, submarginal vein; MV, marginal vein; STV, stigmal vein; PMV, postmarginal vein; F1–3, funicular segments 1–3.

Acronyms used are as follows. ANIC, Australian National Insect Collection, CSIRO Ecosystem Sciences, Canberra, Australia; BMNH, The Natural History Museum, London, UK; IZCAS, Institute of Zoology, Chinese Academy of Sciences, Beijing, China; NCHU, Department of Entomology, National Chung Hsing University, Taichung, Taiwan; NMNS, National Museum of Natural Science, Taichung, Taiwan; KUBT, Insect Museum, Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand; USNM, United Stated National Museum of Natural History, Washington, D.C., USA

Habitus, wing, antenna and SEM images were taken by Nicole Fisher. Habitus images (Figs 1, 2, 7, 8) were
taken using a Canon 5DmkII; wing and antenna images (Figs 3, 4, 9, 10) were captured using a Leica M205 C microscope. SEM images (Figs 5, 6, 11, 12) were photographed using a Zeiss Evo Series Scanning Electron Microscope using Carl Zeiss SmartSEM software. Figures 13–16 were supplied by Dr Michael Gates, Systematic Entomology Laboratory, ARS, USDA.

**Sampling for Molecular Studies.** Seven samples were from Chiayi and Kaohsiung, Taiwan, 12 from Thailand, and 4 from China. The latter two groups of samples were critical point dried material.

**DNA extraction and primer design.** Genomic DNA was extracted by using QuickExtract DNA extraction kit (Epicentre Biotechnologies, Madison, WI). The whole body was dipped in 50 l of QuickExtract Solution and then vortex mix for 15 sec. The sample solution was incubated at 65˚ for 10 min and then transferred to 98˚ for 2 min. For critical point dried samples, we incubated at 37˚ for 24 hours and then transferred to 98˚ for 2 min.

Primers for amplification in COI, Aprost_COI_F (5’-TCCTCGAATAAATAATATAAGATT-3’) and Aprost_COI_R (5’-CAATAATTATTGTGGCTGAAGTA-3’), were designed based on COI sequences of other Aprostocetus species from NCBI Genbank with accession number of HM573640 and HM573860. A second pair of primers was used for critical point dried samples in second round PCR (nested PCR), Aprost_COI_F-2 (5’-GATATAGCWTTCCTCGAATAAATATAAGATT-3’) and Aprost_COI_R2 (5’-ACAGCAATAATTATTGTGGCTG-3’), were designed from the sequences gained from the first round PCR.

Primers for amplification in ITS2, Aprost_ITS2_F (5’-TCCTCGAATAAATAATATAAGATT-3’) and Aprost_ITS2_R (5’-TCTCGCCTGCTCTGAGGTC-3’), were designed based on ITS2 sequences of Aprostocetus aethiops (Zetterstedt) from NCBI with accession number HM573654.

**Amplification and sequence alignment.** PCR reaction for COI was performed in 25 l volume involving 1 l of template DNA, 2.5 l of 10X Taq buffer, 0.5 l of Prime Taq DNA polymerase, 0.2 l of 25 mM dNTP, and 0.5 l of 10 mM of each primer. PCR programming conditions were 94˚ for 2 min as first denaturation, followed by 35 cycles of 94˚ for 40 sec, 51˚ for 40 sec and 72˚ for 40 sec, with a final extension at 72˚ for 10 min. For critical point dried samples, nested PCR processes were additionally used after the first round PCR process. Nested PCR reaction was performed in 25 l volume involving 1 l of first round PCR product, 2.5 l of 10X Taq buffer, 0.5 l of Prime Taq DNA polymerase, 0.2 l of 25 mM dNTP, and 0.5 l of 10 mM of each primer. PCR programming conditions were 94˚ for 2 min as first denaturation, followed by 35 cycles of 94˚ for 40 sec, 53˚ for 40 sec and 72˚ for 40 sec, with a final extension at 72˚ for 10 min. For ITS2 amplification, PCR programming conditions were 94˚ for 2 min as first denaturation, followed by 35 cycles of 94˚ for 30 sec, 59˚ for 40 sec and 72˚ for 30 sec, with a final extension at 72˚ for 10 min. The PCR product was visualized on 1% agarose gel and purified from the gel using QIA quick Gel Extraction Kit (Qiagen, USA). The acquired DNA sequences were aligned using Clustal W method in Bioedit 7.0 (Hall 2004).

**Phylogenetic analyses.** For NJ and ML analyses, the HKY+I+G model (lnL=-1656.8093) was selected as the best model for COI sequences; and for ITS2 sequences, the TPM3 model (lnL=-1176.8631) was selected.

*Aprostocetus Westwood*

*Aprostocetus* is the largest of the tetrastichine genera and it is known from all geographical realms. Species attack a wide variety of hosts, but hosts are often insects inhabiting a variety of plant galls, including Diptera, Hymenoptera, Coleoptera, Coccoidea and even eriophyid mites and nematodes (Graham 1987; La Salle 1994).

Generic keys that distinguish *Aprostocetus* from other tetrastichine genera are available for Australasia (Bouček 1988), North America (LaSalle 1994; Schauff et al. 1997), Europe (Graham 1987, 1991) and India (Narendran 2007). Diagnostic characters include: propodeal spiracle partially covered by a raised lobe or flap on the callus, one of the cercal setae distinctly longer than remaining setae and sinuate or curved, submarginal vein generally with 3 or more dorsal setae, malar sulcus straight or only slightly curved. Keys to species of *Aprostocetus* are known only for Europe (Graham 1987) and India (Narendran 2007).

The “*causalis* group” of *Aprostocetus*

*Aprostocetus causalis* LaSalle & Wu and *A. felix* La Salle, Yang & Lin belong to a small group of Old World
species that are associated with galls. Females are generally yellow to yellow-orange in color, and males have the gaster brown but with a distinctive white patch anteriorly on both the dorsal and ventral surfaces. This group of species is quite difficult to define because, although it does appear to be a distinct group, the only easily recognizable character to support it is the distinctive coloration of the male gaster, which is not found in any other *Aprostocetus* species. Unfortunately, the females are not as easy to recognize, and attempting to key them in Graham’s (1987) key to European *Aprostocetus* would require going 100+ couplets into the key using what are at times very challenging characters even for experts. Although members of this group have a similar biology, association with galls is not at all unusual in *Aprostocetus* and would not serve as a definitive character.

Other Asian species associated with galls, which appear to belong in the *causalis* group, have been confused with, or misidentified as, *Aprostocetus gala* (Walker). This species, originally described as *Tetrastichus gala*, is a New World parasitoid of citrus weevils (Schauff 1987) and thus biologically distinct from the *causalis* group. *Aprostocetus gala* females can vary in coloration (Figs 13–15), and may appear similar in color to members of the *causalis* group. However, there are several species of *Aprostocetus* in which the females are yellow to yellow-orange in color, and *A. gala* is morphologically distinct from the *causalis* group in the males (Fig. 16), which lack the distinct white patch anteriorly on the gaster.

The Old World records in the literature for *A. gala* are misidentifications and need to be corrected. It is likely that these records refer to two additional species in the *causalis* group listed below.

1. **Aprostocetus nigriventris** (Girault)

   Bouček (1988) recorded *A. gala* from Australia, placed *Epitetrastichus nigriventris* Girault 1913 in synonymy with it, and placed *Tetrastichus xanther* Girault 1913 and *Epitetrastichus xanther hilli* Girault 1915 in *Aprostocetus*, where he suggested they might also be synonyms of *A. gala*. However, Australian specimens determined as *A. gala* by Bouček (in ANIC) and examined by JL differ from *A. gala*, and Australian records of *A. gala* should be referred to *Aprostocetus nigriventris* (Girault), which is here removed from synonymy under *A. gala* and treated as a valid species. Further study will be required to determine if *Aprostocetus xanther* (Girault 1913) and *Aprostocetus xanther hilli* (Girault 1915) should also be synonymized with *A. nigriventris*.

2. An Indian species known to attack gall-inducing Cecidomyiidae. *Aprostocetus gala* has also been recorded from India as a parasitoid of gall forming Cecidomyiidae (Diptera): the sorghum midge, *Stenodiplosis sorghicola* (Coquillett) (Kausalya *et al.* 1997; Nwanze *et al.* 1998), blossom midge (*Contarinia* sp.) (David *et al.* 1990), and the mango gall midge, *Erosomyia mangiferae* Felt (Fasih & Srivastava 1990). Examination of specimens in the Natural History Museum, London indicates that these specimens are distinct from *A. gala*, and appear to represent a distinct species in the *causalis* group.

**Aprostocetus causalis** La Salle & Wu, sp. nov.

(Figs 1–6)

**Diagnosis.** *Aprostocetus causalis* belongs to the *causalis* group based on the characters given above, and particularly the distinctive coloration of the male gaster. It can be separated from the other species discussed above by the following characters: forewing with speculum completely closed behind, with the cubital line of setae extending to meet the basal line of setae (Fig. 4); propodeum with a curved parapsyracular carina (Fig. 6); antenna with F1 less than 1.5× longer than wide (Fig. 3). Male with dorsum of mesosoma predominantly dark brown (Fig. 2).

**Female** (Figs 1, 3–6). Length 0.8–1.4 mm. Head generally yellow or yellow-orange, occiput brown. Mesosoma yellow or yellow-orange, anterior face of pronotum brown, except some sutures sometimes brown, particularly the notaulus. Legs and coxae yellow to yellow-orange. Gaster yellow to yellow-orange ventrally, darkened dorsally; darkened areas ranging from a brown transverse stripe posteriorly on all tergites to entire dorsal surface brown. Ovipositor sheaths brown.
Head (Figs 5). Ocellar triangle surrounded by faint grooves. POL 1.2–1.3× as long as OOL. Frontal suture small, v-shaped. Scrobal area without distinct median carina. Torulus placed above level with ventral margin of eye. A broad depression (supraclypeal area) below torulus extending to clypeus. Malar sulcus nearly straight, only slightly curved. Clypeal margin bidentate.
Antenna (Fig. 3) with 3 funicular segments and 3 very small anelli. First and second funicular segments slightly longer than wide, third subquadrate: length/width ratio of F1 1.1–1.3; F2 1.1–1.35; F3 0.9–1.05. Clava 1.7–2.2× longer than wide. C3 short and its end broad, not tapering apically, although with small terminal spine. Scape slightly flattened.

Mesosoma (Fig. 6). Pronotum very short medially in dorsal view. Mid lobe of mesoscutum with very weak median line and a single row of 4–6 adnotaular setae on each side. Mesosternum flat just in front of the trochantal lobes and with precoxal suture. Scutellum with anterior pair of setae located behind middle. Scutellum overhanging dorsellum. Propodeum short medially, subequal in length to dorsellum; with weak median carina and distinct, curved paraspiracular carina. Propodeum with raised lobe of callus partially overhanging spiracle. Callus with 2 setae.

Forewing (Fig. 4) hyaline. Submarginal vein usually with 3 or 4 dorsal setae. Costal cell with line of ventral setae in apical half. Relative length of wing veins to stigmal vein as follows: CC: MV: STV = 2.9–3.1: 3.2–3.8: 1. PMV very short, less than one quarter length of stigmal vein. Speculum small and closed behind by cubital line of setae extending to basal setal line. Wing disk beyond speculum densely pilose.

Gaster distinctly longer (1.3–1.5×) than mesosoma. Hypopygium reaching about half length of gaster. Cercus with 1 setae longer than others and sinuate. Ovipositor sheath slightly protruding, very short in dorsal view.

**Male** (Fig. 2). Length 0.75–1.2 mm. Head brown, face yellow. Thorax brown except sometimes with some slight orange markings; dorsellum yellow. Legs and coxae yellow except sometimes with brown markings. Gaster dark brown, with distinct white patch anteriorly on both dorsal and ventral surfaces, these connected laterally. Antenna with 3 small anelli and 4 funicular segments; F1 quadrate to slightly longer than wide; F2–F4 all distinctly longer than wide; club elongate, 5–6× longer than wide. Each funicular segment and basal club segment with compact subbasal whorls of long setae extending at least to apex of following segment. Ventral plaque small, less than one fifth length of scape, situated near apex of scape.

**Type material.** Holotype ♀, China, Guanxi, Fangcheng, 14.xi.2008, L. Dewei, Wu Yaojun, C. Mingshan, ex galls on *Eucalyptus* spp. [IZCAS]. 57♀, 41♂ Paratypes. 53♀, 34♂, Same data as holotype [18♀, 10♂ IZCAS; 15♀, 10♂ ANIC; 4♀, 2♂ NCHU; 4♀, 2♂ NMNS; 4♀, 2♂ KUBT; 4♀, 4♂ USNM; 4♀, 4♂ BMNH]; 4♀, 7♂, Thailand, Kanchanaburi Prov., Phanomthuan, xi.2008 [3♀, 5♂ KUBT; 1♀, 2♂ ANIC].

**Distribution.** China, Thailand

**Etymology.** The specific name *causalis* is Latin for fortuitous

**Biology.** *Aprostocetus causalis* is a solitary endoparasitoid. In Thailand, the mean longevity of female and male adults fed with honey solution is 18.67 ± 1.93 and 13.33 ± 1.75 days, respectively. The female oviposits in mature larva and pupa of *L. invasa*. Mean developmental time from egg to adult stage is 12.92 ± 0.92 days (Sangtongpraow and Charernsom 2012).

*Aprostocetus felix* La Salle, Yang & Lin, sp. nov. (Figs 7–12)

**Diagnosis.** *Aprostocetus felix* belongs to the *causalis* group of species based on the characters given above, and particularly the distinctive coloration of the male gaster. It can be separated from the other species discussed above by the following characters: forewing with speculum partially closed behind, with the cubital line of setae not quite extending to meet the basal line of setae (Fig. 10); propodeum longer than dorsellum, without a curved paraspiracular carina (Fig. 12); F1 more than 1.5× longer than wide (Fig. 9). Male usually with dorsum of mesosoma predominantly yellow to orange yellow, although sometimes mostly dark brown (Fig. 8).

**Female** (Figs 7, 9–12). Length 1.0–1.6 mm. Head generally yellow or yellow-orange, occiput brown. Mesosoma yellow or yellow-orange, anterior face of pronotum brown, except some sutures sometimes brown, particularly the notaulus; small brown areas sometimes also present, such as mesepimeron dorsally, and a transverse stripe on propodeum posteriorly. Legs and coxae yellow to yellow-orange, except sometimes with some dark markings. Gaster yellow to yellow-orange with a brown transverse stripe posteriorly on each tergite. Ovipositor sheaths brown.

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Head (Fig. 11). Ocellar triangle surrounded by faint grooves. POL 1.4–1.7× as long as OOL. Frontal suture small, v-shaped. Scrobal area without distinct median carina. Torulus placed above level with ventral margin of eye. A broad depression (supraclypeal area) below torulus extending to clypeus. Malar sulcus nearly straight, only slightly curved. Clypeal margin bidentate.

Antenna (Fig. 9) with 3 funicular segments and 3 very small anelli. First and second funicular segments distinctly longer than wide, third slightly longer than wide: length/width ratio of F1 1.6–2.0; F2 1.3–1.5; F3 1.1–1.3. Clava 2.0–2.5× longer than wide. Scape slightly flattened.

Mesosoma (Fig. 12). Pronotum very short medially in dorsal view. Mid lobe of mesoscutum with very weak median line and a single row of 4–7 adnotaular setae on each side. Mesosternum flat just in front of the trochantinal lobes and with precoxal suture. Scutellum with anterior pair of setae located behind middle. Scutellum overhanging dorsellum. Propodeum short medially, slightly longer than dorsellum; with median carina that is split anteriorly and forms a small fovea. Without paraspiracular carina. Propodeum with raised lobe of callus partially overhanging spiracle. Callus with 2 setae.

Forewing (Fig. 10) hyaline. Submarginal vein with 3 dorsal setae. Costal cell with line of ventral setae in apical half. Relative length of wing veins to stigmal vein as follows: CC: MV: STV = 2.5–2.7: 3.33–3.7: 2–3.8: 1. PMV very short, less than one quarter length of stigmal vein. Speculum partially closed behind, with cubital line of setae not quite extending to meet basal setal line. Wing disk beyond speculum densely pilose.

Gaster distinctly longer (1.2–1.3×) than mesosoma. Hypopygium reaching about half length of gaster. Cercus with 1 setae longer than others and sinuate. Ovipositor sheath slightly protruding, very short in dorsal view.

**Male** (Fig. 8). Length 1.2–1.45 mm. Head yellow with brown markings as follows: occiput, ocellar triangle, sometimes lower eye margin near malar sulcus. Thorax yellow to yellow-orange, generally with brown markings restricted to anterior face of pronotum, propodeum, mesepimeron, and along sutures. Dark specimens sometimes with mesosoma almost entirely brown, dorsellum yellow. Legs and coxae yellow, sometimes with some brown markings. Gaster dark brown, with distinct white patch anteriorly on both dorsal and ventral surfaces, these connected laterally. Antenna with 3 small anelli and 4 funicular segments; F1 quadrate to slightly longer than wide; F2–F4 all distinctly longer than wide; club elongate, 5–6× longer than wide. Each funicular segment and basal club segment with compact subbasal whorls of long setae that extend at least to apex of following segment. Ventral plaque small, one quarter to one fifth length of scape, situated near apex of scape.

**Type material.** Holotype ♀: Taiwan, Chiayi, Taibao, 9.i.2011, Yu-Che Lin, host *Quadrastichus erythrinae* [NCHU].
TWO NEW APROSTOCETUS SPECIES

42♀, 37♂ Paratypes. 5♀, 6♂, Same data as holotype [3♀, 4♂ ANIC; 2♀, 2♂ NMNS]. 2♀, 1♂, Taiwan, Miaoli, Houlung, 20.i.2010, Yu-Che Lin, host Quadrastichus erythrinae [NCHU]; 3♀, Taiwan, Taichung, Dali, 29.iii.2010, Yu-Che Lin, host Quadrastichus erythrinae [NCHU]; 7♀, 8♂, Taiwan, Taichung, Dali, 21.iv.2012, Ying-Ying Huang, host Quadrastichus erythrinae [NCHU]; 10♀, 10♂, Taiwan, Chiayi, Taibao, 24.i.2011, Yu-Che Lin, host Quadrastichus erythrinae [NCHU]; 5♀, 3♂, Taiwan, Kaohsiung, Chujin, 10.iii.2012, Ying-Ying Huang, host Quadrastichus erythrinae [NCHU]; 3♀, Taiwan, Taichung, Dali, 21.iv.2012, Ying-Ying Huang, host Quadrastichus erythrinae [NCHU]; 7♀, 8♂, Taiwan, Taichung, Dali, 21.iv.2012, Ying-Ying Huang, host Quadrastichus erythrinae [NCHU]; 10♀, 9♂, Taiwan, Kaohsiung, Chujin, 10.iii.2012, Ying-Ying Huang, host Quadrastichus erythrinae [NCHU].

**Distribution.** Taiwan.

**Etymology.** The specific name felix is Latin for happy, lucky, fortunate.

**Biology.** Aprostocetus felix is a solitary parasitoid collected from mature larvae and pupae of Q. erythrinae. In contrast to other parasitoids found on Q. erythrinae in the early stage of the pest invasion, population levels of this species remain high in cooler times of the year when other parasitoid population levels declined. Its population levels have built up through the years and it now occurs all year round in most areas in Taiwan, where it appears to have the potential to be an effective biological control agent of Q. erythrinae (Yang & Lin, unpublished).

**Discussion**

Despite the fact that *A. causalis* and *A. felix* are similar morphologically, they are clearly genetically distinct. Tables 1 and 2 show within group and between group variation based on CO1 and ITS2 sequence data.

**TABLE 1.** Mean genetic distance within groups using CO1 and ITS2.

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<tr>
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<th>CO1</th>
<th>ITS2</th>
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<tbody>
<tr>
<td><em>A. causalis</em> China</td>
<td>0.007</td>
<td>0.0008</td>
</tr>
<tr>
<td><em>A. causalis</em> Thailand</td>
<td>0.007</td>
<td>0.0008</td>
</tr>
<tr>
<td><em>A. felix</em> Taiwan</td>
<td>0.008</td>
<td>0.0024</td>
</tr>
</tbody>
</table>

**TABLE 2.** Mean genetic distance between groups using CO1 and ITS2.

<table>
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<th></th>
<th>CO1</th>
<th>ITS2</th>
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</thead>
<tbody>
<tr>
<td><em>A. causalis</em> China</td>
<td>-</td>
<td>0.002</td>
</tr>
<tr>
<td><em>A. causalis</em> Thailand</td>
<td>0.007</td>
<td>-</td>
</tr>
<tr>
<td><em>A. felix</em> Taiwan</td>
<td>0.085</td>
<td>0.084</td>
</tr>
</tbody>
</table>

Based on CO1, within group variation is 0.8% for *A. felix* and 0.7% *A. causalis* (both Chinese and Thai populations), and variation between Chinese and Thai populations of *A. causalis* is again 0.7%. By contrast, variation between *A. felix* and *A. causalis* is 8.5% for the Chinese populations and 8.4% for the Thai populations.

Based on ITS2, within group variation is 0.24% for *A. felix* and <0.1% for *A. causalis* (both Chinese and Thai populations), and variation between Chinese and Thai populations of *A. causalis* is 0.2%. By contrast, variation between *A. felix* and *A. causalis* is 4.5% for the Chinese populations and 4.0% for the Thai populations.

These distinct genetic differences are illustrated in figures 17 and 18. Molecular phylogeny of either NJ or ML trees based on both CO1 and ITS genes clearly show distinct monophyly of *A. causalis* (Thailand +China) and *A. felix* (Taiwan), respectively.
FIGURE 17. NJ trees for *A. causalis* and *A. felix* based on CO1 and ITS2 sequence data.

FIGURE 18. ML trees for *A. causalis* and *A. felix* based on CO1 and ITS2 sequence data.

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