Article

Morphological and molecular differentiation of the *Anagrus epos* species complex (Hymenoptera: Mymaridae), egg parasitoids of leafhoppers (Hemiptera: Cicadellidae) in North America

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Abstract

The *Anagrus epos* Girault species complex of the fairyfly wasp genus *Anagrus* Haliday (Hymenoptera: Mymaridae), egg parasitoids of *Erasmoneura* spp., *Erythroneura* spp., and other leafhoppers, is reviewed using both morphological and molecular methods. A new species, *A. vulneratus* Triapitsyn **sp. n.**, is described and illustrated from specimens reared from eggs of the leafhopper *Erasmoneura vulnerata* (Fitch) (Hemiptera: Cicadellidae) on grapevines from Colorado, USA. Discussed and corrected are the earlier published host and distribution records of *A. epos*, which is rediagnosed, and also of *A. daanei* Triapitsyn. Nuclear ribosomal DNA sequence data provides a genetic signature for *A. epos* and within the remainder of the species complex identifies *A. tretiakovae* Triapitsyn as being the most divergent member, confirms *A. vulneratus* as a separate entity, and reveals the closer similarity of specimens from Sonora, Mexico, to *A. vulneratus* rather than *A. epos*. Sequences from individuals identified as *A. daanei* were somewhat heterogeneous and revealed the existence of two distinct rDNA families — one represented by *A. daanei* from the Pacific Northwest and one by specimens from Colorado. Both gene families were represented by specimens from California, USA, and this finding is discussed in relation to recent use of "*A. epos*" from Colorado as a biological control agent in California.

Key words: Chalcidoidea, taxonomy, applied biological control, molecular identification, cryptic species, *Homalodisca vitripennis*

Introduction

Anagrus epos Girault (Hymenoptera: Mymaridae), first described from a collection in Illinois, USA (Girault 1911), is a common and seemingly widespread egg parasitoid of leafhoppers (Hemiptera: Cicadellidae) in North America. Location records for this species also include Colorado, Kentucky, New Mexico, and New York in the USA as well as Baja California and Sonora in Mexico (Triapitsyn 1998). Like many minute parasitoids, identification of members of the genus *Anagrus* Haliday to species is difficult because of their size and a paucity of diagnostic morphological characters. Traditionally, species identifications require skilled preparation of slide-mounted specimens and expert knowledge of the genus. Initially it was believed that eggs of all grape-feeding leafhoppers in North America were attacked by a single species, *A. epos*. However, Pickett *et al.* (1987) first suggested that *A. epos* may be more than one species and later a taxonomic revision, employing high resolution light microscopy (Triapitsyn 1998), confirmed that it was misidentified as several different species, among them at least *A. erythroneurae* Trjapitzin & Chiappini from the *atomus* species group, and also *A. epos*, *A. daanei* Triapitsyn, and *A. tretiakovae* Triapitsyn from the *incarnatus* species group of *Anagrus*. The morphological characters that are used for differentiating the latter three species, which we group in the *A. epos* species complex, can vary to some extent; thus, species limits may be difficult to assess without supporting data from their biology and from DNA sequences.

While commonly collected as a parasitoid of grape-feeding leafhopper species (mainly *Erythroneura* spp.), a recent collection of *A. epos* from the egg mass of *Cuerna fenestella* Hamilton in Minnesota, USA, represented the first time this species had been collected from a proconiine sharpshooter species, i.e., a leafhopper belonging to the tribe Proconiini of the subfamily Cicadellinae (Hoddle & Triapitsyn 2004; Triapitsyn & Rakitov 2005). Mymarid parasitoids from this collection have been reared continuously since June 2004 in the University of California (UC), Riverside Quarantine facility on eggs of the glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar) (Krugner *et al.* 2007). GWSS is an exotic pest in California and a prolific vector of the plant pathogen *Xylella fastidiosa*, which amongst other things is the cause of Pierce's disease in grapes (reviewed by Hopkins 1989). *Anagrus epos* was imported into California as part of a biological control program undertaken against GWSS. The native range of GWSS is southeastern USA and northeastern Mexico (Triapitsyn & Phillips 2000) and GWSS is thought to have invaded California prior to 1990 (Sorensen & Gill 1996), likely as egg masses accidentally imported on ornamental plants from Florida. The search for effective parasitoids in the native range of GWSS (Triapitsyn & Phillips 1996, 2000; Triapitsyn *et al.* 1998, 2003; Hoddle & Triapitsyn 2004; Goolsby *et al.* 2006) has resulted in the discovery of several Mymaridae and Trichogrammatidae (Hymenoptera) species. Among these, the mymarids *Gonatocerus ashmeadi* Girault, *G. fasciatus* Girault, *G. morrilli* (Howard), *G. triguttatus* Girault, and *G. walkerjonesi* Triapitsyn have been mass reared and released in California to suppress GWSS populations (Morse 2006).

The purpose of this study was to determine whether the Minnesota "strain" of *A. epos* in culture at UC Riverside on GWSS, is indeed this species, how it compares to other members of the *A. epos* species complex based on morphology and molecular data, and how these can be separated (i.e., compiling a key). Due to limitations on what is economically practical to rear and mass-release, and also because of restrictions on importing and releasing exotic parasitoids in California without understanding their taxonomy, we needed to refine our knowledge of this species complex. Two approaches were used to determine the identity of different *A. epos* populations: (1) reassessment of key morphological features to determine if subtle differences exist between *A. epos* populations that could indicate species differences and (2) assessment of genetic differences that may exist between populations collected from different regions by comparing ribosomal DNA sequences. Results from the morphological and genetic methods of investigation were evaluated together to establish the identity of the species in the *A. epos* complex.

Materials and methods

Specimen collection. New collections of *Anagrus* egg parasitoids of mainly grape-feeding leafhoppers in Colorado and New Mexico were made by S. Triapitsyn. Specimens of *A. daanei* from Fresno Co., California were made by G. Yokota and sent to us by K. Daane (UC Berkeley, California). Collections of *A. daanei* from a vineyard near Prosser, Washington were made by L. Wright (Washington State University, Prosser, Washington). Collections of *A. erythroneurae* in southern California were made by G. Kenney and K. Tollerup (UC Riverside). Collection of fresh *Anagrus* sp. near *vulneratus* Triapitsyn specimens was difficult due to insecticide treatments of vineyards in Sonora against the vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) (A.A. Fú-Castillo, INIFAP-CECH, Hermosillo, Sonora, Mexico, personal communication). Therefore, ethanol-preserved specimens, collected in Sonora in 1994 by S. Triapitsyn and collaborators, and stored since then at -20°C at the Entomology Research Museum, UC Riverside (UCRC), were used in the molecular analyses. Specimens of *A. tretiakovae* could not be re-collected from their type localities in Arizona because all the organic vineyards there were discontinued due to heavy vine mealybug infestations (S.P. Pavich, Agricultural Consultant, Phoenix, Arizona, personal communication). For the molecular analyses, besides the newly collected specimens from New Mexico, we therefore also used ethanol-preserved specimens at UCRC, collected in Harquahala Valley, Maricopa Co., Arizona in 1994 by S. Triapitsyn and collaborators (Triapitsyn 1998) and ethanol-preserved voucher specimens of *A. daanei* from Okanagan Valley, British Columbia, Canada (Lowery *et al.* 2007). Our attempts to rear egg parasitoids of the leafhoppers on the grapevines in the vineyards in the Tucson, Arizona area failed.

The following method was used to collect parasitoids. Grape leaves from the insecticide-free vineyards were collected and placed in carton boxes and sealed with brown paper tape. In the laboratory, a glass vial (7.5 ml) was partially inserted and secured in the carton's lid in order to allow light to enter and thus attract emerging parasitoids into the vial. Samples were stored for up to 20 days in the laboratory at room temperature, with the vials facing a continuous light source. Emerging parasitoids were removed from the vials every day, labeled, and placed in 95% ethanol for storage at -20°C.

Fresh specimens of the *Anagrus epos* species complex used in the molecular analyses were from the same ethanol-preserved series as listed under "Material examined" sections for the respective species. Additionally, numerous specimens of *A. erythroneurae* (the outgroup) preserved in ethanol (UCRC) have the following collection data: USA, California, Riverside Co.: Temecula, E of Bella Vista Rd., 7.ix.2006, G. Kenney [emerged 8–11.ix.2006 in UCRC from wine grape leaves infested with eggs of *Erasmoneura variabilis* (Beamer)]; Coachella Valley, Oasis, 19.xi.2006, K. Tollerup (emerged in UCRC from table grape leaves infested with eggs of *E. variabilis*). *Anagrus erythroneurae* was studied recently by de León *et al.* (2008) using both morphometric and molecular methods.

Scientific names of the leafhopper hosts in the genera *Erasmoneura* Young and *Erythroneura* Fitch are given according to Dmitriev & Dietrich (2007).

Taxonomic methods. The morphological analysis for this study is based only on females because males of most species within the *Anagrus epos* complex are quite similar (except for the more distinct male of *A. tretiakovae*). Males of North American *Anagrus* differ from females in the normal sexually dimorphic features of genitalia and having a filiform, 13-segmented antenna (flagellum 11-segmented), as well as by the body color being usually at least a little darker and the forewing usually relatively a little wider than for the female. Female antennal characters are used extensively as one of the main diagnostic features for species recognition in the genus and in the keys. Because of this identification of males to species is often difficult or even impossible morphologically without rearing them together or associating them through collecting the sexes together at one time and place. Therefore, often it is not the species that is differentiated but only the females of the species.

Results of the genetic analysis, which follow the taxonomic treatment of the *Anagrus epos* species complex in the text, played a key role in making the identifications along with species separation based on morphology.

For the morphological descriptions we use the terminology of Gibson (1997). All measurements (such as length or length:width for the wings) are given in micrometres (μm). Abbreviations used in the key and description are: F = antennal funicular (female) or flagellar (male) segment; mps = multiporous plate sensillum or sensilla on the antennal flagellar segments (= longitudinal sensillum or sensilla or sensory ridge(s) of authors).

The specimens examined are deposited in following collections: CNCI, Canadian National Collection of Insects, Ottawa, Ontario, Canada; UCRC, Entomology Research Museum, University of California, Riverside, California, USA; and USNM, National Museum of Natural History, Washington, District of Columbia, USA.

DNA amplification and sequencing. DNA was extracted from individual wasps either using Chelex[100] resin as described in Stouthamer *et al.* (1999), or using an EDNA HiSpEx™ Tissue Kit (Saturn Biotech, Perth, Western Australia, Australia), following the manufacturer's protocol. An advantage of the kit is that the wasp's body is preserved intact and, if required, can subsequently be retrieved and slide-mounted for morphological examination. The polymerase chain reaction (PCR) was used to amplify two regions of nuclear ribosomal RNA (rRNA). In separate reactions, a section of the D2 domain of the 28s gene (28sD2) and the entire internal transcribed spacer 2 (ITS2) were amplified using the respective PCR primers, D2-CF (5'-CGTGTTGCTTGATAGTGCAGC-3') paired with D2-CR (5'-TTGGTCCGTGTTTCAAGACGGG-3'; Campbell *et al.* 1993, 2000), and ITS2-forward (5'-TGTGAACTGCAGGACACATG-3'; Campbell *et al.* 1993) paired with ITS4 (5'-TCCTCCGCTTATTGATATGC-3'; White *et al.* 1990). Reactions were performed in 25 μL volumes containing 1x Thermopol PCR buffer (containing 2 mM MgSO$_4$; New England BioLabs, Ipswich, Massachusetts, USA), 200 μM each dNTP, 1U *Taq* polymerase (NEB), and 0.2 μM of each primer.

The presence of amplified products was confirmed by gel electrophoresis and PCR products were cleaned using the Wizard® PCR Preps DNA purification kit (Promega, Madison, Wisconsin, USA). The 28sD2 PCR product was direct sequenced in both directions at the University of California Riverside Genomics Institute, Core Instrumentation Facility using an Applied Biosystems 3730 DNA analyzer with a Big-Dye V3.1® kit (Applied Biosystems, Foster City, California, USA). The ITS2 PCR product required cloning into a plasmid vector (pGEM®-T Easy Vector System; Promega) to facilitate sequencing.

Genetic analysis. All sequences were trimmed (removing the priming regions) and aligned, first using ClustalX version 1.83 (Thompson *et al.* 1997), and then manually in BioEdit version 7.0.5.3 (Hall 1999). Sequences are deposited in GenBank® (Benson *et al.* 2008). Analysis of DNA sequence variation was restricted to the 28sD2 data. Sequences were grouped according to the morphological identification provided by one of the authors (Triapitsyn) and by their state/country of origin. Sequences of specimens from Washington State, and British Columbia, Canada, were grouped together because they belong to the same morphospecies, to form a larger Pacific Northwest population. This created nine "populations", for which intra- and inter-population differentiation was estimated as haplotype diversity (Hd) (Nei 1987, equation 8.4) and the average number of nucleotide differences between all pairs of sequences (κ) (Tajima 1983, equation A3), within and between these "populations", was calculated using DnaSP v. 4.10.7 (Rozas *et al.* 2003). Nucleotide positions with alignment gaps were excluded from the analysis. Simple distance-based relationships between the nine populations were inferred from the 28sD2 alignment using the BootStrap Neighbour-Joining method implemented in ClustalX, with 2000 iterations.

Phylogenetic inference from ITS2 is problematic due typically to large interspecific differences that make alignment of this region difficult and somewhat ambiguous. Cloned ITS2 sequences were compared using two methods. First, sequences were simply compared "by eye" to determine if distinct groups of sequences were present, and similarity between ITS2 sequences was calculated as the proportion of identical residues between sequence pairs, using the Sequence Identity Matrix function in BioEdit. Second, sequences were also grouped using the Neighbour-Joining method implemented in ClustalX. However, we are hesitant to draw phylogenetic inferences from our analysis of ITS2 data but such data were very useful to assist with the taxonomic identifications.

Results

Taxonomy

Anagrus **Haliday**

Chiappini *et al.* (1996) provided a key to the Holarctic species of *Anagrus*, redescribed the Nearctic species, and diagnosed the genus and its three subgenera (i.e., *Anagrella* Bakkendorf, *Anagrus* Haliday s. str., and *Paranagrus* Perkins). All species treated below belong to the subgenus *Anagrus* (*Anagrus*) and its two species-groups, which were diagnosed by Chiappini (1989) and Chiappini *et al.* (1996). Triapitsyn (1998) reviewed the *Anagrus* species found in North American vineyards and orchards. All species of *Anagrus* in the *A. epos* complex belong to the *incarnatus* species group, recognized by the female clava bearing 5 mps, whereas *A. erythroneurae*, used as an outgroup in the molecular analyses only, belongs to the *atomus* species group, recognized by the female clava bearing 3 mps (Trjapitzin & Chiappini 1994). Within the *incarnatus* species group in North America, species in the *Anagrus epos* complex are distinguished by the combination of their minute size (body length usually less than 0.6 mm, often less than 0.5 mm), F2 of the female antenna lacking mps, F3 with or without mps, and F4–F6 bearing mps, mesoscutum with a pair of submedian adnotaular setae, forewing usually with a distinct bare area in the broadest part of disc near its posterior margin, external plate of ovipositor with 2 or 3 distal setae, and also by generally light body color of the females (mostly yellow or light brown). Morphologically, species in the *Anagrus epos* complex, particularly *A. epos* as treated by Triapitsyn (1998), are somewhat similar to the mainly Neotropical species *A. empoascae*

Dozier, which is also known from the Nearctic part of Mexico as well as from Delaware, Florida, Louisiana, and South Carolina in the USA (Triapitsyn 1997, 2002). *Anagrus empoascae* shares some of the morphological features with the named members of the *Anagrus epos* complex but differs from all of them (except for *A. epos* as treated here) by F3 of the female antenna always bearing a mps on both antennae (Triapitsyn 1997). It differs from *A. epos* by the relatively shorter ovipositor (2.2–2.4x as long as protibia in *A. empoascae*, 2.8–3.1x as long as protibia in *A. epos*). Ecologically, however, *A. empoascae* seems to be outside of the *Anagrus epos* complex as it is known primarily as an egg parasitoid of *Empoasca* spp. (Cicadellidae) on weeds and other low vegetation and crops, and also of *Halticus bractatus* (Say) (Miridae) on alfalfa (Triapitsyn 1997, 2002), whereas members of the *Anagrus epos* complex mainly parasitize eggs of *Erasmoneura* spp. and *Erythroneura* spp. on trees, vines, and shrubs, while the natural host of *A. epos* in Minnesota is a proconiine sharpshooter, *Cuerna fenestella*.

Key to females of the named species of the *Anagrus epos* complex in North America

1 Basal one-third of forewing disc beyond venation with 2 well-defined, complete longitudinal rows of setae (Fig. 2) .... *A. tretiakovae* Triapitsyn
1' Basal one-third of forewing disc beyond venation with 1 complete longitudinal row of setae (Figs 4, 6, 8, 10, 12, 13) 2
2(1) Ovipositor at most 2.2x as long as protibia length .... *A. daanei* Triapitsyn
2' Ovipositor at least 2.5x as long as protibia length 3
3(2) F3 usually with a mps (Fig. 9); forewing at least 7.9x as long as wide; ovipositor at least 2.8x as long as protibia length .... *A. epos* Girault
3' F3 usually without mps (Fig. 11), at most (occasionally) with 1 such sensillum on one antenna; forewing at most 6.7x as long as wide; ovipositor at most 2.7x as long as protibia length .... *A. vulneratus* Triapitsyn, **sp. n.**

Anagrus (*Anagrus*) *tretiakovae* **Triapitsyn**
(Figs 1, 2)

Anagrus (*Anagrus*) *tretiakovae* Triapitsyn 1998: 90–93 (description, illustrations, distribution, host associations).
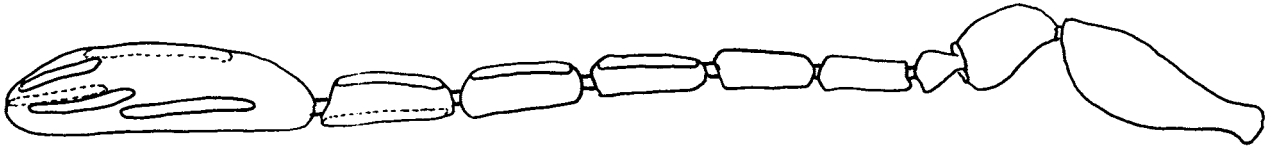*Anagrus tretiakovae* Triapitsyn: Prischmann *et al.* 2007: 43–51; Morse & Stouthamer 2007: 95; Wright & James 2007: 17–18, 20–23.

**Type locality.** Near Stanfield, Pinal Co., Arizona, USA.

Material examined. USA. New Mexico, Bernalillo Co., Albuquerque, Los Ranchos de Albuquerque, 4920 Rio Grande Blvd. NW, Anderson Valley Vineyards, S.V. Triapitsyn, 26.ix.2005 (emerged from Burgundy [red wine] grape leaves infested with various leafhoppers [*Dikrella cockerellii* (Gillette), *Empoasca* sp., *Erasmoneura vulnerata* (Fitch), and *Erythroneura triapitsyni* Dmitriev & Dietrich], preserved upon arrival at University of California, Riverside quarantine 28.ix.2005 or emerged thereafter, S&R # 05–19), 1 male on slide and numerous females and males in ethanol [UCRC]. Also specimens listed by Triapitsyn (1998).

Female diagnosis. F2 and F3 of antenna (Fig. 1) usually without mps; basal one-third of forewing disc beyond venation with two well-defined, complete longitudinal rows of setae in both sexes (Fig. 2); ovipositor 1.8–2.2x as long as protibia length; external plate of ovipositor with 2 distal setae (Triapitsyn 1998).

Hosts. Besides the leafhopper hosts indicated by Triapitsyn (1998) and Williams & Martinson (2000), recent host records of *A. tretiakovae* include *Erythroneura elegantula* Osborn and *E. ziczac* Walsh in Washington (Prischmann *et al.* 2007; Wright & James 2007). The likely hosts of this species in Albuquerque, New Mexico, are *Erasmoneura vulnerata* (Fitch) and *Erythroneura triapitsyni* Dmitriev & Dietrich.
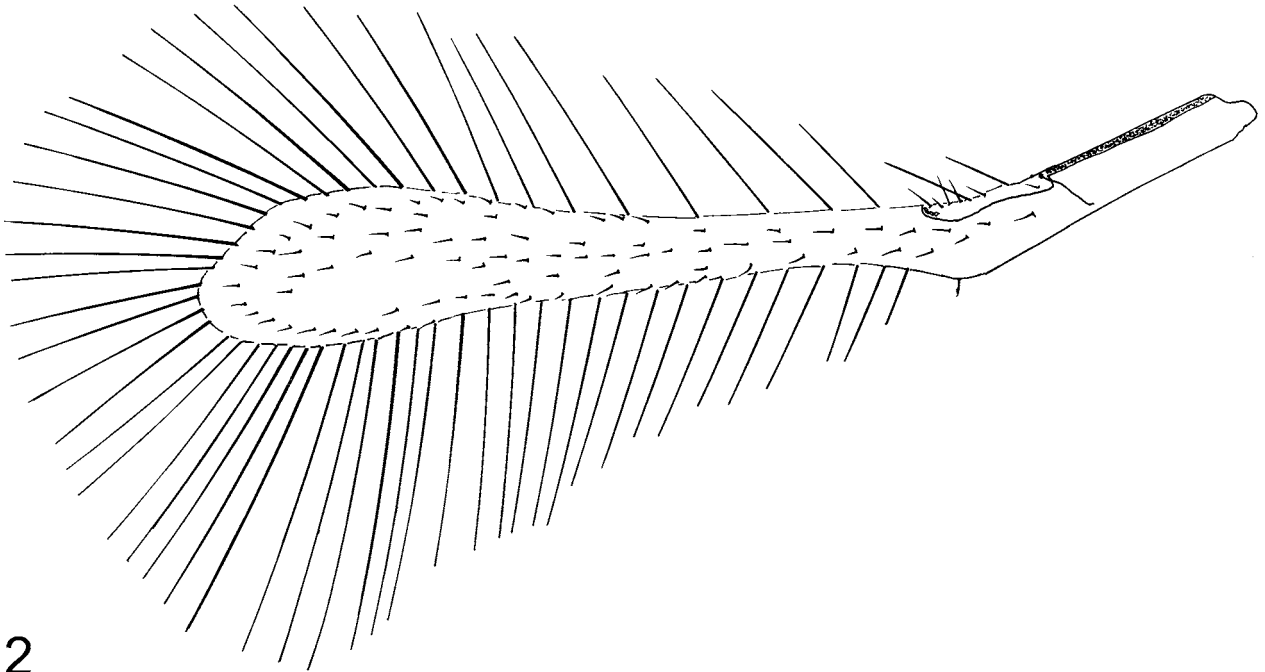
FIGURES 1, 2. *Anagrus tretiakovae* female (Arizona). 1. Antenna. 2. Forewing.

Anagrus (*Anagrus*) *daanei* **Triapitsyn**
(Figs 3–8)

Anagrus (*Anagrus*) *daanei* Triapitsyn 1998: 93–98 (earlier references, description, illustrations, distribution, host associations).
Anagrus daanei Triapitsyn: Prischmann *et al.* 2007: 43–51; Morse & Stouthamer 2007: 95; Lowery *et al.* 2007: 9–11, 13–14; Wright & James 2007: 17–19, 22–23.
*Anagrus* sp. 1 near *A. daanei* Triapitsyn: Morse & Stouthamer 2007: 95 (see "Comments" below).
Anagrus sp. 2 near *A. daanei* Triapitsyn: Morse & Stouthamer 2007: 95 (see "Comments" below).

Type locality. Reedley, Fresno Co., California, USA.
 Material examined. USA. California, Tulare Co., Kingsburg, 19–24.ix.2005, 3–5.x.2005, and 11–14.x.2005, G. Yokota (emerged from grape leaves infested with leafhopper eggs), numerous females and males in ethanol [UCRC]. Colorado, Mesa Co., Palisade, Colo-Color Orchards, 621 37 1/4 Rd., 39°05'39"N 108°21'43"W, 1460 m: 29.viii.2006, S.V. Triapitsyn (emerged 3.viii–6.ix.2006 in University of California, Riverside quarantine from Virginia creeper leaves infested with *Erythroneura anfracta* Beamer, S&R # 06–28–02), 14 females, 6 males [UCRC]; 4.ix.2007, S.V. Triapitsyn (emerged 7–14.ix.2007 in University of California, Riverside quarantine from Virginia creeper leaves infested with *Erythroneura anfracta* Beamer, S&R # 07–39–02), numerous females and males in ethanol [UCRC] and 1 female on a slide [UCRC] (molecular voucher PR–08–193). Washington: Benton Co., near Prosser, Washington State University vineyard, 2.viii.2006 and 7.viii.2006, L. Wright (emerged from *Vitis vinifera* leaves infested with

Erythroneura ?elegantula Osborn), 4 females and 6 males in ethanol [UCRC]. Yakima Co., Sunnyside, Walsh Vineyard, 2.ix.1999, D.G. James (from eggs of *Erythroneura* sp. on cultivated grape), 1 female, 1 male on slides [UCRC]. Also specimens listed by Triapitsyn (1998).
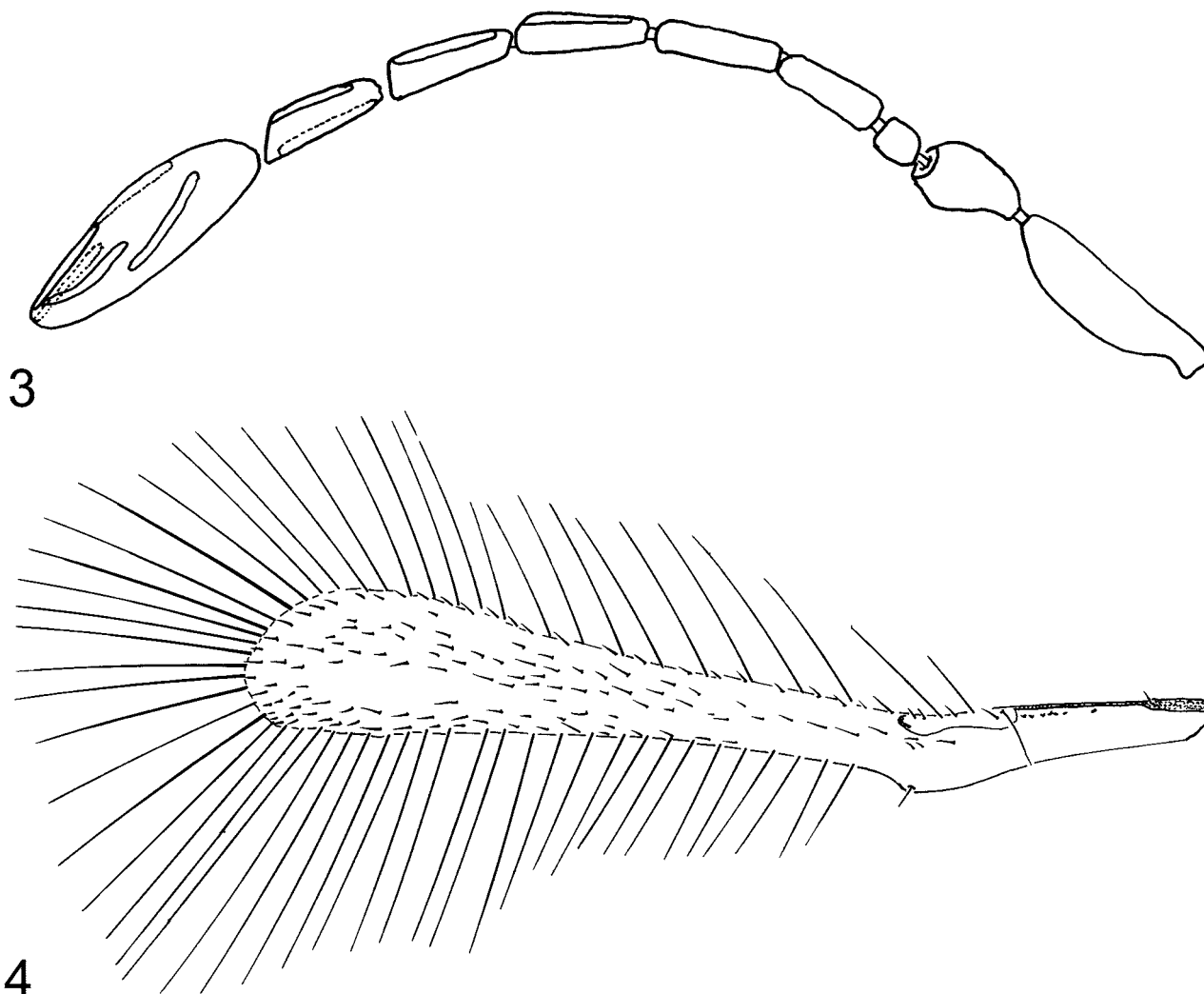

FIGURES 3, 4. *Anagrus daanei* female (California). 3. Antenna. 4. Forewing.

Female diagnosis. Antenna (Figs 3, 5, 7) with F2–F5 subequal in length (F3 and F5 usually slightly shorter), F2 and F3 usually without mps (occasionally F3 has a mps on one antenna); basal one-third of forewing disc beyond venation with one well-defined, complete longitudinal row of setae in both sexes (Figs 4, 6, 8); ovipositor 1.8–2.2x as long as protibia length; external plate of ovipositor usually with 2 distal setae (Triapitsyn 1998).

Hosts. Besides the leafhopper hosts indicated by Triapitsyn (1998) and Williams & Martinson (2000), recent host records of *A. daanei* include *Erythroneura anfracta* Beamer [new record] in Colorado, *E. elegantula* Osborn and *E. ziczac* Walsh in Washington (Prischmann *et al.* 2007; Wright & James 2007), and *E. ziczac* in Okanagan Valley, British Columbia (Lowery *et al.* 2007). Females of *A. daanei* [as *Anagrus* sp. 1 near *A. daanei*] (from Palisade, Colorado) failed to parasitize fresh eggs of *Homalodisca vitripennis* (Germar) under quarantine laboratory conditions (Morse & Stouthamer 2007).

Comments. Based on preliminary molecular data, Morse & Stouthamer (2007) identified separate forms from Colorado and Washington (referred to as *Anagrus* sp. 1 near *A. daanei* and *Anagrus* sp. 2 near *A. daanei* respectively) that were morphologically indistinguishable from specimens of *A. daanei* from California. However, the results of our expanded molecular analyses imply that these three forms likely fall within one, rather heterogenous species, *A. daanei* (see below).
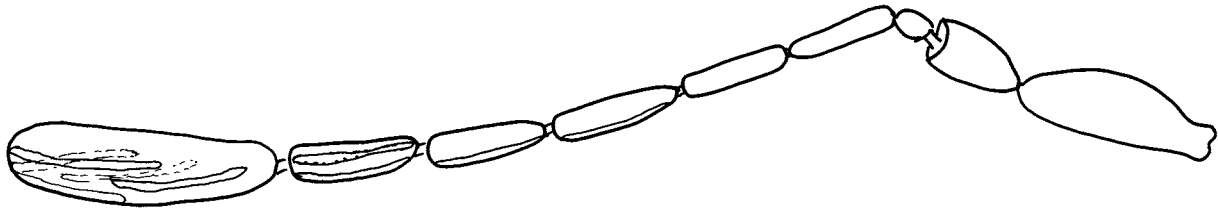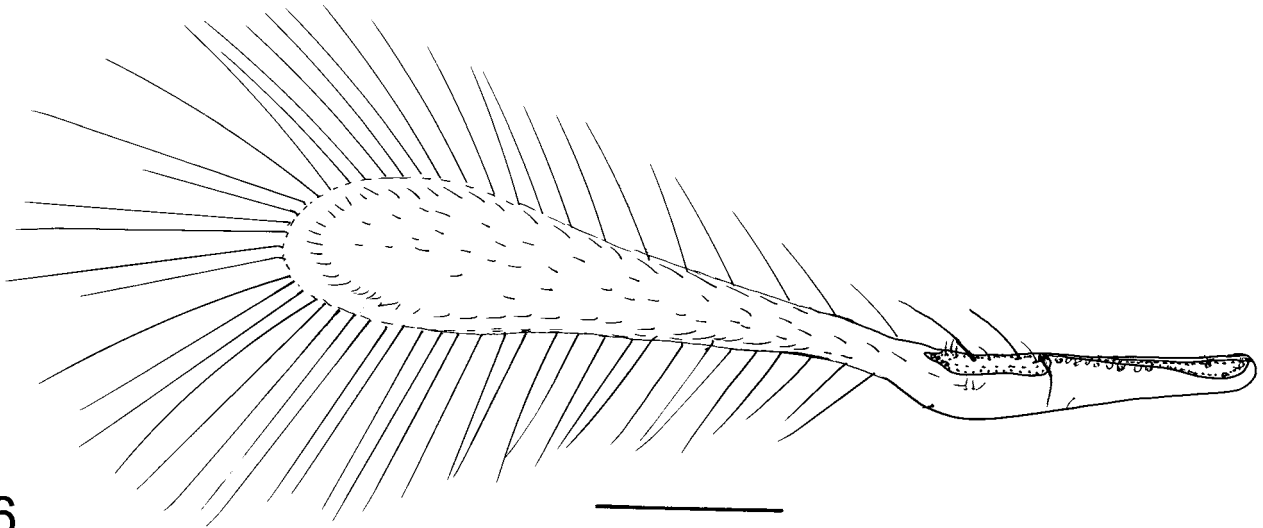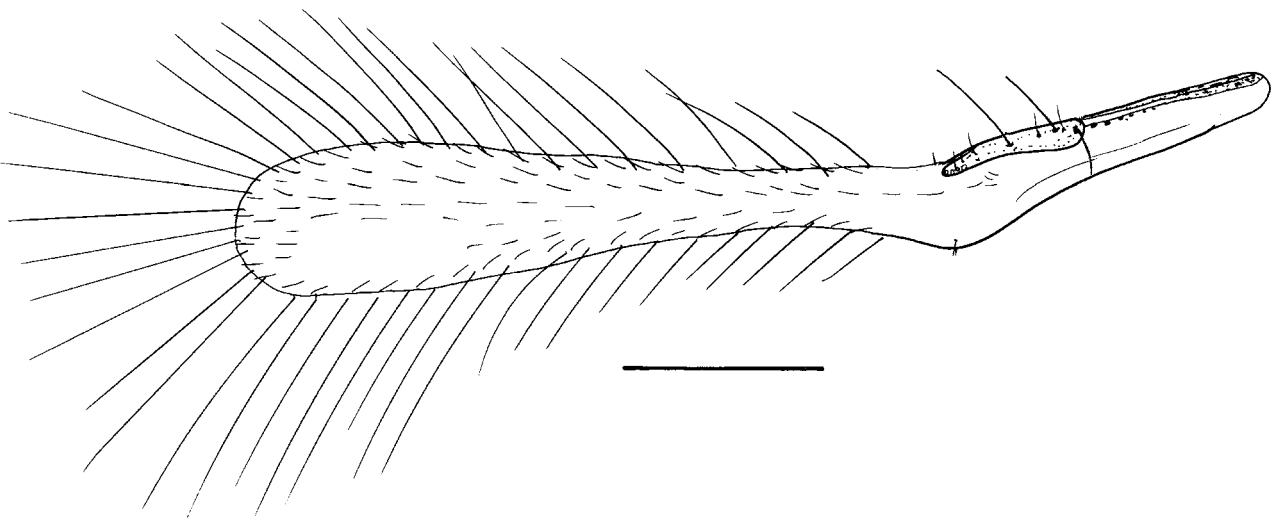
FIGURES 5, 6. *Anagrus daanei* female (Colorado). 5. Antenna. 6. Forewing. Scale bars = 0.1 mm.

FIGURES 7, 8. *Anagrus daanei* female (Washington). 7. Antenna. 8. Forewing. Scale bars = 0.1 mm.

*Anagrus* (*Anagrus*) *epos* **Girault**
(Figs 9, 10)

*Anagrus epos* Girault 1911: 292–293; Triapitsyn 2006: 7–9; Morse & Stouthamer 2007: 94–96.
Anagrus (*Anagrus*) *epos* Girault: Triapitsyn 1998: 100–100 (in part, type material and non-type specimens from Illinois only).

Type locality. Centralia, Marion Co., Illinois, USA.

Material examined. The specimens listed by Triapitsyn (1998) from Illinois only and Triapitsyn (2006) from Minnesota and also the following specimens: USA, California, Riverside Co., Riverside, University of California at Riverside quarantine laboratory, from colony on *Homalodisca vitripennis* (Germar) eggs on leaves of *Euonymus japonica*, reared during vi–ix.2004 by V.V. Berezovskiy & S.V. Triapitsyn and from ix.2004 to xii.2006 by R. Krugner; originally from: USA, Minnesota, Clay Co., ca. 4 mi. SEE of Glyndon, Bluestem Prairie (Nature Conservancy Preserve, moist tallgrass prairie), 46.85521°N 96.47353°W, 31.v–1.vi.2004, R.A. Rakitov (ex. egg masses of *Cuerna fenestella* Hamilton on *Solidago* sp. and *Zigadenus* sp.; emerged in UCR quarantine 8–14.vi.2004, collected and colony established by S.V. Triapitsyn & V.V. Berezovskiy), numerous females and males in ethanol [UCRC].
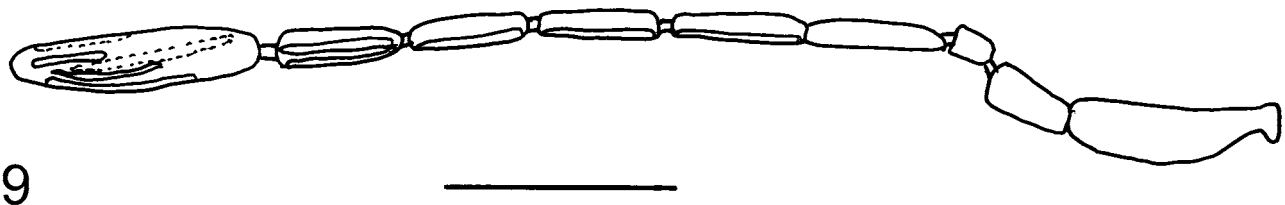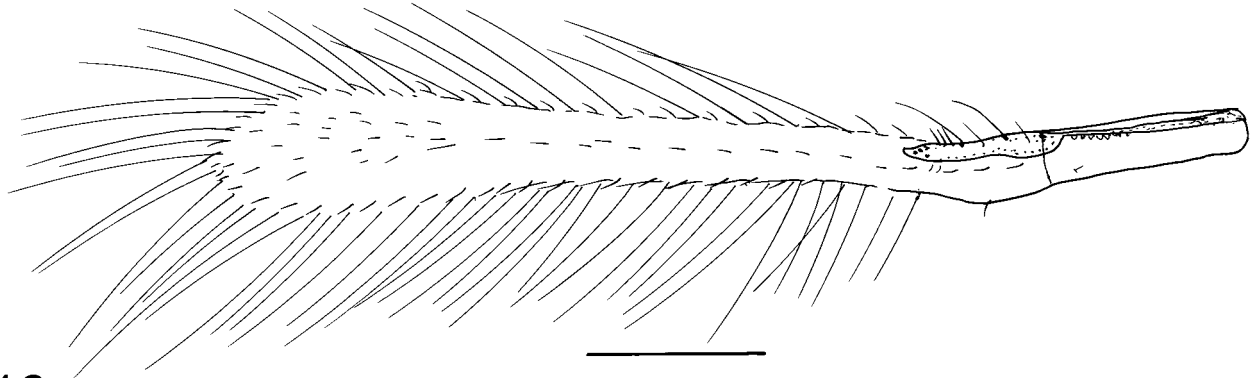
FIGURES 9, 10. *Anagrus epos* female (Minnesota). 9. Antenna. 10. Forewing. Scale bars = 0.1 mm.

Female diagnosis. F3 of antenna (Fig. 9) usually with a mps; basal one-third of forewing disc beyond venation with one well-defined, complete longitudinal row of setae in both sexes (Fig. 10); forewing 7.9–8.6x as long as wide; ovipositor 2.8–3.1x as long as protibia length; external plate of ovipositor with 3 distal setae.

Hosts. *Cuerna fenestella* Hamilton the natural host in Minnesota (Triapitsyn & Rakitov 2005; Triapitsyn 2006), *Homalodisca vitripennis* (Germar) (Triapitsyn 2006), and several other factitious hosts listed by Krugner *et al.* (2007).

Comments. The record of *A. epos* from New Mexico (Triapitsyn 1998) was mostly likely erroneous due to a misidentification of a very similar species whose identity is not clear, although it may belong to either *A. vulneratus* or *A.* sp. near *vulneratus*. Records of *A. epos* from Baja California and Sonora are referred to below as *A.* sp. near *vulneratus*. The identities of the specimens of *A. epos* reared from eggs of *Erythroneura aclys*

McAtee and *E. bistrata* McAtee in Kentucky and of those reared from eggs of *E. bistrata* and *E. comes* (Say) in New York (Triapitsyn 1998) need to be verified using molecular methods.

Anagrus (*Anagrus*) *vulneratus* Triapitsyn, sp. n.
(Figs 11–15)

Anagrus epos Girault: González *et al*. 1988: 23–25 (misidentification, in part [specimens from Grand Junction, Colorado]).
Anagrus (*Anagrus*) *epos* Girault: Triapitsyn 1998: 100–103 (misidentification, in part [specimens from Grand Junction, Colorado]).
Anagrus new species: Morse & Stouthamer 2007: 95.

Type material. Holotype female [UCRC ENT 014814] on slide labeled: "USA: Colorado, Mesa Co., Grand Junction, Colorado State University Western Colorado Research Center – Orchard Mesa, 3168 B1/2 Road [39°02'31''N 108°27'58''W, 1450 m], 29.viii.2006, S.V. Triapitsyn. Emerged 3.ix.2006 at University of California, Riverside quarantine from wine grape leaves infested with *Erythroneura vulnerata* Fitch S&R # 06–28–01". Paratypes: same data as holotype, 1 female and 1 male on slides [UCRC]; same data except emerged 30.viii.2006, 1 female on point [UCRC]; same data except emerged 1.ix.2006, 1 male on point [UCRC]; same data except emerged 4.ix.2006, 2 females on points [CNCI, USNM]; same data except emerged 5.ix.2006, 7 females and 2 males on points [UCRC]; same data except emerged 7.ix.2006, 3 females on points and 1 male on slide [UCRC]. USA, Colorado, Mesa Co., Grand Junction: 16.vii.1986, D. González (from eggs of *Erasmoneura vulnerata* (Fitch) on grape leaves), 10 females on slides [UCRC]; Colorado State University Western Colorado Research Center – Orchard Mesa, 3168 B1/2 Road, 39°02'31''N 108°27'58''W, 1450 m: 29.viii.2006, S.V. Triapitsyn (sweeping wine grapes [nursery] infested with *E. vulnerata*), 1 male on point [UCRC]; 4.ix.2007, S.V. Triapitsyn (emerged 14.ix.2007 at University of California, Riverside quarantine from wine grape [nursery] leaves infested with *E. vulnerata*, S&R # 07–39–01), 1 female on slide [UCRC] (molecular voucher PR–08–192).

Additional material examined (all in ethanol, UCRC). Same data as holotype except emerged 2.ix.2006, 2 females and 1 male; emerged 5.ix.2006, 1 female; emerged 6.ix.2006, 2 females. USA, Colorado, Mesa Co.: Grand Junction, Colorado State University Western Colorado Research Center – Orchard Mesa, 3168 B1/2 Road, 39°02'31''N 108°27'58''W, 1450 m, 4.ix.2007, S.V. Triapitsyn (emerged 7–12.ix.2007 at University of California, Riverside quarantine from wine grape [nursery] leaves infested with *E. vulnerata*, S&R # 07–39–01), numerous females and males. Palisade, Garfield Estates vineyard, 29.viii.2006, S.V. Triapitsyn (emerged 6.ix.2006 at University of California, Riverside quarantine from wine grape leaves infested with *E. vulnerata*, S & R # 06–28–03), 1 female.

Description. FEMALE. Length 430–590 µm. General body color yellow to light brown except transverse trabecula, stemmaticum, anterior half of mesoscutum, and basal metasomal terga notably darker (brown), eyes and ocelli pink; appendages: scape, pedicel, and F1 light brown, the remaining flagellar segments brown, legs light brown.

Antenna (Fig. 11) with scape 2.6–2.8x as long as wide; F1 subglobular or subcylindrical, less than half length of pedicel; F2 and F3 subequal (F3 usually slightly shorter) and each a little shorter than following funicular segments; F4–F6 subequal (F5 usually slightly shorter than F4 and F6); F1 and F2 without mps, F3 usually without mps but occasionally with 1 mps on one antenna, F4 and F5 with 1, F6 with 2 mps each; clava 3.0–3.3x as long as wide, a little longer than combined length of the two preceding segments, with 5 mps.

Mesosoma about 0.7x as long as metasoma. Mesoscutum with a pair of submedian adnotaular setae. Forewing (Figs 12, 13) 6.3–6.7x as long as wide; disc hyaline, with 1 complete row of setae extending from apex of venation to wing apex and 2–4 additional, irregular rows of discal setae, usually leaving a small bare area in the broadest part of disc near posterior margin (Fig. 13) but often without a distinct bare area (Fig. 12); distal macrochaeta 1.8–1.9x as long as proximal macrochaeta; longest marginal seta 2.2–2.7x greatest wing width. Hind wing (Fig. 12) about 22x as long as wide; longest marginal seta about 6.6x greatest wing width.

Ovipositor anteriorly extending to mesophragma and posteriorly exserted beyond apex of gaster by 0.11–0.14x own length. External plate of ovipositor with 2 or 3 distal setae. Ovipositor 2.5–2.7x as long as protibia length.
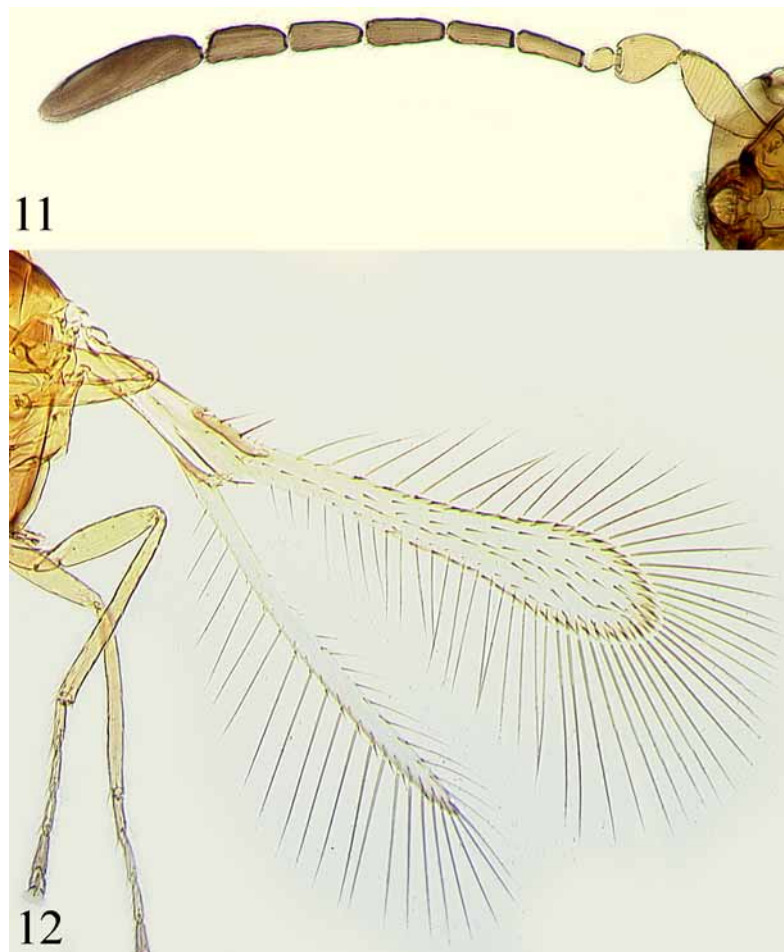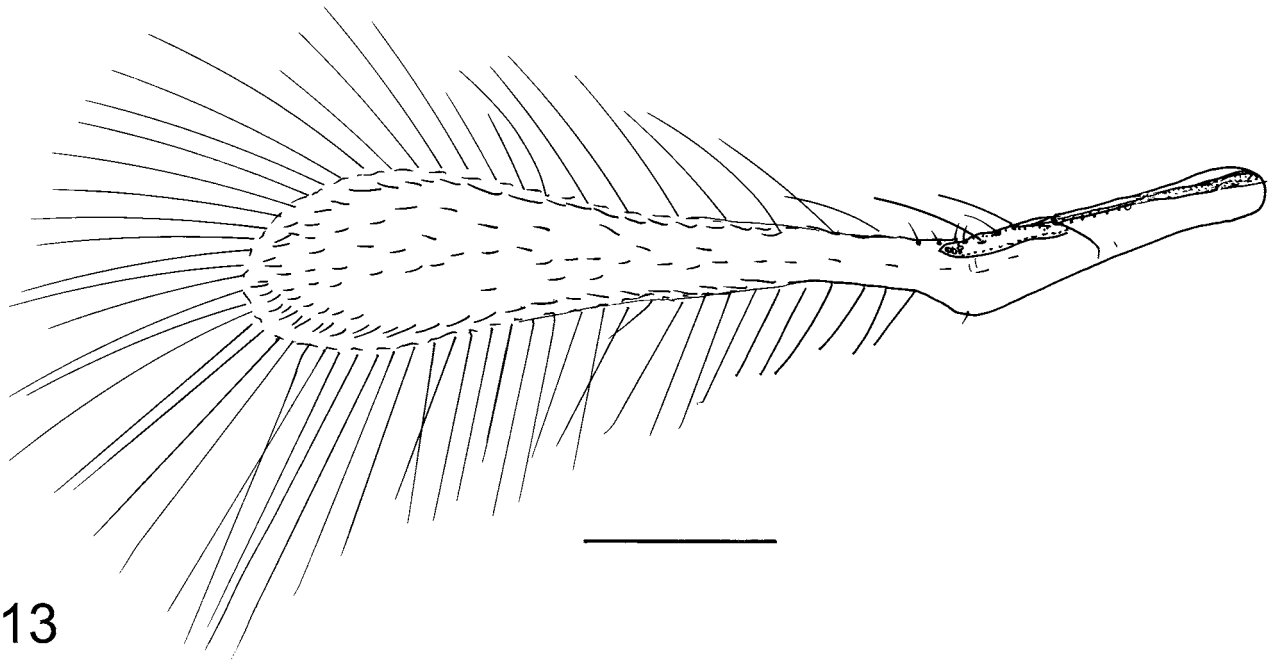

FIGURES 11, 12. *Anagrus vulneratus* female (paratype, Colorado). 11. Antenna. 12. Wings.

Measurements (µm) of the holotype. Body: 590; ovipositor: 279. Antenna: scape: 70; pedicel: 34; F1: 14; F2: 40; F3: 39; F4: 46; F5: 43; F6: 46; Clava: 100. Forewing: 515:82; longest marginal seta: 182. Hind wing: 484:22; longest marginal seta: 145.

MALE. Length 430–515 µm. Similar to female but general body color darker (dorsum of mesosoma and metasoma brown to dark brown except anterior scutellum light brown and lobes of posterior scutellum yellow); appendages pale light brown except F2–F11 brown. Antenna as in Fig. 14. Forewing usually slightly wider than in female (6.2–6.5x as long as wide), with disc notably more setose and with a relatively smaller bare area, if any. Genitalia (Fig. 15) elongate, with hooked digiti.

Female diagnosis. This new species belongs to the *incarnatus* species group as defined by Chiappini *et al.* (1996). Females differ from *A. daanei* and *A. tretiakovae* by a relatively longer ovipositor, as indicated in the key, and from *A. epos* primarily by forewing proportions (6.3–6.7x as long as wide in *A. vulneratus*, 7.9–8.6x as long as wide in *A. epos*) and shorter ovipositor (2.5–2.7x as long as protibia in *A. vulneratus*, 2.8–3.1x as long as protibia in *A. epos*), and also by F3 usually lacking a mps, although females of both species may occasionally have a mps on F3 of one antenna but lack one from the other. All these species are also genetically distinct (see below). Females of *A. vulneratus* differ from *A. empoascae* in usually lacking a mps on F3 and also in having a relatively longer ovipositor. Females of *A. empoascae* always bear a mps on F3 of both antennae, and the ovipositor is 2.2–2.4x as long as the protibia (Triapitsyn 1997). Males of *A. vulneratus* are very similar to those of *A. daanei* and *A. epos*.
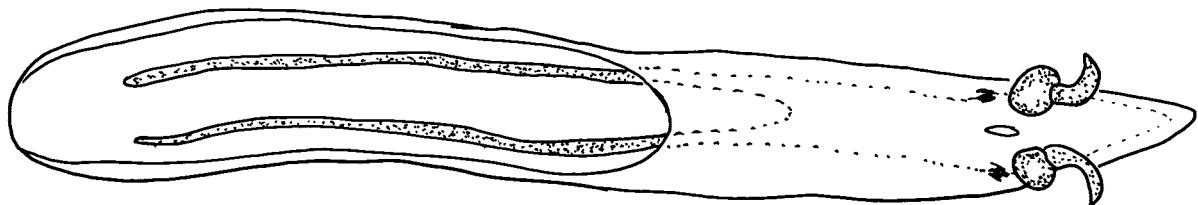
Etymology. The specific name is an adjective referring to the likely leafhopper host of this species.
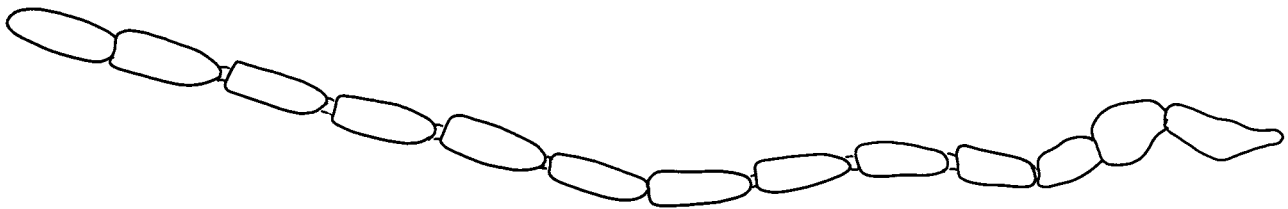
FIGURES 13–15. *Anagrus vulneratus* (paratypes, Colorado). 13. Female forewing. 14. Male antenna. 15. Male genitalia, dorsal view. Scale bars = 0.1 mm.

Host. *Erasmoneura vulnerata* (Fitch), most likely. Females of *A. vulneratus* [as *Anagrus* new species] from Grand Junction, Colorado, failed to parasitize fresh eggs of *Homalodisca vitripennis* (Germar) under quarantine laboratory conditions (Morse & Stouthamer 2007).

Comments. Zimmerman *et al.* (1996) likely referred to this species as "*Anagrus epos* Girault", but this is now impossible to verify because no voucher specimens of their study are available. Interestingly, and contrary to the earlier report by Zimmerman *et al.* (1996), we did not find any specimens of *Erythroneura ziczac* Walsh on grapevines in Grand Junction and Palisade, Mesa County, Colorado. The grapevines in the commercial and experimental vineyards there were predominantly infested by *E. vulnerata*, whereas the following deltocephaline leafhoppers were also present in much smaller numbers: *Balclutha* sp., *Circulifer tenellus* (Baker), and *Colladonus* sp.

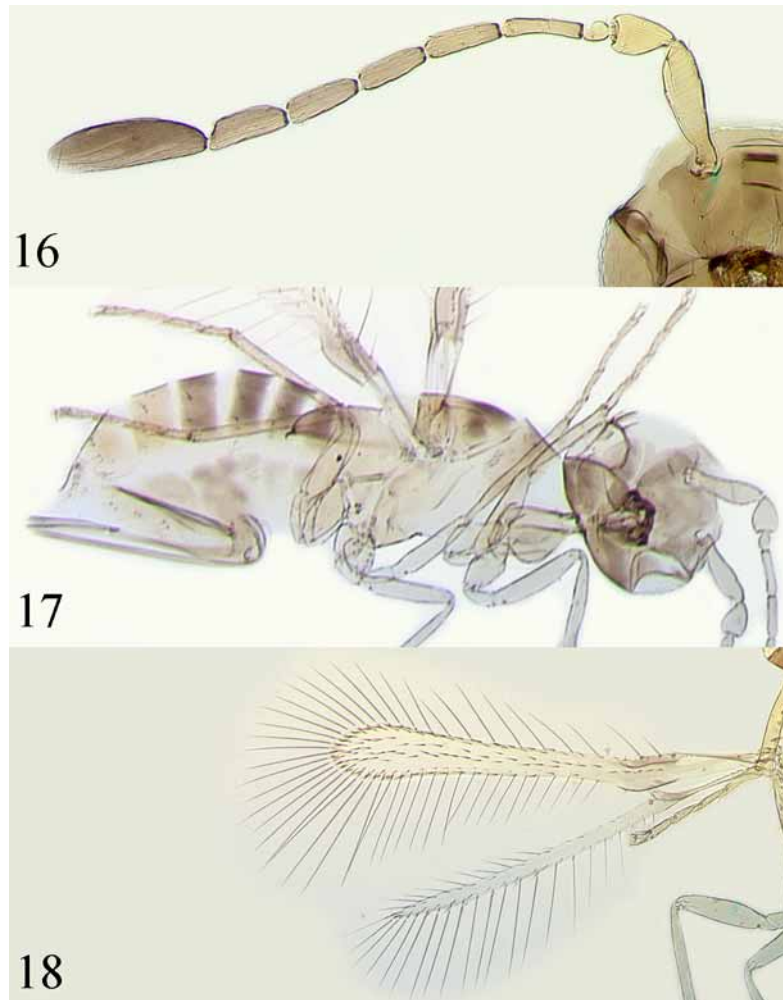***Anagrus*** (***Anagrus***) **sp. near *A. vulneratus* Triapitsyn**
[Not included in the key]
(Figs 16–18)

Anagrus epos Girault: González *et al.* 1988: 23–25 (misidentification, in part [specimens from Sonora]).
Anagrus (*Anagrus*) *epos* Girault: Triapitsyn 1998: 100–103 (misidentification, in part [specimens from Baja California and Sonora]).
Anagrus sp.: Morse & Stouthamer 2007: 95.

FIGURES 16–18. *Anagrus* sp. near *vulneratus* female (Sonora). 16. Antenna. 17. Body (lateral view). 18. Wings.

Material examined. MEXICO, Sonora, W of Hermosillo (Carretera a Bahía Kino km. 12.6), Campo Experimental INIFAP-CECH "Costa de Hermosillo", 27.vii.1994, D. González, S.V. Triapitsyn, D. Powell, A. Fú-Castillo (from eggs of *Erasmoneura variabilis* (Beamer) on grape leaves, University of California, Riverside quarantine S&R # 94–33), 1 female, 1 male on slides and numerous females and males in ethanol [UCRC]. Also 1 male from Baja California and numerous specimens of both sexes from Sonora listed by Triapitsyn (1998).

    Host. *Erasmoneura variabilis* (Beamer).

    Comments. According to the molecular data presented below, specimens from Sonora from eggs of *Erasmoneura variabilis* on grape that were listed under *A. epos* by Triapitsyn (1998) likely belong to a separate species. Morphologically, females are quite similar to *A. vulneratus* from Colorado, including the antenna (Fig. 16), which often has a mps on F3, and the forewing (Fig. 18), which often lacks a bare area on the broadest part of the disc. The ovipositor (Fig. 17), however, is slightly shorter than in *A. vulneratus* from Colorado, and the external plate of the ovipositor has 3 distal setae. Since the Mexican specimens are

genetically somewhat different from *A. vulneratus* from Colorado we prefer to call them *A.* sp. near *A. vulneratus*. As noted by Triapitsyn (1998), *A.* sp. near *A. vulneratus* (as *A. epos* from Sonora) is very similar morphologically to *A. empoascae* whose forewing, however, has a small, more or less defined bare area in the broadest part; also, the latter species parasitizes mainly eggs of *Empoasca* spp. on weeds and other low vegetation and crops (Triapitsyn 1997, 2002). Their species status needs to be further investigated using molecular methods and cross-breeding experiments but that is beyond the scope of this study.
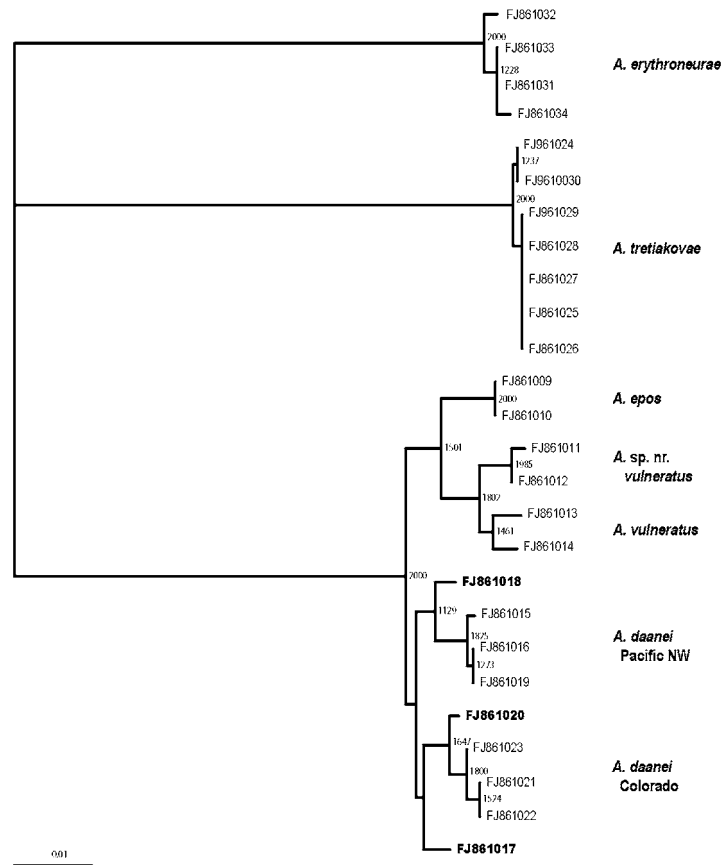

FIGURE 19. Relationships between North American *Anagrus* populations inferred from 28sD2 rDNA sequences. Bootstrap Neighbour-Joining tree with 2000 iterations, produced using ClustalX version 1.83. Sequences in bold represent three *A. daanei* specimens from California.

Results of the molecular analyses

The aligned 28sD2 sequences produced a sequence matrix 607bp long (GenBank accessions FJ861009–FJ861034). Within this matrix, 27 positions included alignment gaps and were excluded from analysis. The outgroup, *A. erythroneurae*, was, as expected, the most divergent population studied (Table 1). Within the *A. epos* species complex, differences in the 28sD2 region ranged from less than 0.2% (or a mean of 1.0 nucleotide substitutions over 580bp) in the most similar groups (*A. tretiakovae* populations from Arizona and New Mexico), to 12.4% (or a mean of 72.0 nucleotide substitutions) in the most divergent populations (*A. epos* from Minnesota and *A. tretiakovae* from Arizona) (Table 1). Indeed, the populations of *A. tretiakovae* were highly divergent from the populations of other members in the *A. epos* species complex, showing a minimum of 11.8% divergence (or a mean of 68.2 nucleotide substitutions; Table 1; Figure 19). Intra-population sequence polymorphism in 28sD2 was surprisingly high, with seven of the nine populations having more than one haplotype, and a maximum of 0.9% divergence (or a mean of 5.3 nucleotide substitutions) between specimens of *A. daanei* from California (Table 1). Indeed, this level of divergence was similar to pair-wise levels of divergence between all three geographic populations of *A. daanei* (Table 1).

TABLE 1. DNA sequence variation in a 580bp fragment (alignment gaps excluded) of 28sD2 of the *Anagrus* study populations. In the rows and columns below, average number of pairwise differences (κ) are shown within (diagonal element, to the left of the division) and between nine population groups (below diagonal). The diagonal element also displays haplotype diversity (Hd) to the right of the division. Population names were applied in retrospect to the findings of the current study.

	A. epos (MN)	A. sp. nr. vulneratus (SO)	A. vulneratus (CO)	A. daanei (CO)	A. daanei (WA/BC)	A. daanei (CA)	A. tretiakovae (NM)	A. tretiakovae (AZ)	A. erythroneurae (CA)
A. epos (MN; n = 2)	0.000/0.000								
A. sp. nr. vulneratus (MX; n = 2)	8.500	1.000/1.000							
A. vulneratus (CO; n = 2)	10.000	6.000	4.000/1.000						
A. daanei (CO; n = 4)	11.667	14.167	13.167	0.667/0.667					
A. daanei (WA/BC; n = 3)	10.667	12.167	13.667	9.000	0.667/0.667				
A. daanei (CA; n = 3)	10.333	12.500	13.000	5.333	5.444	5.333/1.000			
A. tretiakovae (NM; n = 5)	71.600	71.100	69.600	69.267	71.067	68.200	0.600/0.600		
A. tretiakovae (AZ; n = 2)	72.000	71.500	70.000	69.667	70.667	68.333	0.400	0.000/0.000	
A. erythroneurae (CA; n = 4)	73.250	72.750	70.250	69.917	69.917	69.250	69.650	69.250	1.500/0.833

Abbreviations: Canada: BC=British Columbia; Mexico: SO=Sonora; USA: AZ=Arizona, CA=California, CO=Colorado, MN=Minnesota, NM=New Mexico, WA=Washington.
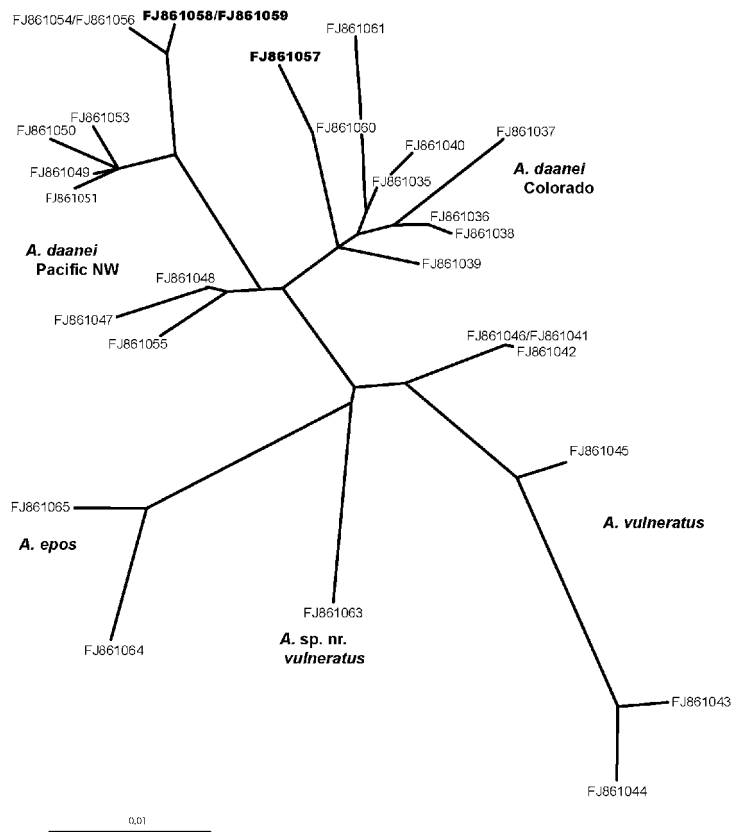
FIGURE 20. Simple Neighbour-Joining tree highlighting the multiple gene-family origin of copies of the ITS2 region in individuals from select members of the *Anagrus epos* species complex. Sequences in bold represent three ITS2 clones from a single specimen of *A. daanei* from California.

Like 28sD2, sequences of the ITS2 region of *A. erythroneurae* and *A. tretiakovae* (GenBank accessions FJ861068–FJ861073 and FJ861066–FJ861067 respectively) were very divergent from the ITS2 of the other populations studied and they were excluded from further consideration. Cloned sequences of the ITS2 of the remaining members of the *A. epos* species complex ranged in length from 621–659bp and their alignment produced a matrix 674bp long (GenBank accessions FJ861035–FJ861065). The species divisions identified by the 28sD2 were again apparent (Fig. 20). Variation between cloned sequences was relatively high within populations and even within an individual. Most notably, clones from a single specimen of *A. vulneratus* and a single specimen of *A. daanei* from California were 5.9% and 5.1% dissimilar respectively (i.e. the proportion of identical residues between each sequence pair was 0.941 and 0.949) (Fig. 20). This is perhaps unsurprising given that sequencing of the ITS2 region required cloning for all populations except *A.* sp. nr. *vulneratus*.

Discussion

The success or failure of several biological control projects against insect pests and weeds has hinged on the correct taxonomic identification of the target and its natural enemies (Gordh & Beardsley 1999). Incorrect understanding of the taxonomy and subsequent interrelationships between the target and its natural enemy guild are serious impediments to an efficacious biological control program. For example, *Trichogramma minutum* Riley and *T. platneri* Nagarkatti are important commercially available biological control agents that are morphologically indistinguishable but reproductively incompatible (Nagarkatti 1975). Experimental work and subsequent modeling with these two species of *Trichogramma* Westwood has indicated that because pre-mating isolation mechanisms are absent (e.g., pre-mating courtship behaviors that prevent coupling of males and females from different species), severe negative effects on biological control can occur. Negative effects

manifest themselves because females that mate with males from different species fail to produce female offspring. This occurs because *Trichogramma*, like *Anagrus*, are haplodiploid, i.e. fertilized eggs produce female offspring and unfertilized eggs produce male offspring. Thus, in situations where incompatible interspecific matings occur, both species fail to produce females and the potential population growth of both parasitoid species is reduced to levels below the growth rate expected for either species in the absence of the other (Stouthamer *et al.* 2000a, b).

For many years, the mymarid wasp *Anagrus epos* had been regarded as the only species of egg parasitoid affecting good natural control of leafhopper species in North American vineyards (Triapitsyn 1998). However, recent research has suggested that in many areas earlier identifications of this species were erroneous and that *A. epos* is in fact a complex of closely related cryptic species (Triapitsyn 1998; Williams & Martinson 2000; Wright & James 2007; Prischmann *et al.* 2007). This study provided new support for the presence of cryptic species from DNA sequence data and identified two further members of this species complex. *Anagrus* specimens reared from eggs of *Erasmoneura vulnerata* on grapevines in Colorado were identified as a new species, *A. vulneratus*. Morphological and molecular evidence also place specimens originally diagnosed as *A. epos* from Sonora, Mexico, close to the new species. Because at present we cannot be certain of the species status of these specimens, we refer to them as *A.* sp. nr. *vulneratus*.

Anagrus epos was redescribed by Chiappini *et al.* (1996) based on a poorly preserved type series, which may contain more than one species. The inherent problem of recognizing *A. epos* was again tackled by Triapitsyn (1998) with a further redescription of this species based on two specimens collected in 1995 from the type locality (Centralia, Illinois), and which closely resembled the lectotype and Girault's (1911) original description. Whilst DNA sequence data are unavailable for specimens from the type locality, the Minnesota "strain" examined in the present study is morphologically indistinguishable from the material used by Triapitsyn (1998) and we confidently classify it as *A. epos*. Of the species described here and in Triapitsyn (1998), which were previously misidentified as *A. epos*, the most divergent is *A. tretiakovae*. The DNA sequences of both 28sD2 and ITS2 place *A. tretiakovae* relatively distant from the remainder of the complex (Table 1; Figs 19, 20), and this is also reflected in the morphology (basal third of the forewing disc of *A. tretiakovae* with an extra well defined, complete longitudinal row of setae as compared to the other species). Morphological diagnosis of the remaining members of the *A. epos* species complex is much more ambiguous, with the division of character states open to personal interpretation. This highlights the importance of integrating data from other sources such as DNA sequences in selecting characters and defining character states.

The relatively high level of intraspecific variation in the 28sD2 and ITS2 of *A. daanei* and *A. vulneratus* was somewhat unexpected. In eukaryotes, ribosomal RNA genes are multi-copy loci that cluster in nucleolar organizing regions (NOR) on one or more of the chromosomes. Typically, these NORs consist of several to several thousand tandomly repeated units, preceded by an external transcribed spacer and interspersed by a non-transcribed spacer region. Each coding unit is further divided into three genes (18s, 5.8s and 28s) separated by two internal transcribed spacers (ITS1 and ITS2) that are known to evolve at varying rates (Hillis & Dixon 1991). It is generally accepted that concerted evolution results in the homogenization of these repeats (Elder & Turner 1995), resulting in much lower variation within species or populations than between. However, intra-population variation in these regions is clearly evident in the 28sD2 sequences of conspecific individuals (Table 1), and even within different ITS2 clones isolated from a single individual (Fig. 20). This is particularly marked in the population of *A. daanei* from California, which displayed a level of intra-population variation in the 28sD2 similar to that between it and each of the *A. daanei* populations from Colorado and the Pacific Northwest. These latter populations displayed much lower intra-population variation (as would be expected under concerted evolution) but are more divergent from each other than they are from the California population (Table 1). This initially led Morse & Stouthamer (2007) to propose that these might in fact be different species. Interestingly, of the three Californian specimens sequenced, two were genetically more similar to the Colorado population, whereas the third was much closer to specimens from the Pacific Northwest (Fig. 19).

A similar pattern was even more evident in the ITS2 clones of one of the three Californian specimens. When sequencing cloned PCR product, a very small amount of inter-clonal variation may be expected as a result of errors made by *Taq* polymerase during amplification (Tindall & Kunkel 1988). However, of three clones sequenced from one Californian individual, two clearly grouped with clones isolated from Pacific Northwest specimens, whereas the third was very different, but highly similar to ITS2 sequences of specimens from Colorado (Fig. 20). A second California specimen yielded only clones that grouped with the Colorado sequences. The rather heterogenous nature of rRNA across the geographic range of *A. daanei* may be viewed as evidence against concerted evolution. However, it is also possible that this apparent mixture of ITS2 clones from different gene families is a reflection of the recent history of these populations. Cloned ITS2 sequences of the Colorado and Pacific Northwest populations formed distinct groups and no intermediates were isolated. In 1985 and 1986, several different populations of "*A. epos*" were imported from Colorado and released against *Erasmoneura variabilis* in central California's San Joaquin Valley (Pickett *et al.* 1987; González *et al.* 1988). It seems probable that some of these releases were in fact *A. daanei*. Prior to these introductions, there may have been two distinct genetic *A. daanei* types (perhaps even species), a "Pacific coastal" type with a range including British Columbia, Washington State and California; and an "inland" type centered in Colorado. Following the release of "*A. epos*" from Colorado in the mid 1980s, hybridization of the two types may have occurred. With such a recent hybridization event, concerted evolution is unlikely to have operated for long enough to homogenize the gene families, and thus resulting in the "mixed bag" that is the current California population of *A. daanei*. Indeed, the level of divergence in the ITS2 regions may be sufficient to suppress recombination between the homologous sequences (Petit *et al.* 1991) and greatly slow, or even prevent, homogenization of the gene families (Muir *et al.* 2001). Unfortunately, our sample is very small and therefore a much more extensive sampling effort, with collections from multiple locations across all three regions, would be required before any strong conclusions can be drawn with respect to this hypothesis.

In similar taxa where morphological identification is difficult, the size of the ITS2 PCR product and restriction fragment length polymorphisms produced by digesting the ITS2 product with appropriate restriction enzymes, has been used as a tool for identifying species (e.g., *Trichogramma*, Stouthamer *et al.* 1999). However, in the *A. epos* species complex, inter- and intra-specific differences in the size of the ITS2 were somewhat overlapping. Furthermore, intra-specific variation in the ITS2 makes it difficult to identify restriction enzymes that work in a consistent manner across all specimens of a single species. Therefore, as a relatively quick and easy method of identifying members of this species complex, we advocate amplifying and sequencing the 28sD2 gene. Sequences can then simply be compared to those deposited in GenBank. If this approach is coupled with a non-destructive DNA extraction method, such as that provided by the EDNA HiSpEx™ Tissue Kit used for some of the material in the present study, extracted specimens can be recovered and slide-mounted for morphological examination, should the DNA sequence reveal something novel.

Only limited attempts have been made to date to release the Minnesota strain of *Anagrus epos* as part of the biological control program against *Homalodisca vitripennis* in California, largely due to the difficulty in rearing large numbers of this insect. So far, no recoveries of this species have been made (D.J.W. Morgan, California Department of Food & Agriculture, Riverside, California, personal communication). Should this insect establish in California and appear to provide improved biological control of glassy-winged sharpshooter over endemic and other introduced egg parasitoids, it might be productive for search further within the *A. epos* complex for species capable of attacking the glassy-winged sharpshooter in California.

Identification of *A. vulneratus* (from Colorado) and *A.* sp. nr. *vulneratus* (from Sonora) as separate entities from *A. epos* may also be important for potential biological control programs against other grape-feeding leafhopper pests such as *Erythroneura* spp. and *Erasmoneura* spp., particularly against the variegated leafhopper, *Erasmoneura variabilis*, in California.

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