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The genus *Nipponaetes* (Hymenoptera: Ichneumonidae: Cryptinae) in Costa Rica, with a reassessment of the generic limits

GAVIN R. BROAD[1, 4], NINA M. LAURENNE[2] and DONALD L.J. QUICKE[3, 4]

[1]Centre for Ecology and Hydrology, Monks Wood, Abbots Ripton, Huntingdon PE28 2LS, UK; e-mail: gabro@ceh.ac.uk

[2]Finnish Museum of Natural History, Zoological Museum, Entomological Division, P.O. Box 17 (P. Rautatiekatu 13), FIN-00014 University of Helsinki, Finland; e-mail: nina.laurenne@ helsinki.fi

[3]Department of Biological Sciences, Imperial College London, Silwood Park Campus, Ascot, Berks. SL5 7PY, UK; e-mail: d.quicke@imperial.ac.uk

[4]Department of Entomology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK

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Abstract. The endemic Costa Rican genus *Zurquilla* Gauld, 1997, is transferred from the ichneumonid subfamily Tryphoninae (tribe Oedemopsini) to the Cryptinae (tribe Phygadeuontini) and synonymised with *Nipponaetes* Uchida, 1933. This decision is justified using morphological and molecular (28S D2-3 ribosomal DNA sequence) evidence. We briefly discuss the phylogenetic utility of characters that led to the type species of *Zurquilla* being described as a tryphonine and provide a host record that indicates that *Nipponaetes* is a parasitoid of spider egg sacs.

INTRODUCTION

Amongst the Hymenoptera material collected by DLJQ and NML on a trip to Thailand in May 2001 was an unfamiliar female phygadeuontine (Cryptinae: Phygadeuontini). The specimen was identified as *Nipponaetes haeussleri* (Uchida, 1933), *Nipponaetes* being a small genus of the old world tropics placed by Townes (1970) in his subtribe Rothneyiina of the tribe Phygadeuontini. Whilst we were identifying the specimen, recollection of the description of the tryphonine genus *Zurquilla* Gauld, 1997, indicated that this genus might be synonymous with *Nipponaetes*. Examination of type material of the type species of the genus, *Z. hansoni* Gauld, 1997, in The Natural History Museum (London) has leant support to this hypothesis. Here we justify the reclassification and synonymy of the genus *Zurquilla* and present limited host information based on specimens in The Natural History Museum (London). The genus *Nipponaetes*, represented by the species *N. haeussleri*, is also recorded from Thailand for the first time.

To establish the taxonomic position of the Thai specimen of *N. haeussleri*, and as part of ongoing projects investigating the higher-level phylogeny of the Ichneumonidae and of the Cryptinae in particular (Belshaw et al., 1998; Quicke et al., 2000; Laurenne et al., 2002), a 28S D2–3 ribosomal DNA sequence was obtained from this specimen. An estimate of relationships was derived from phylogenetic analysis of a set of sequences from 30 ichneumonids of the subfamilies Cryptinae, Ichneumoninae, Tryphoninae, Brachycyrtinae, Banchinae, Neorhacodinae, Ctenopelmatinae, Orthocentrinae, Orthopelmatinae and Xoridinae. As we consider the species of *Nipponaetes* and *Zurquilla* to represent the same genus (see Discussion) there are two alternative hypotheses: (1) that the genus *Zurquilla* was mistakenly described in the Tryphoninae and in fact belongs in the Cryptinae, or (2) *Nipponaetes* is an aberrant tryphonine and does not belong in the Cryptinae. By sequencing a taxonomically wide-ranging selection of cryptines and tryphonines we are able to refute the second of these hypotheses.

MATERIAL AND METHODS

Specimens

The taxa used in our phylogenetic analyses were chosen to represent, as far as specimen availability would allow, the tribes of cryptines and tryphonines, together with outgroups. All three genera of the *Rothneyia* group were sequenced, as were two oedemopsine genera and representatives of seven of the eight tribes of Tryphoninae (no material of the Ankylophonini was available). Table 1 details the provenance and European Molecular Biology Institute nucleotide sequence database (EMBL) accession numbers of ichneumonids used for DNA extraction. All molecular vouchers will be deposited in The Natural History Museum (London) (BMNH) except for the specimen of *Hyperacmus crassicornis* (Gravenhorst, 1829), which is deposited in the National Museums of Scotland (Edinburgh). Identifications were carried out by GRB, corroborated using the collections of the The Natural History Museum and American Entomological Institute (Gainesville) (AEI). Although type material of *N. haeussleri* was not examined, we are confident of the identification judging by the original description (Uchida, 1933) and the specific characters given by Momoi (1970). The taxa used in the analyses are a subset of the taxa being sequenced as a more wide-ranging analysis of ichneumonid and, particularly, cryptine relationships (Laurenne et al. in preparation).

Of the *Rothneyia* group of cryptines (= Rothneyiina Townes, 1970), we were able to examine the morphology of four female specimens of *N. haeussleri* (one light-trapped in Thailand, three reared from spider egg sac(s) in India, in the BMNH as detailed below); one female specimen of *N. inelegans* (Seyrig, 1952)

TABLE 1. Taxa included in study, provenance of specimens and EMBL accessions numbers for D2–3 28S rDNA sequences.

Subfamily Tribe	Species	Provenance	EMBL accession number
Cryptinae			
Phygadeuontini	*Nipponaetes haeussleri* (Uchida, 1933)	Thailand	AF423138
	Hyparcha sp.	Malaysia: Cameron Highlands	AY389165
	Rothneyia sp.	Malaysia: Cameron Highlands	AY222795
	Chirotica sp.	Belize	AF423157
	Paraphylax sp.	no data	AJ302867
	Bentyra sp.	Malaysia	AF423165
	Palpostilpnus sp.	Malaysia: Sabah	AF423137
	Stilpnus pavoniae (Scopoli, 1763)	England: Silwood Park	AY222805
Hemigasterini	*Cubocephalus anatorius* (Gravenhorst, 1829)	England: Hilbre Island	AY222801
Cryptini	*Buathra laborator* (Thunberg, 1822)	Turkey	AY222800
	Microstenus canaliculatus Szépligeti, 1916	Malaysia: Cameron Highlands	AY222802
	Nematopodius debilis (Ratzeburg, 1852)	England: Silwood Park	Z97921
Ichneumoninae			
Alomyini	*Alomya semiflava* Stephens, 1835	England: Silwood Park	AY222808
	Misetus oculatus (Wesmael, 1845)	England: Silwood Park	AF418559
Ichneumonini	*Amblyteles armatorius* (Forster, 1771)	England: Silwood Park	AY222803
Tryphoninae			
Oedemopsini	*Oedemopsis ryitoi* Gauld, 1997	Costa Rica	AY222807
	Thymaris sp.	Malaysia: Sabah	AY222809
Eclytini	*Eclytus exornatus* (Gravenhorst, 1829)	Finland: Värriö	AY222797
Sphinctini	*Sphinctus gastoni* Gauld, 1997	Costa Rica	AY389166
Phytodietini	*Netelia* (*Monomacrodon*) *bicolor* (Cushman, 1934)	Burma	AY222806
Idiogrammatini	*Idiogramma* sp.	Caucasus	AY389167
Tryphonini	*Tryphon rutilator* (Linnaeus, 1761)	Turkey	AY389168
Exenterini	*Excavarus sibiricola* Kasparyan, 1990	Russia: Primorje	AY389164
Brachycyrtinae	*Brachycyrtus* sp.	no data	Y18585
Ctenopelmatinae	*Euryproctus* sp.	Germany: Bayerische Wald	AY222798
Neorhacodinae	*Neorhacodes enslini* (Ruschka, 1922)	England: Chippenham Fen	Z97910
Banchinae	*Sjostedtiella* sp.	Togo	AY222796
Orthopelmatinae	*Orthopelma* sp.	Turkey: Bolu Bolu	AY222799
Orthocentrinae	*Hyperacmus crassicornis* (Gravenhorst, 1829)	Scotland: Wester Ross	AY222804
Xoridinae	*Odontocolon dentipes* (Gmelin, 1790)	England: Silwood Park	AJ302924

from Madagascar in the AEI; six undescribed species of *Nipponaetes* in the collections of the AEI; the female holotype and male paratype of *Zurquilla hansoni*; an undescribed species of *Hyparcha* Townes, 1970 (Malaise-trapped in the Cameron Highlands, Malaysia); and three described species of *Rothneyia* Cameron, 1897, from South East Asia in the BMNH, together with two unnamed specimens Malaise-trapped in the Cameron Highlands, Malaysia.

Laboratory protocols

A hind leg of each insect sample was homogenised in 100 µl TE-buffer (1M Tris, 5M NaCl, 0.5M EDTA), incubated in 37°C overnight with proteinase K, which was followed by sodium acetate/ethanol precipitation and re-suspension in 50 µl TE buffer. The PCR conditions were 30 cycles of 98°C denaturation (15 s), 50°C annealing (30 s) and 72°C extension (40 s) with an initial denaturation of 3 min at 93°C and a final extension of 3 min. The D2–3 region of the 28S rDNA gene was amplified using the following primers: forward 5'-AGA GAG AGA GTT CAA GAG TAC GTG-3' , reverse 5'-TAG TTC ACC ATC TTT CGG GTC-3'.

Phylogenetic analysis

The phylogenetic reconstruction was carried out using the program POY that optimizes characters straight to the tree without a priori alignment (Wheeler & Gladstein, 2000). The data set was split into 18 blocks to speed up the analysis and these blocks were anchored in conserved regions where there was no length variation. The xoridine *Odontocolon dentipes* (Gmelin, 1790) was specified as the outgroup because the Xoridinae have consistently appeared as basal to the Tryphoninae and Cryptinae in molecular analyses (Belshaw et al., 1998; Belshaw & Quicke, 2002). The POY command line used was the following: norandomizeoutgroup, noleading, random 50, multibuild 6, treefuse, fuselimit 50, slop 5, checkslop 3, buildmaxtrees 2, maxtrees 2, holdmaxtrees 50, driftspr, drifttbr, numdriftspr 5, numdrifttbr 5, fitchtrees, seed -1.

TABLE 2. Summary of results of phylogenetic analyses of relationships of *Nipponaetes* from 28S rDNA sequence alignments under different gap: substitution parameters.

Gap:substitution ratio	Tree length	Number of most parsimonious trees	Sistergroup of *Nipponaetes*
1.5:1	1756	4	*Brachycyrtus/Hyperacmus*
1.75:1	3636	1	*Hyparcha*
1.8:1	4574	1	*Hyparcha*
2.0:1*	945	21	*Rothneyia*
2.0:1*	945	9	*Hyparcha*
2.5:1	2006	7	*Hyparcha*
3.0:1	1060	8	*Hyparcha*

*two islands of most parsimonious trees were recovered from analysis with the gap:substitution cost ratio set at 2.0:1

The data were analysed with gap:substitution ratios set at 1.5:1, 1.75:1, 1.8:1, 2:1, 2.5:1 and 3:1 in order to explore the sensitivity of the results to alignment parameters. POY searches were run on a cluster at the Centre of Scientific Computing (University of Helsinki) using 8 processors of a 128 processor (type R12000, based on S2MP acrhitecture) set-up.

RESULTS

Fig. 1 shows the strict consensus trees resulting from analyses with each of the six gap:substitution ratios. *Nipponaetes haeussleri* groups with the Cryptinae + Ichneumoninae in all analyses except that with the very low gap:substitution cost ratio 1.5:1. With the majority of gap:substitution costs, it emerges as the sister taxon to the rothneyiine genus *Hyparcha* (Table 2), though with the 2:1 analysis two islands of optimal trees were found and in one of these *Nipponaetes* grouped with the other rothneyiine genus, *Rothneyia*. The representative oedemopsine genera, *Oedemopsis* Tschek, 1869, and *Thymaris* Förster, 1869, group with the other tryphonine genera. Thus we reject the hypothesis that *Nipponaetes* (including *Zurquilla*) is a tryphonine.

DISCUSSION

The presence of a complete sternaulus and fused first metasomal tergite and sternite, with the spiracles placed well behind the middle of the segment, support the molecular evidence that *Nipponaetes* is a cryptine. It can be classified in the tribe Phygadeuontini based on the complete propodeal carination and the form of the sternaulus (ending above the lower hind corner of the mesopleuron). Comparison of specimens of *N. haeussleri* with the holotype female and a paratype male of *Zurquilla hansoni* (BMNH) leads us to believe that these two species are congeneric. Within the Oedemopsini (Tryphoninae), apomorphies of *Zurquilla* are, according to Gauld (1997), a single bulla in vein 2*m-cu* of the forewing and the presence of a more or less complete posterior transverse carina on the mesosternum. Both of these features are found in the rothneyiine genera and some chiroticine genera of the Phygadeuontini. Gauld (1997) also listed four features that indicated an oedemopsine affinity for *Z. hansoni*: (1) clypeus with a subapical fringe of stout hairs; (2) the characteristic mandible, with the upper tooth about four times the length of the lower; (3) the un-notched ovipositor; and (4) the long, deeply impressed and posteriorly convergent notauli. The form of the clypeus would be the most convincing tryphonine feature but the fringe of setae is not of the regularly spaced, dense form found in typical oedemopsine genera. In fact, all of these features are found separately or in combination in the rothneyiine and various chiroticine genera, although only with *Nipponaetes* displaying such a disparity in mandibular tooth size (Townes, 1970; Gauld, 1984; examination of specimens in BMNH). *Zurquilla* lacks such oedemopsine features as the membranous area on the lower valve of the ovipositor, a synapomorphy of the tribe in Gupta's (1988) phylogeny, and the presence of glymmae on the first tergite, a presumably plesiomorphic character found in most tryphonines. *Zurquilla hansoni* is mostly pale in coloration, including the head, which is a fairly unusual colour pattern for the Phygadeuontini. However, several species of *Stilpnus* from Costa Rica (GRB: pers. observ.) are extensively pale; at least two undescribed species of *Nipponaetes* in the AEI are almost entirely creamy coloured; several undescribed species of the *Chirotica* group in the BMNH have pale heads; and *N. haeussleri* has large, pale, triangular marks on the malar space. The pale mesopleuron reduces the visibility of the sternaulus of *Z. hansoni* but it is present and complete, best viewed from below. The number of shared character states between *Zurquilla* and *Nipponaetes*, the presence of at least one cryptine apomorphy (the long, strong sternaulus) and the absence of any convincing tryphonine apomorphies leads us to synonymise *Zurquilla* under *Nipponaetes*. The genus now comprises the following five species, with distributions taken from Gupta (1987) and Yu & Horstmann (1997).

***Nipponaetes* Uchida, 1933**

Potia Seyrig, 1952
Thalops Townes, 1958
Zurquilla Gauld, 1997, **syn. nov.**
fessus (Townes, 1958): Micronesia
haeussleri (Uchida, 1933): India, China, Philippines, Korea, Japan, Ryukyu archipelago, Thailand
hansoni (Gauld, 1997): Costa Rica
inelegans (Seyrig, 1952): Madagascar
striatus Momoi, 1970: Ryukyu archipelago

In addition, undescribed species are represented by specimens in the AEI from Malaysia and the Philippines and Gauld (1984) records an undescribed species from Australia. Although *Nipponaetes* has previously been considered to be a genus of the Old World tropics (Tow-

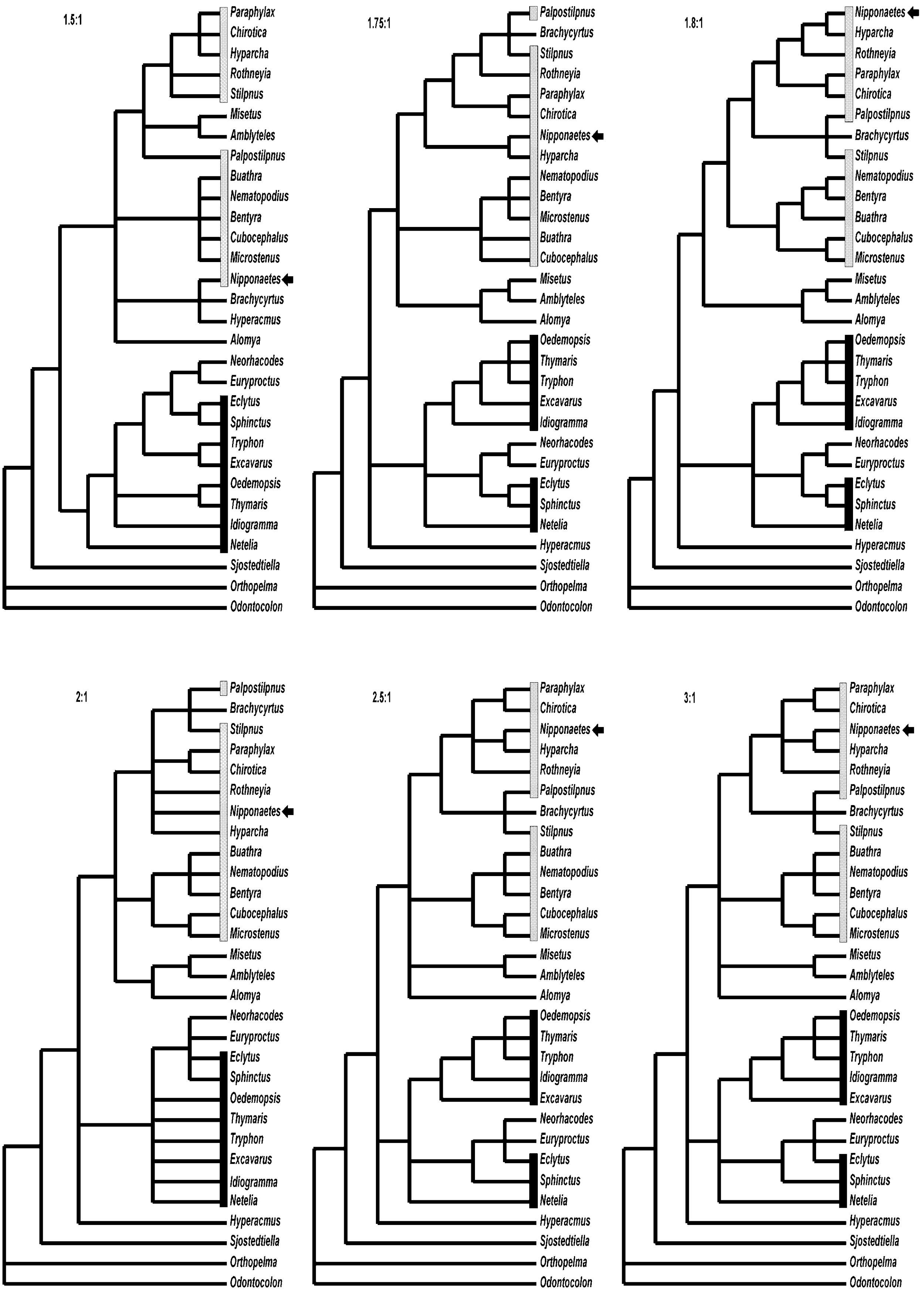

Fig. 1. Trees from the POY analysis with relative gap: substitution costs 1.5, 1.75, 1.8, 2, 2.5 and 3. Taxa belonging to the Cryptinae are indicated by black bars and taxa belonging to the Tryphoninae are indicated by grey bars. The position of *Nipponaetes* is indicated with an arrow in each tree.

nes, 1970; Gupta, 1987), synonymisation of *Zurquilla* results in a pan-tropical distribution.

Nipponaetes can be distinguished from all other ichneumonid genera by the following combination of features:

1. complete sternaulus, ending above posterior ventral corner of the mesopleuron;
2. first tergite and sternite fused, spiracle far behind mid-length of segment;
3. complete or narrowly interrupted posterior transverse carina on the mesosternum;
4. single bulla in fore wing vein 2*m-cu*;
5. upper mandibular tooth much longer then lower tooth;
6. notauli long, deeply impressed and posteriorly convergent.

Biology

The only host information previously presented for *Nipponaetes* is the assertion, without any supporting information, that it is a parasitoid of *Cydia molesta* (Busck, 1916) (Lepidoptera: Tortricidae) in Japan (Uchida, 1933). However, there is doubt associated with this record as neither the holotype nor paratype was accompanied by host data in Uchida's original description, unlike other species in the paper. A species of Ichneumoninae subsequently described by Uchida and sharing the same specific name, *Phaeogenes haeussleri* Uchida, 1935, is a parasitoid of tortricid fruit moths (Garman, 1940) and clearly there is some potential for confusion. We found three previously unidentified specimens of *N. haeussleri* in the "Chiroticina" drawers of the Cryptinae collections in the NHM. These specimens, all females, are labelled as "ex spider eggs", from "India: Orissa, Cuttack 1981" and presented by the Commonwealth Institute of Entomology. The form of the ovipositor, tapered to a point, lacking nodus and obvious teeth, compares well with other cryptine genera with known spider egg sac associations (Fitton et al., 1987; Schwarz & Shaw, 2000).

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