Eur. J. Entomol. **102**: 617–624, 2005
ISSN 1210-5759

Molecular and morphological phylogeny of the parasitic wasp genus *Yelicones* (Hymenoptera: Braconidae: Rogadinae)

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Key words. Molecular phylogeny, Braconidae, Rogadinae, *Yelicones*, 28S rRNA, combined analysis, biogeography

Abstract. Phylogenetic relationships of the braconid wasp genus *Yelicones* Cameron are studied using the D2–D3 region of the nuclear 28S rRNA gene, both alone and simultaneously with morphology. The results support a morphology-based phylogeny, presented elsewhere, with *Yelicones* being divided into two major groups corresponding to the New and Old World faunas. The African and Asian species largely form separate clades except for *Yelicones wui* Chen & He from China which is associated with the Afrotropical species. Potential molecular synapomorphies are illustrated.

INTRODUCTION

Yelicones Cameron is a cosmopolitan and highly distinctive genus of parasitic rogadine braconid wasps. The genus is recognised by its robust legs, highly modified and distinctive tarsi with the telotarsus extremely enlarged and the other tarsal segments greatly reduced (Roman, 1910) and by its tridentate mandibles although these are often difficult to observe when they are closed (Quicke & Kruft, 1995; Areekul & Quicke, in press a). As in all other Rogadinae sensu stricto, *Yelicones* sp. are koinobiont endoparasitoids of Lepidoptera larvae, specifically Pyralidae and Crambidae (Shenefelt, 1975; Areekul & Quicke, in press a). *Yelicones* mummify the host before pupating within the host (Quicke & Chishti, 1997; Quicke & Shaw, in press).

The genus was known for many years after its original description (Cameron, 1887) from only a few specimens collected in the Neotropics (Shenefelt, 1975). However, over the last 40 or so years, many more species have been described, extending the known range to the Indo-Australian (including Oceania), Afrotropical, East Palaearctic and Oriental regions [Fischer, 1961, 1962 (as *Pectenopius* Fischer, a junior synonym of *Yelicones*); Togashi, 1980; Papp, 1985, 1989, 1991, 1992; Belokobylskij, 1993a,b; Quicke & Kruft, 1995; Chen & He 1997; Quicke et al., 1996, 1997, 1998; Quicke & Chishti, 1997; Areekul & Quicke, 2002; Areekul & Quicke, 2004a,b, in press a]. Thus the genus is now known to be widely distributed throughout the Old and New Worlds and has recently been recorded from the West Palaearctic (Spain: Shaw, 1998).

Recently, Areekul & Quicke (in press a) revised *Yelicones* species from North, Central and South America. Sixty-three species were described as new and a species level morphological phylogeny based on 116 characters was presented for the world fauna. The results showed a nearly perfect division into two large groups comprising the Old World (OW) and the New World (NW) species, respectively. In the preferred tree in which colour characters were excluded, nearly all the OW species formed a monophyletic group nested within a basal grade of NW taxa, though within the OW clade species from the Australian, African and Oriental regions were largely intermixed, probably due to a lack of phylogenetic signal. However, inclusion of colour characters had a major impact on tree topology in terms of whether the OW or NW species were derived groups: when colour characters were included in the analysis, the NW species appeared to be derived from within a paraphyletic OW grade.

The above conflict in topologies between analyses including or excluding colour characters could be the result of the latter being more homoplastic at higher taxonomic levels, particularly as none of the outgroup taxa are very close to *Yelicones*. However, the fit of the colour characters on the trees from the simultaneous analysis of colour and morphological characters was higher than the fit of the morphological characters. This suggested that colour character data should not be discounted immediately, though it is also possible that colour characters might be evolving differently from morphological ones, especially when they are involved in aposematic patterns (Areekul & Quicke, in press b). Here we attempt to resolve the conflict by using molecular data in addition to morphological data. Due to the rarity of this taxon, very few fresh specimens were available for sequencing. A total of 11 species, 3 from the NW and 4 each from Africa and Asia were sequenced. We were not able to obtain fresh material from Australia.

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TABLE 1. Species included in this study, collection localities and EMBL/GenBank accession numbers.

Taxon	Localities	Voucher depository*	Genbank accession no.
Y. fisheri Areekul & Quicke	Tollara Province, Madagascar	CAS	AJ784318
Y. spectabile Areekul & Quicke	Tollara Province, Madagascar	CAS	AJ784319
Y. variegates Areekul & Quicke	Ambilanivy, Madagascar	CAS	AJ784320
Y. kibaleiensis Areekul & Quicke	Kibale, Uganda	NHM	AJ784321
Y. belokobylskiji Quicke, Chishti & Chen	Thailand	NHM	AJ784322
Y. siamensis Areekul & Quicke	Chonburi, Thailand	NHM	AJ784323
Y. wui Chen & He	China	—	AY167659
Y. nipponensis Togashi	Taiwan	NHM	AJ784324
Y. delicatus (Cresson)	Florida, USA	NHM	AJ784327
Y. zitanae Areekul & Quicke	La Selva Biological Station, Costa Rica	ESUW	AJ784325
Y. belshawi Areekul & Quicke	Rio Mogi Guaçu Luís, Brazil	DCBU	AJ784326
Pseudoyelicones limonensis Areekul & Quicke	Quepos Province, Costa Rica	INBC	AJ784929
Bulborogas van Achterberg	Columbia	NHM	AJ784930
Rogas Nees	Amani, Tanzania	NHM	AJ784931
Conspinaria Schulz	Taiwan	NHM	AJ509015
Spinaria Brullé	Malaysia	NHM	AJ784960
Aleiodes dispar (Haliday)	Silwood Park, UK	NMS	AJ784935
Aleiodes nigricornis Wesmael	Scotland, UK	NMS	AJ784934
Aleiodes praetor (Reinhard)	Silwood Park, UK	NMS	AJ784936
Stiropius Cameron	Costa Rica	none	AJ784961
Tebennotoma Enderlein	Taiwan	NHM	AJ784933
Clinocentrus Haliday	Silwood Park, UK	none	AJ784962
Mesocentrus Szépligeti	Canberra, Australia	NHM	AJ784932

* Abbreviations: CAS – California Academy of Sciences, San Francisco; DCBU – Universidade Federal de São Carlos, São Carlos, São Paulo, Brazil; ESUW – University of Wyoming, Laramie, Wyoming; INBC – Instituto Nacional de Bioversidad (INBio), Santo Domingo de Heredia, Costa Rica; NHM – Natural History Museum, London; NMS – National Museums of Scotland, Edinburgh.

MATERIAL AND METHODS

Laboratory protocols

A middle leg was removed from each individual (7 preserved in ethanol and 3 from recently collected dry pinned specimens). Sequence data for *Y. wui* Chen & He came from Chen et al. (2003). After drying, the tissue was ground in 50 µl of 5% (w/v) Chelex (Bio-Rad, Hercules, CA, USA)/TE containing 12 µg/ml proteinase K, and digested for 100 min at 56°C. Proteinase K was then heat-inactivated by incubation at 96°C for 15 min, and the samples stored at –20°C without separating the supernatant prior to PCR amplification.

Samples were thawed on ice, briefly vortexed and the Chelex pelleted by centrifugation at 16,300 g for five min prior to removal of 2 ml of supernatant as template for each PCR reaction. PCR reactions were carried out in a 25 µl final volume using *pureTaq* ready-to-go PCR beads (Amersham Biosciences, Buckinghamshire, UK) with 400 nM of each of 28S forward (GCG AAC AAG TAC CGT GAG GG; Hancock et al., 1988) and 28S reverse (TAG TTC ACC ATC TTT CGG GTC; Campbell et al., 1993) primers. The PCR program for all the amplifications had an initial 3 min denaturation at 80°C, followed by 40 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. A 10 min extension followed the final cycle. The PCR products were cleaned with the wizard SV gel (Promega, Madison, WI, USA) and PCR clean up system and then sequenced using the dideoxy terminator cycle sequencing (Applied Biosystems, Inc., Foster City, CA, USA) with an ABI 3700 automated DNA sequencer according to manufacturers instructions.

All species were sequenced in both directions and all pherogram interpretations checked manually.

Taxa investigated

The species included in this study are listed in Table 1, together with their provenances, depositories and EMBL/GenBank sequence accession numbers. The twelve outgroup taxa chosen for this study were as follows: *Aleiodes nigricornis* Wesmael, 1838, *Aleiodes praetor* (Reinhard, 1863), *Aleiodes dispar* (Haliday, 1833), *Pseudoyelicones limonensis* Areekul & Quicke, *Rogas* Nees, 1818, *Spinaria* Brullé, 1846, *Conspinaria* Schulz, 1906, *Bulborogas* van Achterberg, 1995, *Stiropius* Cameron, 1911, *Clinocentrus* Haliday, 1833, *Tebennotoma* Enderlein, 1912, *Mesocentrus* Szépligeti, 1900, and of which *Tebennotoma* was scored from the literature (van Achterberg, 1995). The use of multiple outgroups reflects the general lack of understanding of rogadine relationships (see Zaldivar-Riverón et al., 2004), and in particular the lack of an obvious sister taxon to *Yelicones*, though *Bulborogas* and *Pseudoyelicones* van Achterberg, Panteado-Dias & Quicke species are similar, perhaps convergently, in body form, being robust with swollen femora and shortened tarsi (see Areekul et al., 2004). Three species of *Aleiodes* have been included because this is the largest and morphologically most diverse genus of the Rogadinae sensu stricto (Chen & He, 1997). Zaldivar-Riverón et al. (2004) studied the venom apparatus of 46 species of *Aleiodes*, six different types of venom apparatus are recognised which partially corresponded with the different subgenera. The three species chosen here represent groups with markedly different types of venom apparatus: *A. nigricornis* belongs to the subgenus *Aleiodes* (sensu van

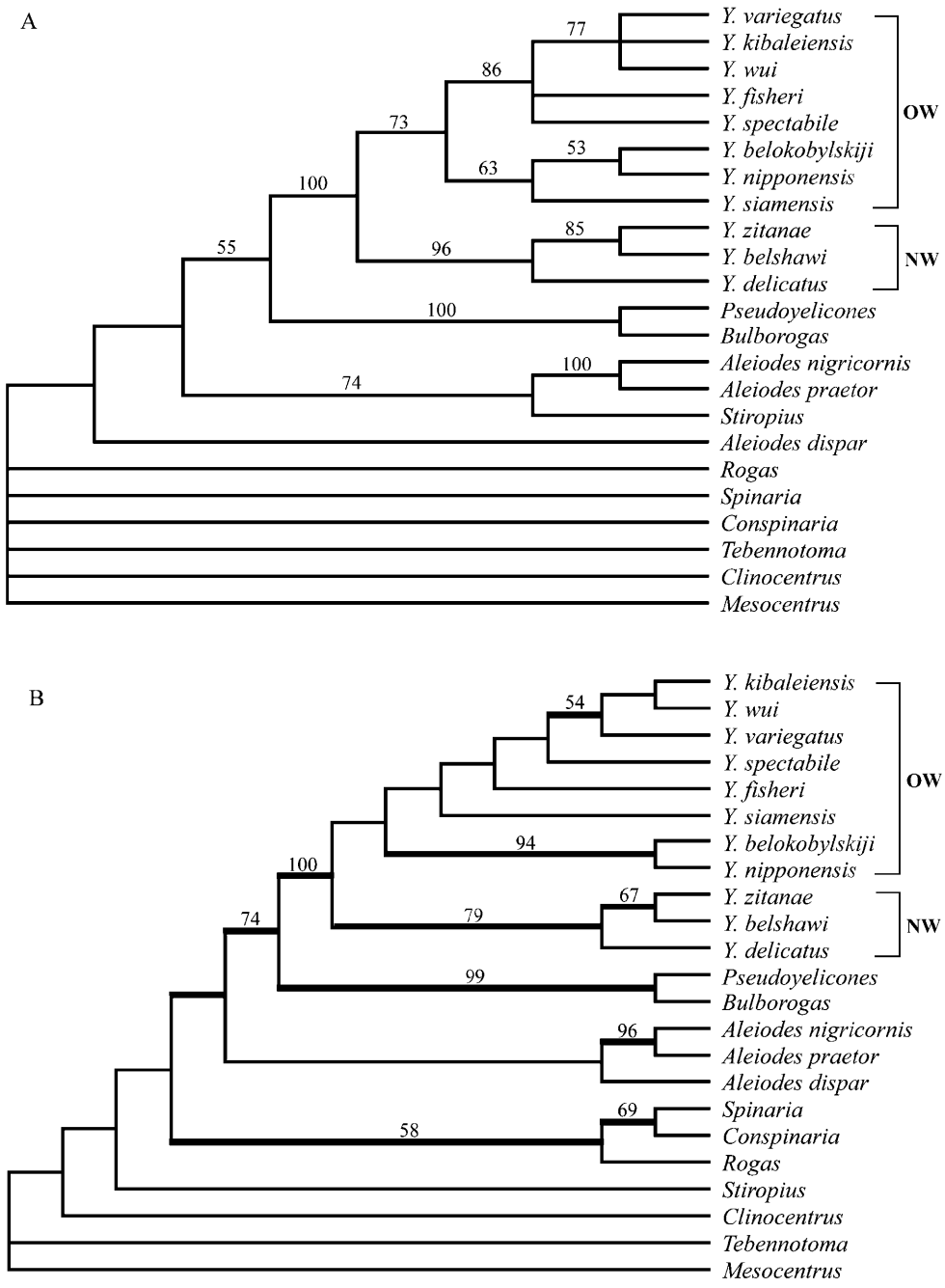

Fig. 1. A – strict consensus of 2 MPTS from molecular-based phylogeny of *Yelicones*; B – successive approximations weighting tree from the combined analysis. Clades congruent with the strict consensus of MPTs from unweighted analysis are shown in bold. Bootstrap values greater than 50% from unweighted analyses are indicated

Achterberg, 1991), *A. praetor* belongs to the subgenus *Neorhogas* Szépligeti and *A. dispar* belongs to the subgenus *Heterogamus* Wesmael. Three members of the *Rogas* group of genera (*Rogas*, *Conspinaria* and *Spinaria*) were included as they appear to form the sister group of the *Aleiodes* group and were expected to help show whether *Yelicones*, *Bulborhogas* and *Pseudoyelicones* form a natural group. The betylobraconine, *Mesocentrus* Szepligeti, was included because betylobraconines also have shortened fore tarsi and robust femora (van Achterberg, 1995) and it is not certain that they are not derived rogadines and thus could be related to *Yelicones*. Finally, *Stiropius* of the Stiropiini and *Clinocentrus* and *Tebennotoma* of the Clinocentrini were included as these tribes are putatively most basal within the Rogadinae, the former because of their parasitization of leaf-mining microlepidoptera larvae (Gracillariidae and Lyonetiidae), and the latter because of their more exserted ovipositors. Trees were rooted with *Tebennotoma*.

Phylogenetic analysis

Sequences were aligned by eye and analyses performed with several ambiguous regions excluded and with remaining gaps treated as uninformative (see Fig. 2). The alignment used with excluded regions indicated is available from http://www.treebase.org/treebase/ (study accession number S1356; matrix accession number M2396). Maximum parsimony analyses were implemented in PAUP* version 4.0b10 (Swof-

ford, 1998) using 10,000 random additions with tree bisection reconnection (TBR) branch swapping holding a maximum of one tree. Relative branch support was assessed by bootstrapping (Felsenstein, 1985) using 500 replicates, each based on 100 random additions.

Eighty morphological characters were used in a combined analysis of molecular and morphological data. The morphological characters used are a subset of those used by Areekul & Quicke (in press a) that are informative for the *Yelicones* species investigated here. See appendix 1 for character definitions and appendix 2 for the data matrix.

Successive approximations weighting (SAW: Farris, 1969) using the maximum value of the retention index as the reweighting function (see Quicke et al., 1999; Gauthier et al., 2000) was applied to the combined molecular plus morphological data set to obtain a more resolved tree.

RESULTS

Molecular results

Parsimony analysis of 28s rRNA sequence data based on 564 alignable base pairs resulted in 2 most parsimonious trees (MPTs), the strict consensus of which is shown in Fig. 1A (tree length = 386, CI = 0.642, RI = 0.681). The analysis supports the morphological-based phylogeny of Areekul & Quicke (in press a) in that *Yelicones* species are divided into 2 large clades, corresponding to OW and NW species, with high bootstrap support (100) (Fig. 1A). Many clear sequence features separate the OW and NW species including several insertions/deletions (indels) that were excluded from or were uninformative in our analyses (Fig. 2, fragments 1, 2, 4, 6). Additionally, a clear resolution of the OW group was obtained, separating *Yelicones* into largely African and Asian clades (see Fig. 2, fragments 1, 2, 4, 5). The problematic taxon, *Y. wui* from China, being consistantly recovered with the African clade. *Yelicones delicatus* from North America has a distinctive sequence relative to other *Yelicones*, with many unique features such as a two base insertion in fragment 6, a deletion in fragment 1 and a 1 base substitution in fragment 3 (Fig. 2). The outgroup taxa are not well resolved.

Combined analysis

The combined analysis of morphological and molecular data sets resulted in 6 MPTs (tree length = 787, CI = 0.478, RI = 0.544). The strict consensus of these was less resolved than that from the molecular analysis of *Yelicones* relationships (see thickened branches in Fig. 1B). The NW species were recovered as monophyletic but the OW species were hardly resolved. However, there is more resolution among the basal taxa, with the Rogadini + Yeliconini recovered as monophyletic in addition to the *Rogas* genus group (*Spinaria*, *Conspinaria* and *Rogas*) being recovered as monophyletic. A more resolved tree was found following successive approximations weighting (SAW): congruence between the SAW tree and the strict consensus tree of 6 MPTs is indicated in bold (Fig. 1B). The OW and the NW species again form two separate clades. However, the Asian species are not recovered as monophyletic except for a clade comprising *Y. nipponensis* + *Y. belokobylskiji*. *Y. wui* was recovered with the otherwise monophyletic African taxa. The SAW tree shows additional resolution among the outgroup taxa compared with the strict consensus tree from molecular data alone including the three *Aleiodes* species recovered as monophyletic. *Pseudoyelicones* and *Bulborogas* formed a clade with 100% bootstrap support as found with the molecular data, and again they formed a sister group to *Yelicones* with slightly decreased bootstrap support (99%) (Fig. 1).

DISCUSSION

Our molecular-based phylogeny supports the phylogeny based on morphological characters and indicated that *Yelicones* species are divided into 2 large clades, the OW and NW species groups. DNA sequences show strong signals separating the OW and NW, and also differentiate African from Asian species [except for the problematic *Y. wui* (Fig. 1A)]. In morphology-based analysis including colour characters (Areekul & Quicke, in press a), *Y. wui* was recovered as sister group to *Y. variegatus* from Madagascar, and within the NW clade in analysis excluding coloration. *Y. wui* has more or less the same colour pattern as many species found in Africa (e.g. *Y. variegatus* and *Y. kibaleiensis*), their ground-plan body colour being yellow with brown to dark brown distributed throughout the body. This might lead to the recovery of *Y. wui* closely related to *Y. variegatus* and *Y. kibaleiensis* in morphology-based phylogeny with colour characters included. There are a few species from Asia that have more or less the same colour pattern as *Y. wui* (e.g. *Y. elegans* and *Y. koreanus*) but the lack of specimens of these taxa for DNA sequencing prevented us from telling whether these also belong to the same group.

In the morphology-based phylogeny (Areekul & Quicke, in press a), *Y. delicatus* was often recovered between the OW and NW groups, especially in analysis including coloration. Its morphology is rather intermediate between that of OW and NW species, (e.g., it has an almost complete occipital carinae and the maximum length of eye is more than 1.8 times the width of eye, both of which are characteristic of most OW species). However, the 28S rDNA data clearly show that it belongs to the same monophyletic group as the other two NW species examined (note the apparent synapomorphic substitutions in fragments 3 and 6 and the possibly synapomorphic "AAA" insertion in fragment 6 (Fig. 2).

The molecular and combined analyses presented here do not resolve the initial question (based on morphological analyses including and excluding colour characters) as to whether NW or OW *Yelicones* species are basal (forming a paraphyletic grade) (Areekul & Quicke, in press a). Instead they both point to a reasonably well-supported scenario with both NW and OW groups being monophyletic. However, our taxon sampling was poor especially for the NW clade, and additional taxon sampling will be required fully to resolve this matter.

The molecular phylogeny also confirms a close relationship between *Pseudoyelicones* and *Bulborogas* as was suggested by Areekul et al. (2004) based on venom appa-

Taxon	Part of 28S rDNA sequence fragments		
	Fragment 1		Fragment 2
Y. fisheri	AGCCGCATTTAT**TATATA**TTA----------TGCGT		G**A**GTTA**T**TATTT--**TT**ATAG
Y. spectabile	AGCCGCATCTAT**TATATA**----------TGCGT		G**A**GTTA**T**TATTT--**TT**ATAG
Y. variegatus	AGCCGCATTTAT**TATATA**----------TGCGT		G**A**GTTA**T**TATTT--**TT**ATAG
Y. kibaleiensis	AGCCGCATTTAT**TATATA**----------TGCGT		G**A**GTTA**T**TATTT--**TT**ATAG
Y. wui	AGCCGCATTTAT**TA**T**ATA**----------TGCGT		G**A**GTTA**T**TATTT--**TT**ATAG
Y. belokobylskiji	AGCCGCATATATATATA**CATACTATATATTATAAA**TGCGT		GGGTCACTATTT**TATT**ATAG
Y. siamensis	AGCCGCA--TATATATA**CATACTATATATTATAAA**TGCGT		GGGTTACTATTT---**T**ATAG
Y. nipponensis	AGCCGCAAATATATATA**CATACTATATATTATAAA**TGCGT		GGGTTACTATTT---**T**ATAG
Y. delicatus	AGCCGCATT-ATA----------TGCGT		G**A**GTTACTATTT----ATAG
Y. zitanae	AGCCGCATTTATATTTAATATA----------TGCGT		GGGTTACTATTT----ATAG
Y. belshawi	AGCCGCATTTATATTTAATATA----------TGCGT		GGGTTACTATTT----ATAG
P. limonensis	AGCCGCAAATTTTATTTAATT----------TGCGT		GGGTTACTACTT-------G
Bulborogas sp.	AGSCGSAAATKAATA----------TGCGT		GGGTTACTACTT---GGGAG
Rogas sp.	AGCCGCAATTATA----------TGCGT		GGGTTACTACTT----GTAG
Spinaria sp.	AGCCGCAATTATA----------TGCGT		GAGTTACTACTT----GTAG
Conspinaria sp.	AGCCGCAAGTATA----------TGCGT		GAGTTACTACTT----GTAG
A. dispar	AGCCGCATATTTA-TA----------TGCGT		GGGTTACTACTT----GTAG
A. nigricornis	AGCCGCTAAATTTATTA----------AGCGT		GGGTTACTACTT----GTAG
A. praetor	AGCCGCTAAATTTATTAA----------AGCGT		GGGTTACTACTT----GTAG
Stiropius sp.	AGCCGCATA-ATTTTTA----------TGCGT		GGGTTACTACTT----GTAG
Tebennotoma sp.	AGCCGCAT--ATTTATTATT----------TGCGT		GAGTTACTACTT----GTAG
Clinocentrus sp.	AGCCGCAC--ATTTTA----------TGCGT		GGGTTACTACTT----GTAG
Mesocentrus sp.	AGCCGCATATTTATTA----------TGCGT		GGGTTACTACTT----GTAG
	Fragment 3	Fragment 4	Fragment 5
Y. fisheri	CATTA**A**TGTTTC--CTGA-CCGAC	ACGGC**T**TAAT--GATTAGCC	AATTT**T**AT**TA**-----GTAGTA
Y. spectabile	CATTG**A**TGTTTT--CTGA-CCGAC	ACGGC**T**CAATT-GAT-AGCC	AATTT**T**AC**TA**-----GTAGTA
Y. variegatus	CATTG**A**TGTTTTTTCTGA-CCGAC	ACGGC**T**CAATT-GAT-AGAC	AATTT**T**AC**TA**-----GTAGTA
Y. kibaleiensis	CATTG**A**TGTTTT--CTGA-CCGAC	ACGGC**T**CAATT-GAT-AGAC	AATTT**T**AC**TA**-----GTAGTA
Y. wui	CATTG**A**TGTTTT--CTGA-CCGAC	ACGGC**T**CAATT-GAT-AGAC	AATTT**T**AC**TA**-----GTAGTA
Y. belokobylskiji	CATTG**A**TGTTTT--CTGA-CCGAC	ACGGC**T**CAATT**T**GAT-AGCC	AATTTCATA------GTAGTA
Y. siamensis	CATTG**A**TGTTTT--CTGA-CCGAC	ACGGC**T**CAATT**T**GAT-AGCC	AATTTCATA------GTAGTA
Y. nipponensis	CATTG**A**TGTTTT--CTGA-CCGAC	ACGGC**T**CAATT**T**GAT-AGCC	AATTTCATA------GTAGTA
Y. delicatus	CATTGGTGTTTT--CT**A**A-CCAAC	ACGGCCCAATT-GAT-AGAC	AATTTCATA------GTAGTA
Y. zitanae	CATTGGTGTTTT--CT**A**A-CCGAC	ACGGCCCAATT-GAT-AGAC	AATTTCATA------GTAGTA
Y. belshawi	CATTGGTGTTTT--CT**A**A-CCGAC	ACGGCCCGATT-GAT-AGAC	AATTTCATA------GTAGTA
P. limonensis	CCTTGGTGTTTT--CTGATCCGAC	ACGGCCCAAGT-GAT-AGTC	AATTCAATTACTA--GTAGTA
Bulborogas sp.	CCTTGATGTTTT--CTGA-CCGAC	AGGGGTCAAGT-GAT-TGGC	AATTTTTTTAATTAAGTAGTA
A. dispar	CCTTGGTGTTTT--CTGA-CCGGC	ACGGCCCAAGT-GAT-AGCC	AATTACTGATA----GTA---
A. nigricornis	CCTTGGTGTTTT--CTGA-CCGGC	ACGGCCCAAGT-GAT-AGCC	AATTTACTGATA---GTA---
A. praetor	CCTTGGTGTTTT--CTGA-CCGGC	ACGGCCCAAGT-GAT-AGTC	AATTTACTGATA---GTA---
Rogas sp.	CCTTGGTGTTTT--CT**A**A-CCGGC	ACGGCCCAAGT-GGT-AGTC	AATTTCTG-------ATAGTA
Spinaria sp.	TCTTGGTGCTTT--CT**A**A-CCGGC	ACGGCCCAAGT-TATTAGTC	AATTTTTG-------GTAGTA
Conspinaria sp.	CTTTGGTGTATT--CTGG-CCGGC	ACGGCCCAAAT-GGT-AGCC	AATTTCTG-------GTAATA
Stiropius sp.	CCTTAGTGTTTC--CTGA-CCGGC	ACGGCCTAAGT-GAT-AGCC	AATTTCTTA------GTAGTA
Tebennotoma sp.	CTCTGATGTTTT--CTGA-TCAAC	ACGGCTCAGAA-GGGCAGAC	AATTACTG-------GTATTA
Clinocentrus sp.	CCTTGGTGTTTT--CTGA-CCGAC	ACGGCCCAAGT-GGT-AGCC	AATTTCTG-------GTAGTA
Mesocentrus sp.	CCTTGGTGTTTT--CTGA-CCGGC	ACGGCCCAAAT-GGT-AGTC	AATTTCTG-------GTAATA
	Fragment 6		
Y. fisheri	TTA-AACGGTGTTCTAGGACTGGC---TAAA**A**-**T**TTAA----TTA		
Y. spectabile	TTA-AACGGTGTTCTAGGACTGGC---TAAA**A**-**T**TTAA----TTA		
Y. variegatus	TTA-AACGGTGTTCTAGGACTGGC---TAAA**A**-**T**ATAA----TTA		
Y. kibaleiensis	TTA-AACGGTGTTCTAGGACTGGC---TAAA**A**-**T**ATAA----TTA		
Y. wui	TTA-AACGGTGTTCTAGGACTGGC---TAAA**A**-**T**ATAA----TTA		
Y. belokobylskiji	TTA-AATGGTATTCTAGGACTGGC---TAAA**A**-**T**TTAA----TTA		
Y. siamensis	TTA-AACGGTGTTCTAGGACTGGC---TAAA**A**-**T**TTAA----TTA		
Y. nipponensis	TTA-AACGGTGTTCTAGGACTGGC---TAAA**A**-**T**TTAA----TTA		
Y. delicatus	TTA-AAC**A**GTGTTCTAGGACTGGC---TAAA**AA**-TTAA**AAAA**TTA		
Y. zitanae	TT**T**-AAC**A**GTGTTCTAGGACTGGC---TAAA**A**--TTAA**AAA**-TTA		
Y. belshawi	TT**T**-AAC**A**GTGTTCTAGGACTGGC---TAAA**A**--TTAA**AAA**-TTA		
P. limonensis	TTG-TACGGTGTTCTAGAACTGGC---TA------TAA**GAA**-TTA		
Bulborogas sp.	TTG-TACGGTGTTCTAGAACTGGC---TAAA---TTAT**TAAA**TTA		
A. dispar	TTG-TACGGTGTTCTAGGACTGGCT--AAAA---TTAA----TTA		
A. nigricornis	TTG--ATGGTATTCTAGGACTGGCT--AAAA---TTAA----TTA		
A. praetor	TTG-TACGGTATTCTAGGACTGGCT--AAAA---TTAA----TTA		
Rogas sp.	TTGTTACGGTGTTCTAGGACTGGCT--TATA---ATAA----TTA		
Spinaria sp.	CTGTTATGGTATTCTTGGACTGGCTGAAAAA---ATAA----TTA		
Conspinaria sp.	TTGTTACAGTATTTTAGAACTGGCT--TAAA---ATTA----TTA		
Stiropius sp.	TTG-TACGTGATTCTAGGACTGGCTT-TAAA---TTT-----TTA		
Tebennotoma sp.	TTGTTACGGTGTTCTAAGACTGGCT--TAAA---TAAA----TTA		
Clinocentrus sp.	TTG-TMCGGTGTTCTGGGACTGGCT--TAAA---TTAA----TTA		
Mesocentrus sp.	TTGTTACGGTGCTCTAGGACTGGCT--AAAA---TTAA----TTA		

Fig. 2. Six fragments of the D2–D3 region, 28S rRNA arranged by eye showing marked differences between the Old and New World species and between two OW groups (indicated in bold). The dark bars indicate ambiguously alignable sequence blocks excluded from phylogenetic analysis. The fragments correspond to positions in the alignment depicted in Belshaw et al. (1998) as follows; fragment 1 = bases 284–318; fragment 2 = bases 71–87; fragment 3 = bases 252–268; fragment 4 = bases 177–200; fragment 5 = bases 330–349; fragment 6 = bases 390–416.

ratus morphology. Although *Pseudoyelicones* and *Bulborogas* appear as a sister group to *Yelicones* in both analyses (Fig. 1A, B), the bootstrap support for this in the purely molecular tree (Fig. 1A) is only marginal. The secondary venom duct insertion in *Pseudoyelicones* and *Bulborogas* is modified (hard) and thus resembles that of members of the *Rogas* groups of genera (Zaldivar-Riverón et al., 2004) whereas secondary venom duct of *Yelicones* is unmodified, suggesting a more basal position, (unless this modification has been secondarily lost). Unfortunately, the hosts and mummification biology of *Pseudoyelicones* and *Bulborogas* are unknown, though features of *Yelicones* mummies (Quicke & Shaw, in press) suggest that *Yelicones* is probably less derived than the *Rogas* group.

ACKNOWLEDGEMENTS. We thank C. Godoy (INBio, Costa Rica), S.-H. Yen (National Sun Yat-Sen University, Taiwan), B. Fisher (California Academy of Science, USA), M. Sharkey (University of Kentucky, USA) and M. Shaw (National Museums of Scotland, UK) for providing material or permission to sequence material in their care.

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Appendix 1. Morphological characters used in cladistic analysis, subset from Areekul & Quicke (in press) that are informative for this study.

Head

1. Antennae: 0 = with more than 36 flagellomeres; 1 = with 30–36 flagellomeres; 2 = with 29 or fewer flagellomere (ordered).

2. Third flagellomere: 0 = less than 1.5′ longer than wide; 1 = more than 1.5′ longer than wide.

3. First flagellomere: 0 = more than 1.3′ longer than the second; 1 = less than 1.3′ longer than the second.

4. Maximum length of eye: 0 = more than 1.8′ maximum width of eye (in lateral view); 1 = less than 1.8′ maximum width of eye.

5. Eyes: 0 = glabrous; 1 = distinctly but weakly setose; 2 = strongly setose (Fig. 481) (ordered).

6. Eyes: 0 = emarginate; 1 = not emarginate.

7. Shortest distance between posterior ocellus and eye/maximum distance across posterior ocelli: 0 = more than 0.7; 1 = less than 0.7.

8. Face: 0 = densely punctured but without marked transverse striation (Figs 26, 30, 36, 90, 260); 1 = transversely striate, at least between antennal sockets (Figs 116, 170, 290); 2 = entirely smooth with only sparse small punctures at the bases of setae (Figs 66, 108, 128, 146, 194, 320); 3 = granulate.

9. Face, median carina: 0 = absent; 1 = present but incomplete (Figs 170, 188, 493); 2 = present and complete.

10. Width of face: 0 = less than 1.35× height of eye; 1 = more than 1.35× height of eye.

11. Occipital carina: 0 = complete medio-dorsally (Figs 38, 123, 448); 1 = medially narrowly absent (Fig. 285); 2 = medially widely absent (Figs 233, 380, 442, 461); 3 = completely absent (ordered).

12. Gena: 0 = less than 0.2′ height of eye; 1 = 0.2–0.4′ height of eye; 2 = more than 0.4′ height of eye (ordered).

13. Mandibles: 0 = bidentate; 1 = tridentate (Figs 54, 76, 146, 220, 254).

Mesosoma

14. Mesoscutum: 0 = coarsely rugulose; 1 = shiny with more or less dense, deep punctured; 2 = almost entirely smooth; 3 = punctate-rugulose.

15. Notauli: 0 = present and complete (Figs 39, 46, 148, 386, 394, 405); 1 = absent on posterior half of mesoscutum (Figs 462, 495); 2 = almost completely absent (ordered).

16. Notauli: 0 = smooth (Fig. 155); 1 = finely punctate (Fig. 1489); 2 = crenulated (Figs 39, 148).

17. Mesoscutum: 0 = without a mid-longitudinal carina; 1 = with a mid-longitudinal carina.

18. Precoxa suture: 0 = present; 1 = absent.

19. Precoxal suture (if present): 0 = well developed, wide, extending most of length mesopleuron (Figs 125, 223); 1 = narrow, occupying most of length of mesopleuron (Figs 53, 112, 434); 2 = narrow and restricted to antero-medial part of mesopleuron (Figs 63, 119).

20. Scutellum: 0 = with fewer than 50 setae; 1 = with 50–100 setae; 2 = with more than 100 setae (ordered).

21. Scutellum medio-posteriorly: 0 = smooth; 1 = punctured; 2 = rugulose; 3 = formed into a strong keel.

22. Metanotum: 0 = simple (Fig. 443); 1 = with mid-posterior pit (Figs 453, 484).

23. Number of carinae in scutellus sulcus between two outer ones: 0 = more than 5 (Figs 387, 426, 433, 463); 1 = 4 or 5 (Fig. 444); 2 = 3 (Fig. 196) (ordered).

24. Propodeum: 0 = simple; 1 = with inverted "U"-shaped carina antero-medially (Figs 198, 210, 217, 229); 2 = with inverted "V/Y"-shaped carina antero-medially (Figs 362, 491, 457). State 2 appears to arise from state 1 via a narrowing and loss of the gap between the carinae at the anterior end of the propodeum, therefore we have treated it as ordered in relevant analyses (ordered).

25. Propodeum: 0 = antero-submedially foveate/rugose (Figs 138, 186, 390, 396); 1 = antero-submedially smooth (Figs 48, 57, 72, 105, 210, 224).

26. Propodeum, sublateral carinae: 0 = without distinct sublateral carinae (Figs 48, 138); 1 = distinct on posterior half but not complete (Figs 167, 288, 435); 2 = with well-developed, continuous sublateral carinae (Figs 57, 81, 224, 229) (ordered).

27. Propodeum: 0 = postero-medially without "A"-shaped arrangement of carinae (Figs 47, 74, 156); 1 = postero-medially with "A"-shaped arrangement of carinae (Figs 113, 288, 426).

28. Propodeum, mid-longitudinal carina: 0 = absent; 1 = present.

29. Propodeum: 0 = without spine; 1 = with a pair of spines postero-laterally.

30. Mid-ventral suture of mesoscutum: 0 = smooth; 1 = crenulated.

Fore wing

31. Fore wing vein r arising: 0 = before middle of pterostigma; 1 = beyond middle of pterostigma.

32. Fore wing vein 1-SR+M: 0 = sinuous, markedly curving towards wing tip after arising from 1-M (Figs 64, 82); 1 = straight or with simple curve, not curving anteriorly after arising from 1-M (Figs 24, 35, 88, 114, 133).

33. Fore wing subdiscal cell: 0 = less than 5.5′ longer than maximally wide; 1 = more than 5.5′ longer than maximally wide (excluding veins).

34. Fore wing vein 1-CU: 0 = more than 0.5′ 2-CU1; 1 = 0.3–0.49′ 2-CU1; 2 = less than 0.3′ 2-CU1 (ordered).

35. Fore wing vein 2-CU1: 0 = more than 2.3′ 3-CU1; 1 = 2.0–2.3′ 3-CU1: 2 = less than 2.0′ 3-CU1 (ordered).

36. Fore wing vein 3-SR: 0 = longer than r-m (Figs 88, 126): 1 = shorter than r-m (Figs 98, 106).

37. Fore wing vein r: 0 = longer than 3-SR (Figs 35, 106, 114, 133); 1 = shorter than 3-SR (Fig. 64).

38. Pterostigma: 0 = length less than or 3′ width; 1 = length more than 3′ width.

39. Fore wing m+cu1: 0 = straight apically; 1 = curved apically (Fig. 82).

40. Fore wing length: 0 = less than 4.0 mm; 1 = 4.0–6.0 mm; 2 = more than 6.0 mm (ordered).

41. Fore wing CU1a: 0 = not weak; 1 = weakened.

42. Fore wing CU1b: 0 = present; 1 = absent.

Hind wing

43. Hind wing marginal cell: 0 = evenly setose; 1 = with glabrous patch.

44. Hind wing vein SR: 0 = more or less straight; 1 = weakly sinuous; 2 = strongly sinuous.

45. Hind wing vein SR: 0 = straight or curving anteriorly at apex; 1 = curving posteriorly at apex.

46. Hind wing vein 2-SC+R: 0 = strongly longitudinal (Figs 5, 6, 7); 1 = weakly longitudinal (Fig. 430); 2 = interstitial; 3 = short transverse (Fig. 5); 4 = long transverse (Fig. 6) (ordered).

47. Hind wing m+cu: 0 = absent; 1 = present.

48. Hind wing vein m+cu (when present): 0 = curving strongly towards base of wing (Fig. 364): 1 = curving distinctly but weakly towards base of wing; 2 = more or less straight (Fig. 82); 3 = curving distinctly away from base of wing (Fig. 42).

49. Hind wing vein m+cu (when present): 0 =antefurcal (Figs 4,5); 1 = interstitial (Fig. 6); 2 = weakly but distinctly postfurcal by up to 0.1′ length of vein 1r-m (Fig. 230); 3 = strongly postfurcal by approximately 0.2′ 1r-m (Fig. 7) (ordered).

50. Hind wing M+CU: 0 = less than 1.0′ 1-M; 1 = 1.0–1.5′ 1-M; 2 = more than 1.5′ 1-M (ordered).

51. Hind wing vein 2-M: 0 = running straight to wing margin; 1 = curving parallel to wing margin at apex.

Legs

52. Fore tibia: 0 = simple; 1 = with a mid-longitudinal smooth or longitudinally striate line; 2 = with a well-developed mid-longitudinal ridge.

53. Fore tarsus: 0 = normal; 1 = fore tarsus shorter than fore tibia; 2 = fore tarsus much shorter than fore tibia (ordered).

54. Hind basitarsus: 0 = more than 0.4′ longer than tibia; 1 = less than 0.4′ tibia.

55. Hind basitarsus: 0 = not flat and not enlarged; 1 = flat and enlarged.

56. Tarsal claws: 0 = not pectinate; 1 = strongly pectinate.

Metasoma

57. T1 sculpture: 0 = striate; 1 = rugose, rugulose; 2 = punctured to smooth.

58. T1 spiracle: 0 = more than 0.75 distance between posterior margin of dorsope and posterior margin; 1 = less than 0.75 distance.

59. T1 dorsal carinae: 0 = uniting before the level of spiracles (Figs 13, 29, 65); 1 = uniting at or behind the level of spiracles (Figs 89, 307, 337, 379).

60. Dorso-lateral carinae of first tergite: 0 = well-developed; 1 = half length of T1; 2 = absent (ordered).

61. T2: 0 = with large, smooth mid-basal area (Figs 13, 59, 163); 1 = without or with only trace of mid-basal area (Fig. 43).

62. T2: 0 = largely striate (Figs 29, 43, 65); 1 = at most basal half striate (Figs 13, 49); 2 = completely smooth or at most with a few striae very close to base (Figs 75, 89, 253) (ordered).

63. T2 mid-longitudinal carina: 0 = present; 1 = absent.

64. T2 mid-longitudinal carina (if present): 0 = complete (Figs 29, 43, 65); 1 = nearly complete or just beyond half-length (Figs 181, 193); 2 = present but not extending beyond mid-length (Figs 13, 199, 253) (ordered).

65. T2 width/length: 0 = less than 2.0; 1 = 2.0–2.5; 2 = more than 2.5 (ordered).

66. Second suture: 0 = deep and crenulated (Figs 29, 43, 65); 1 = deep and smooth (Figs 13, 49, 83); 2 = shallow but distinct (Fig. 151); 3 = obsolescent.

67. Second suture: 0 = straight (Figs 151, 175); 1 = weakly to strongly sinuate (Fig. 83).

68. Length of T2/T3: 0 = 0.63– 1.0; 1 = more than 1.0.

69. T3: 0 = largely striate; 1 = half striate; 2 = hardly striate (ordered).

70. If T3 not completely striate then: 0 = smooth; 1 = sparsely punctured; 2 = densely punctured; 3 = granulate; 4 = transverse carinae.

71. Ovipositor tip: 0 = weakly sclerotised; 1 = strongly sclerotised, dark.

Internal anatomy

The following characters of the venom apparatus are taken from Zaldivar-Riverón et al., (2004) and Areekul et al. (2004).

72. Insertion of secondary venom duct: 0 = anterior or medially on reservoir; 1 = posterior on venom reservoir.

73. Base of secondary venom duct: 0 = without internal filaments: 1 = few or numerous internal filaments.

74. Secondary venom duct insertion on to reservoir: 0 = not recessed; 1 = recessed, with well defined and numerous internal filaments.

75. Secondary venom duct: 0 = simple or with no evident sculpture; 1 = spiral sculpture; 2 = “hexagonal” type sculpture.

76. Secondary venom duct: 0 = considerably long, at least 2× as long as the venom reservoir length; 1 = distinctively short, not more than 2× as long as wide.

77. Secondary venom duct: 0 = branched into tertiary and subsequent gland ducts, without insertion of venom gland; 1 = not branched, with two venom gland bunches, one at its middle and the other at its end.

78. Secondary venom duct: 0 = without flange; 1 = with a distinct flange.

79. Tertiary venom duct: 0 = parallel-sided; 1 = medially expanded.

80. Tertiary venom duct: 0 = symmetric; 1 = asymmetric.

Received January 7, 2005; revised and accepted July 8, 2005