Molecular insights into the phylogenetic structure of the spider genus *Theridion* (Araneae, Theridiidae) and the origin of the Hawaiian *Theridion*-like fauna

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The Hawaiian happy face spider (*Theridion grallator* Simon, 1900), named for a remarkable abdominal colour pattern resembling a smiling face, has served as a model organism for understanding the generation of genetic diversity. *Theridion grallator* is one of 11 endemic Hawaiian species of the genus reported to date. Asserting the origin of island endemics informs on the evolutionary context of diversification, and how diversity has arisen on the islands. Studies on the genus *Theridion* in Hawaii, as elsewhere, have long been hampered by its large size (> 600 species) and poor definition. Here we report results of phylogenetic analyses based on DNA sequences of five genes conducted on five diverse species of Hawaiian *Theridion*, along with the most intensive sampling of Theridiinae analysed to date. Results indicate that the Hawaiian Islands were colonised by two independent Theridiinae lineages, one of which originated in the Americas. Both lineages have undergone local diversification in the archipelago and have convergently evolved similar bizarre morphs. Our findings confirm para- or polyphyletic status of the largest Theridiinae genera: *Theridion*, *Achaearanea* and *Chrysso*. Convergent simplification of the palpal organ has occurred in the Hawaiian Islands and in two continental lineages. The results confirm the convergent evolution of social behaviour and web structure, both already documented within the Theridiidae. Greater understanding of phylogenetic relationships within the Theridiinae is key to understanding of behavioural and morphological evolution in this highly diverse group.

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Introduction

Islands, by their nature, are isolated, well defined geographically, and have distinct boundaries. These properties have been used to examine the ecological and evolutionary underpinning of biodiversity, with much focus on the formation of communities through colonisation and evolution. Moreover, because island ecosystems tend to be simpler than those on continents, they are generally more amenable to elucidating mechanisms underlying the formation of biotic assemblages. In particular, recent studies have highlighted the interplay between ecological and evolutionary processes in shaping island biodiversity, allowing intriguing insights into biodiversity dynamics (Losos *et al*. 1998; Losos & Schluter 2000; Beheregaray *et al*. 2004; Gillespie 2004; Emerson & Kolm 2005). However, in order to develop general principles, and hence be able to extrapolate to continental systems, we must compare and contrast patterns of diversification across multiple lineages.

The Hawaiian Islands, being the world's most isolated archipelago, have been a primary focus of studies on the evolution and adaptive radiation of multiple lineages (Simon 1987; Wagner & Funk 1995). However, understanding of the interplay between colonisation and adaptation in shaping species diversity has frequently been hampered by the inability to identify an initial source of colonists, in large part because taxa that have undergone adaptive radiation have often changed morphologically to such an extent that affiliations with mainland

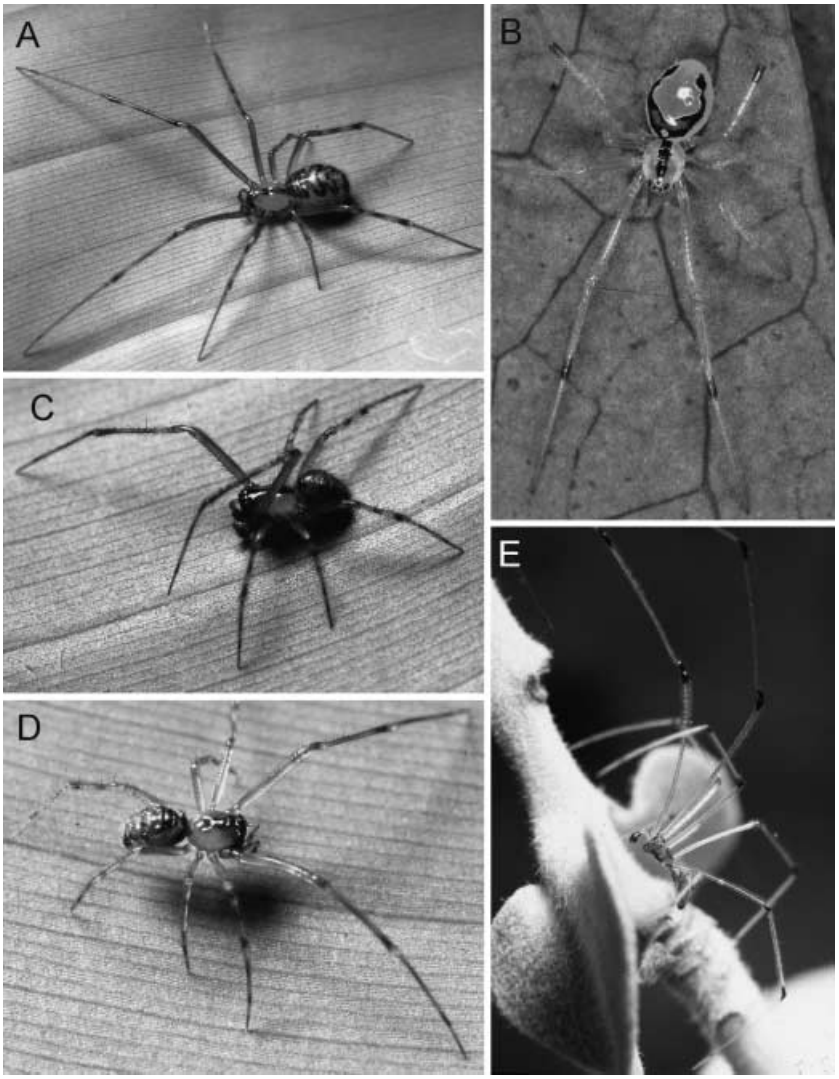

Fig. 1 A–E. —A. *Theridion kauaiensis*, female, Hawaiian I., Oahu, Mt. Tantalus. —B. *T. grallator*, female, Hawaiian I. —C. *T. posticatum*, male, Hawaiian I., Oahu, Mt. Tantalus. —D. *T. acutitarse*, male, Hawaiian I., Oahu, Mt. Tantalus. —E. unknown genus sp., male, Hawaiian I., Kauai (photo credits: A, C, D G. Hormiga; B, R. Gillespie; E, D. Preston).

taxa are not apparent. The similarities between the biotas of Hawaii and groups in the islands of the South Pacific led to a general belief that much of the biota in these different islands was derived from the western Pacific and Australasian region (Zimmerman 1948; Mueller-Dombois & Fosberg 1998). Yet recent studies have shown that many plant (Carr 1987; DeJoode & Wendel 1992; Howarth *et al.* 1997; Pax *et al.* 1997; Vargas *et al.* 1998; Baldwin & Wessa 2000; Ballard & Sytsma 2000; Ganders *et al.* 2000; Lindqvist & Albert 2002; Wanntorp *et al.* 2002; Carlquist *et al.* 2003) and bird (Fleisher & McIntosh 2001) lineages colonised from the east.

Spiders, in particular those that build webs, provide a huge advantage for interpreting mechanisms underlying adaptive radiation as their webs and sedentary lifestyle provide ready access to information on both their behaviour and ecological affinities. Accordingly, a number of studies have used spiders lineages as models for the investigating various aspects of the evolutionary process (Hormiga *et al.* 2003; Blackledge & Gillespie 2004; Gillespie 2004; Arnedo & Gillespie 2006; Garb & Gillespie 2006). Additional studies have used populations within a single species to examine similar questions, with most studies to date focusing on the happy face spider (*Theridion grallator* Simon, 1900) (Fig. 1). This species, one of the most popular arthropods in the islands and an icon of the native Hawaiian fauna, gets the vernacular name from the remarkable abdominal colour patterns that in some individuals resemble a human smiling face. This species is a model organism for the study of independent evolution of genetic diversity. A similar array of genetically controlled colour morphs have evolved repeatedly on the different islands (Gillespie & Tabashnik 1989; Oxford & Gillespie 1996a, 1996b, 1996c; Gillespie & Oxford 1998; Oxford & Gillespie 2001). Development

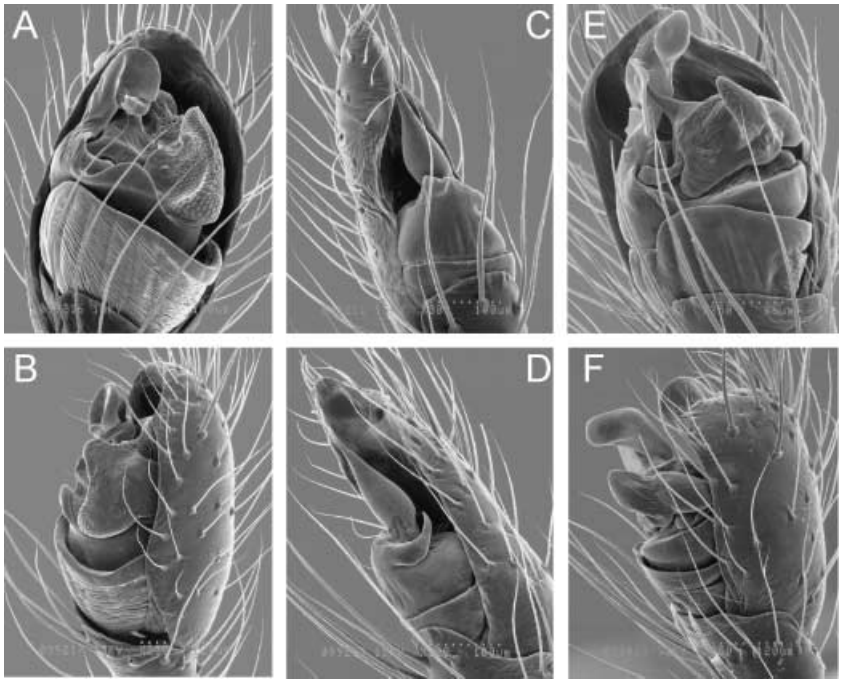

Fig. 2 A–C. SEM micrographies of right male palp tarsus: A and B, Unknown genus sp.: —A. retrolateral; —B. dorsal; C and D, *T. acutitarse*: —C. retrolateral; —D. dorsal; E and F, *T. grallator*: —E. retrolateral, —F. dorsal.

of such population-level studies is expected to provide insights into the generation of diversity at the molecular level.

However, population-level studies require understanding of the evolutionary relationships of the species in question. The Hawaiian happy face spider is only one of about nine, or more, *Theridion* species (and one subspecies) endemic to the islands (Simon 1900) (Figs 1–3). This assemblage of Hawaiian spiders is very poorly known and none of the species have been mentioned in the literature since their original description, other than in catalogues. An understanding of the evolutionary history of the lineage, in particular whether it emanated from one or multiple sources, and the identity of these sources, is necessary for establishing the evolutionary context of diversification of the genus within the islands, and how communities of multiple species within this genus have arisen.

Part of the reason for the paucity of studies is the overall phylogenetic status of the genus. *Theridion* Walckenaer 1805, is a cosmopolitan genus that ranks as one of the largest spider genera with more than 600 species described to date (Platnick 2006). Its magnitude is largely due to poor definition; it constitutes a 'dumping ground' for theridiids with no trace of a colulus and that do not fit other more defined genera (Forster *et al*. 1990b). The paraphyletic status of the genus was already implicitly recognised by Levi & Levi (1962), who considered theridiid genera such as *Chrysso* O.P. Cambridge, 1882 and *Thymoites* Keyserling 1884 as specialised offshoots of *Theridion*. Levi acknowledged vast genitalic differences between some of the species assigned to *Theridion*, but considered them of doubtful phylogenetic significance and inadequate to separate species groups. Considering larger genera easier to work with, Levi (1957, 1959, 1963; Levi & Levi 1962) organised *Theridion* species into several groups, mainly involving American species. Subsequent authors have elevated some of these species groups to genera (Wunderlich 1991, 1995a, 1995b; Yoshida 2001), while some of the genera subsumed in *Theridion* by Levi have since been removed from synonymy (Wunderlich 1987, 1995a). In addition, a number of new, small (several monotypic) genera containing *Theridion*-like species have been established (e.g., Wunderlich 1991, 1995a, 1995b; Yoshida 2001; Saaristo 2006). Recent work therefore has added to the confusion around the definition and the limits of *Theridion* and its affinities with other theridiid genera, which has been further aggravated by the lack of an explicit phylogenetic hypothesis based on a quantitative cladistic analysis of the group. Recent efforts have been made to remedy the situation through studies that have focused on the internal phylogentic structure of the family Theridiidae using morphological and behavioural data (Agnarsson 2003, 2004, 2006a) and DNA sequences from five gene fragments (Arnedo *et al*. 2004; Agnarsson 2006b). Not surprisingly, none of the cladistic analyses reported in these studies recovered the monophyly of the sampled *Theridion* species. However, all studies strongly supported monophyly of the theridiid species without any trace of a colulus (Theridiinae Sundevall 1833 *sensu* Agnarsson 2004), including *Theridion* and many other genera, and suggested the genus *Anelosimus* as the sister group to this clade.

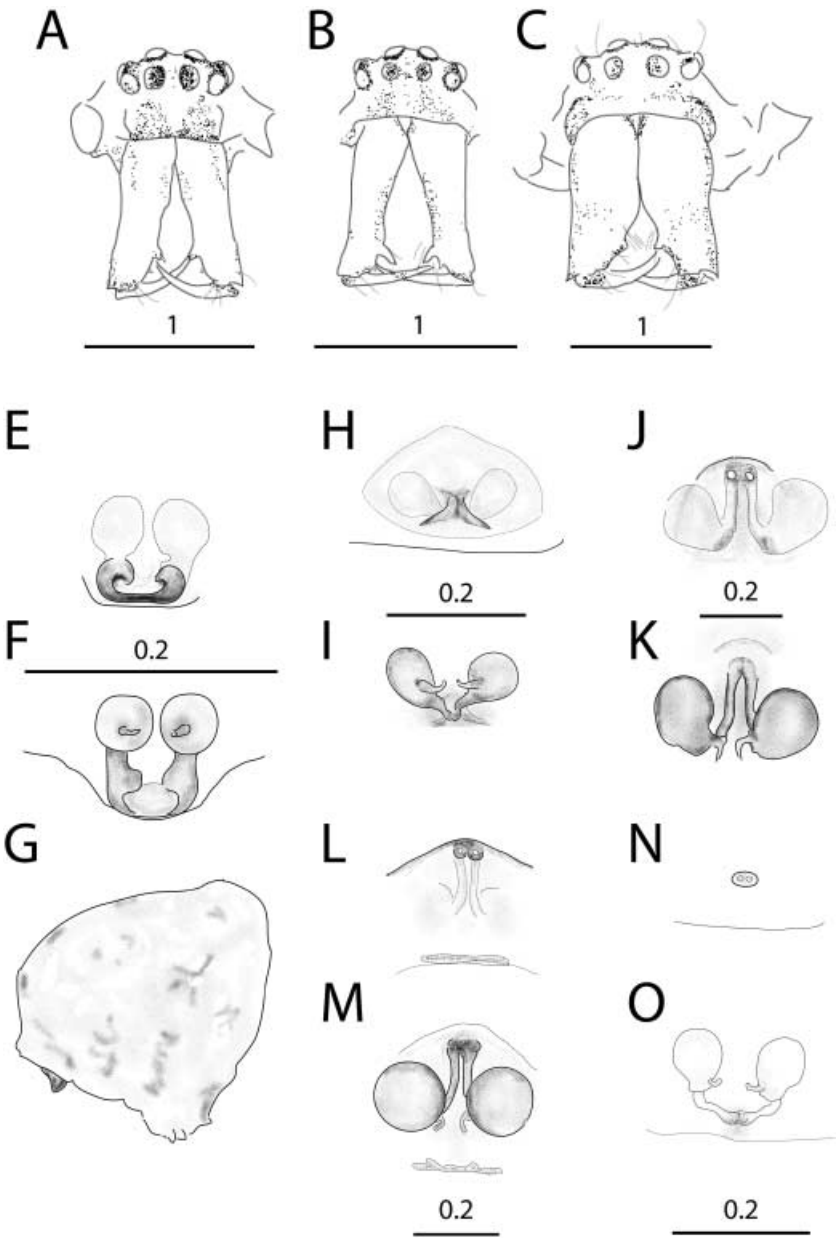

Fig. 3 A–O. A–C, Drawings of male anterior carapace and chelicerae, frontal view: —A. Unknown genus sp.; —B. *T. acutitarse*; —C. *T. grallator*. E–G, Drawings of unknown genus sp. female vulva: —E. ventral; —F. dorsal; —G. lateral (left, whole abdomen included). H and I, Drawings of *T. acutitarse* female vulva: —H. ventral; —I. dorsal. J and K, Drawings of *T. kauaiense* female vulva: —J. ventral; —K. dorsal. L and M, Drawings of *T. posticatum* female vulva: —L. ventral; —M. dorsal. N and O, Drawings of *T. grallator* female vulva: —N. ventral; —O. dorsal. Vulva were removed and treated with KOH 30% before observation. Scale bars in mm.

As will be apparent from this discussion, understanding of the colonisation history of the Hawaiian *Theridion* requires a broad sampling of genera across the subfamily Theridiinae, and cannot be limited to the genus *Theridion*. Although most of the Hawaiian *Theridion* seem to be closely related (Simon 1900), at least two appear morphologically distinct: *Theridion acutitarse* Simon, 1900 (Figs 1–3), and an undescribed species from Kauai and Oahu (Figs 1–3). The aims of the present work are to use molecular data to assess the number of independent colonisations necessary to account for the current diversity of *Theridion* in the Hawaiian Islands and in so doing to provide preliminary information on the phylogenetic structure of the genus *Theridion* and related genera to improve the delimitation of natural units in one of the largest spider genera.

Materials and methods

Taxonomic sampling

A total of 55 terminal taxa, containing 17 out of the 32 theridiid genera of the subfamily Theridiinae currently recognised, were included in the analysis (Platnick 2006). Larger genera (e.g., *Theridion*) were represented by more than one species, and three specimens of unknown or doubtful generic identity were included (see Table 1). The genera not represented in the analysis are mostly species poor (many

Table 1 Taxonomic and geographical information of the specimens included in the present study and GenBank accession number of the gene fragments sequenced for each specimen.

Genus	Species	Locality	Code	cox1	16S	18S	28S	H3
Ariamnes	*attenuata*	Guyana: S of Gunns Landing	MS101	AY231033	AY230946	AY230901	AY231078	AY230993
Anelosimus	*eximius**	Guyana: S of Gunns Landing	MS95	AY231031	AY230956	AY230899	AY231089	AY230991
Anelosimus	*rupununi*	Ecuador: Morona Santiago: rd. between Limón-Patuca	016A$^+$	EF050316	EF458144	EF050199	EF050256	EF050363
Achaearanea	*tepidariorum*	USA: NC: Macon Co., Highlands B.S.	MS15	AY231029	AY230955	AY230897	AY231088	AY230989
Achaearanea	***acoreensis***	**USA: HI: Kauai, Mohihi rd., Kohua tr. head**	X20	EF449594	EF449620	EF449547	EF449569	EF449528
Achaearanea	***trapezoidalis****	**French Guiana: Commune Regina, Les Nouragues F.S.**	235A	EF449595	EF449621	—	EF449570	—
Ameridion	sp.	Costa Rica: Cartago, Cerro de la Muerte	MS83	AY231030	AY230944	AY230898	AY231076	AY230990
Ameridion	***petrum***	Costa Rica: Cartago, Reserva Forestal de Rio Macho	MS82	EF449596	EF449622	EF449548	EF449571	—
Ameridion?	sp.	Malaysia: Pahang: Gunung Brinchang	102A	EF050270	EF458143	—	EF050205	EF050330
Arctathea	**n. sp.**	**French Guiana: Commune Regina, Les Nouragues F.S.**	226A	EF449597	EF449623	—	EF449572	—
Chrysso	sp.	Colombia: Iguaque	MS2	AY231036	—	AY230904	AY231093	AY230996
Chrysso	***albipes***	**Japan: Hokkaido: Kamishishoro-cho**	A72	EF449598	EF449624	EF449549	EF449573	EF449529
Coleosoma	*acutiventer*	Ecuador: Morona Santiago: km 7 from Limón towards Gualaceo	037A	EF050286	EF050181	—	EF050222	EF050339
Echinotheridion	*otlum*	Ecuador: Napo: Jatun Sacha	036A	EF050291	EF050179	DQ842141	EF050227	EF050344
Exalbidion	pallisterorum	Costa Rica: Puntarenas: Monteverde, road to Las Torres	164A	EF050296	EF050182	—	EF050232	—
Exalbidion?	sp.	Malaysia: Pahang: Tanah Rata	101A	EF050295	EF050183	EF050190	EF050231	EF050346
Helvibis	cf. *longicauda*	Guyana: S of Gunns Landing	MS98	AY231044	AY230965	AY230913	AY231099	AY231003
Keijia	***alabamensis***	**USA: TN: Sevier Co., GSMNP, Chinquapin ridge**	S9509	EF449602	EF449628	EF449553	EF449578	EF449532
Keijia	*mneon*	USA: HI: Kauai, Hanalei	X43	AY231037	AY230960	AY230905	AY231094	AY230997
Keijia	***punctosparsa***	**USA: TN: Blount Co., GSMNP, Tabcat cr. & Maynard cr.**	9862	EF449615	EF449640	EF449564	EF449589	EF449543
Meotipa	sp.	Malaysia: Pahang: Gunung Brinchang	104A	EF050306	EF050180	—	EF050245	—
Neottiura	*bimaculata**	Slovenia: 500 m N of Cmice	X51	AY231047	AY230967	AY230916	AY231101	AY231005
Nesticodes	*rufipes**	USA: HI: Oahu, Hawaii Kai	X6	AY231049	AY230968	AY230918	AY231102	AY231007
Rugathodes	*sexpunctatus**	USA: NC: Haywood Co, Cataloochee	X98	AY231054	—	AY230923	AY231106	AY231011
Rugathodes	***aurantius***	**USA: NC: Swain Co., GSMNP, Andrews bald**	X33	EF449600	—	EF449551	EF449575	—
Rugathodes	sp.	Costa Rica: Guanacaste: Playa Hermosa	149A	EF050314	EF458145	—	EF050254	—
Simitidion	***Simile****	**Costa Rica: Guanacaste: Playa Hermosa**	145A	—	EF449626	—	EF449576	—
Takayus	***lyricus***	**USA: NC: Macon Co., Highlands B.S.**	X37	EF449608	EF449634	EF449558	EF449584	EF449537
Theridiinae	sp.	Malaysia: Pahang: Gunung Brinchang	103A	EF050323	EF458146	—	EF050263	—
Theridion	***acutitarse***	**USA: HI: Kauai: Koke'e S.P., Nualolo tr.**	K71	EF449601	EF449627	EF449552	EF449577	EF449531
Theridion	***albidum***	**USA: NC: Macon Co., Cole gap**	X36	EF449603	EF449629	EF449554	EF449579	—
Theridion	*calcynatum*	Ecuador: Napo: Caucheras, Yenayacu	035A	EF050322	—	EF458147	EF050262	EF050368
Theridion	***differens***	**TN: Sevier Co, GSMNP, NW Chimneys picnic area**	9613	EF449604	EF449630	EF449555	EF449580	EF449533
Theridion	***flavonotatum***	**USA: NC: Macon Co., Highlands B.S.**	X32	EF449605	EF449631	EF449556	EF449581	EF449534
Theridion	cf. *frondeum*	USA: NC: Macon Co, Highlands B.S.	X31	AY231060	AY230953	AY230929	AY231086	AY231016
Theridion	***glaucescens***	**USA: NC: Macon Co., Highlands B.S.**	X35	EF449606	EF449632	EF449557	EF449582	EF449535
Theridion	*grallator*	USA: HI: Hawaii, Puu Makaala	X67	AY231061	AY230952	AY230930	AY231085	AY231016
Theridion	***kauaiense***	**USA: HI: Kauai, Kokee S.P., Mohihi rd., Koua tr. head**	K5	EF449618	EF449642	EF449567	EF449592	EF449545
Theridion	*longipedatum*	Colombia: Iguaque	X54	AY231062	AY230954	AY230931	AY231087	—
Theridion	***melanosticum***	**USA: HI: Maui**	X97	EF449610	EF449636	EF449560	EF449585	EF449539
Theridion	***melanurum***	**UK: England: Yorkshire**	A25	EF449609	EF449635	EF449559	—	EF449538
Theridion	***murarium***	**USA: TN: Sevier Co., GSMNP, along Porters cr.**	9617b	EF449611	EF449637	EF449561	—	EF449540
Theridion	*nigroannulatum*	Ecuador: Morona Santiago: 6.6 km N of Limón to Méndez	055A$^+$	EF050324	EF050178	EF050201	EF050264	EF050369
Theridion	***pennsylvanicum***	**USA: NC: Macon Co., Blue Valley**	X34	EF449612	EF449638	EF449562	EF449586	EF449541
Theridion	***pictipes***	**USA: NC: Swain Co., GSMNO, Indian cr.**	X153	EF449614	EF449639	EF449563	EF449588	EF449542
Theridion	***pictum***	**Austria, Innsbruck**	MS96	EF449613	—	——	EF449587	—
Theridion	***posticatum***	**USA: HI: Oahu: Koolaus: Puamoho tr.**	OK77	EF449619	EF449643	EF449568	EF449593	EF449546
Theridion	***theridioides***	**Australia**	X150	EF449616	EF449641	EF449565	EF449590	EF449544
Theridion	*varians*	UK: England: Yorkshire	A34	AY231063	AY230976	AY230932	AY231111	AY231017
Theridula	*opulenta**	USA: NC: Macon Co, Horse cove	MS4	AY231064	AY230977	AY230933	AY231112	—
Theridula	***emertoni***	**USA: NC: Macon Co., Highlands B.S.**	MS26	EF449617	—	EF449566	EF449591	—
Thymoites	*unimaculatus*	USA: MA: Middlesses Co., Pepperell	A58	AY231066	AY230978	AY230935	AY231114	AY231019
Tidarren	*sisyphoides**	USA: SC: Pickens Co., L. Issaqueena	MS21	AY231067	AY230979	AY230936	AY231115	AY231020
Unknown genus	**sp.**	**USA: HI: Oahu: Koolaus, Waimano trail**	OK3	EF449599	EF449625	EF449550	EF449574	EF449530
Wamba	***crispulus***	**USA: TN: Blount Co., GSMNP, Cades cove**	9613	EF449607	EF449633	—	EF449583	EF449536

An asterisk after the species name indicates it is the type species of the genus. Accession numbers starting with EF denote new sequences produced in the present study. — denotes that the specific sequence could not be obtained. 016A$^+$: 16S gene fragment obtained from specimen 268A, 055A$^+$: cox1 gene fragment obtained from specimen 56A.

monotypic) and with restricted distributions, with the exception of *Molione* Thorell 1892 (five species) and *Paidiscura* Archer 1950 (four species). Twenty species of *Theridion* were sampled representing all species groups proposed by Levi (1957, 1959). The genera *Argyrodes* and *Anelosimus* were selected as outgroups based on former studies that suggested *Anelosimus* as the sister group to Theridiinae and *Argyrodes* as part of the sister clade to *Anelosimus* plus the 'lost colulus' clade (Agnarsson 2004, 2006c; Arnedo *et al*. 2004). All trees were rooted at the branch joining *Argyrodes* to the remaining taxa. The following endemic Hawaiian *Theridion*-like species representing the range of somatic and genitalic diversity observed were included in the analysis: *T. kauaiense* Simon, 1900 (Fig. 1A); *T. grallator* (Fig. 1B); *T. posticatum* Simon, 1900 (Fig. 1C); *T. acutitarse* Simon, 1900 (Fig. 1D) and an undescribed species of uncertain affinities (Fig. 1E). The species *T. kauaiense* and *T. posticatum*, share a common genitalic pattern, which also closely resembles that of *T. grallator* (Figs 2 and 3). The last species is characterised by a slender body shape, long appendages and a particular colouration (greenish with white, yellow, black or red spots on abdomen). The species *T. acutitarse* shows a remarkable simplification of male genitalia with a reduction or loss of most of its sclerites (Fig. 2). Finally, we also include an undescribed species of uncertain generic affinities found in the field by the authors and subsequently rediscovered in museum collections where it had been labelled by former researchers as either belonging to a new genus or questionably as a member of the genus *Chrysso* (hereafter referred to as unknown genus sp.) (Figs 1–3). All taxa and localities analysed in the present study are listed in Table 1.

Molecular characters

Protocols for specimen handling, DNA extraction, amplification and sequencing of the mitochondrial gene fragments cytochrome oxidase I (cox1) and 16S rRNA (16S) and the nuclear gene fragments 18S rRNA (18S), 28S rRNA (28S) and Histone 3 A (H3) followed those of Arnedo *et al*. (2004). About 2.5 kb from five different gene fragments were obtained from each terminal. GenBank accession numbers of the new sequences obtained in the present study along with sequences available from previous studied are listed in Table 1. Vouchers were deposited at the Essig Museum of Entomology at UC Berkeley and at the Department of Zoology of the University of British Columbia.

Phylogenetic analysis

The ribosomal gene fragments sequenced showed differences in length, suggesting the occurrence of insertion/deletion events during the evolution of these sequences. The information on indel events is fundamental to assess positional homology in sequences of different length, and it can be incorporated into phylogenetic analyses in two different ways. The most widely used method involves the use of gaps to align nucleotides assumed to be homologous among the terminals. In this case, either automatic alignment programs (e.g., CLUSTAL) or manual alignments based on additional information (e.g., secondary structure in ribosomal genes) are used to build static alignments that are subsequently analysed using standard phylogenetic inference methods. Alternatively, insertion/deletion events can be included as one of the possible transformations along with nucleotide transformations during cladogram optimisation (Wheeler 1996). In this approach, referred as direct optimisation, alignments are dynamic and depend upon a particular topology. In this study, sequences were analysed using both static alignments and direct optimisation.

Automatic alignment methods are superior to manual implementations because of their objectivity and repeatability (Ogden *et al*. 2005). However, these methods are dependent upon parameters that require the explicit but somehow arbitrary selection of specific values. A way to circumvent this problem is use Sensitivity analysis (Wheeler 1995), exploring the data under a range of parameter values to assess the robustness of the results to changes in analytical assumptions.

Static alignments

For each ribosomal gene, multiple sequence alignments were constructed with CLUSTAL X (Thompson *et al*. 1997). The effects of alternative gap opening (GOP) and extension (GEP) costs were examined by constructing alignments under different cost combinations, from very gappy to more consolidated alignments (Hedin & Maddison 2001). The following combinations were explored (GOP : GEP): 8 : 2, 8 : 4, 20 : 2, 24 : 4 and 24 : 6 (in all cases transition weight was fixed to 0.5). Congruence among data partitions as measured by the ILD (Mickevich & Farris 1981) and the RILD (Wheeler *et al*. 1998) was used to select across the different parameter cost combinations assayed (Wheeler 1995; Wheeler & Hayashi 1998; Giribet 2003). Comparisons were limited to the protein coding genes, with each of the ribosomal genes examined separately (15 comparisons) to avoid an overwhelmingly large number of analyses (125 comparisons). The combined data matrix was constructed by concatenating the protein coding genes with the preferred static alignment of each ribosomal data set in WINCLADA v.1.00.08 (Nixon 2002). Gaps were incorporated into the analyses as separate presence/absence characters, according to a set of rules based on gap overlapping and sharing of the 5′ and/or the 3′ termini (Simmons & Ochoterena 2000). This coding scheme allows incorporation of indel information in phylogenetic reconstruction using not only parsimony but also Bayesian inference methods, while minimising the effect of increasing the weight of overlapping multiple non-homologous gaps that result from scoring gaps as an additional state (Pons & Vogler 2006). The

program GapCoder (Young & Healy 2002) was used to facilitate the automatic recoding of the alignments based on the simple method proposed by Simmons *et al*. (2001).

Parsimony analyses of the static matrices were conducted with the program TNT v.1.0 (Goloboff *et al*. 2003) based on heuristic searches consisting of 1000 iterations of Wagner trees constructed with random addition of taxa and subsequent TBR branch swapping, holding five trees per iteration and up to a total maximum of 10 000. When the number of replicates finding optimal trees was less than 10%, the number of replicates was increased to 1000. Clade support was assessed via jackknife resampling (Farris *et al*. 1996) using 1000 replicates with individual heuristic searches consisting of 15 iterations of Wagner tree construction using random addition of taxa, holding five trees per iteration and an overall maximum of 10 000.

The online version (Posada 2006) of the program ModelTest v.3.06 (Posada & Crandall 1998) was used to select the substitution model that best fit the data with the fewest parameters (including branch lengths as parameters), as indicated by the Akaike information criterion (AIC) (Akaike 1973), which allows the comparison of multiple nested models and accounts for model selection uncertainty (Posada & Buckley 2004).

Bayesian inference analyses were performed with MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003) and run at Cornell's CBSU computer cluster (http://cbsuapps.tc.cornell.edu/mdiv.aspx). Unlinked nucleotide substitution models selected by ModelTest were specified for each gene fragment and a standard discrete model was implemented for the gaps scored as absence/presence data. The substitution estimates were allowed to vary independently between each partition. Two independent runs with four simultaneous MCMC (Markov Chain Monte Carlo) chains (one cold and three heated), each starting with random starting trees, were carried out simultaneously, sampling 1000 generations until the standard deviation of the split frequencies of these two runs dropped below 0.01 (10 million generations). The program TRACER v.1.3 (Rambaut & Drummond 2003) was used to ensure that the Markov chains had reached stationarity by examining the effective sample size (ESS) values and also to determine the correct number of generations to discard as a *burn-in* for the analysis.

Maximum likelihood analyses were performed with Phyml v.2.4 (Guindon & Gascuel 2003), implementing the model of nucleotide substitution selected by ModelTest and using the Bayesian consensus tree as starting tree. Clade support was assessed through bootstrap resampling (Felsenstein 1985) using 500 replicates.

Direct optimisation

Direct optimisation analyses were performed with the computer program POY v.3.0 (Wheeler *et al*. 1996–2003) under the maximum parsimony criterion. The 16S, 18S and 28S gene fragments were spliced into 8, 10 and 5 regions, respectively, of about 100 bp flanked by a series of about 10 nucleotides long conserved motifs to speed up computation time (Giribet 2001). Protein-coding genes were incorporated into the analyses as prealigned fragments. Analyses were run on a Linux cluster of 38 processors at clock speeds between 1 and 2.4 GHz at Harvard University (darwin.oeb.harvard.edu) with PVM software and the parallel version of POY (commands -parallel -dpm -dpmacceptratio 1.5 in effect). The heuristic search strategy involved 100 replicates of random addition of taxa (buildmaxtrees 2 -multirandom -replicates 100). Each replicate was followed by two rounds of tree fusing (-treefuse -fuselimit 10 -fusemingroup 5) and tree drifting (-numdriftchanges 30 -driftspr -numdriftspr 10 -drifttbr -numdrifttbr 10), holding up to five trees per round (-maxtrees 5) and using the command -fichtrees, which saves the most diverse cladograms found for each island. A final round of refinement was accomplished by pulling together all cladograms obtained in the searches run under the different gap and transversion cost combinations assayed (see below) and performing an additional round of treefusion (-treefuse -fuselimit 100 -minterminals 50). In all searches, cladograms found within 0.5% of the minimum tree length (-slop 5 -checkslop 10) were examined to avoid finding of suboptimal trees due to tree length miscalculations (POY uses some shortcuts to speed up tree evaluation) and the maximum number of trees saved was set to 50 (-holdmaxtrees 50). Clade support was assessed by means of Jackknife proportions using 100 randomly resampled matrices, with a probability of character deletion of 1/e (default option).

Sensitivity of the results to particular assumptions of the analyses were investigated using the following combinations of gap opening (GOC), gap extension (GEC) and transversion (TV) and transition (TS) costs (GOC : GEC : TV : TS): 1 : 1 : 1 : 1, 2 : 1 : 2 : 1, 2 : 1 : 2 : 2, 4 : 1 : 2 : 1, 4 : 1 : 2 : 2, 8 : 1 : 4 : 2. Congruence among data partitions as measured by the ILD (Mickevich & Farris 1981) was used to select across the different parameter cost combinations assayed (Wheeler 1995; Wheeler & Hayashi 1998; Giribet 2003).

Results

Static alignments

Characteristics of the static alignments obtained under the different GOP and GEP combinations explored, along with results of the parsimony analyses of the separated and combined searches are summarised in Table 2. Ribosomal alignments obtained with parameter combinations (GOP : GEP) 24 : 4, 8 : 2 and 20 : 2 for the 16S, 18S and 28S, respectively, were selected as the best alignments based on maximum congruence with protein coding genes. Uniformly weighted parsimony of the concatenated data matrix of the preferred alignment of each ribosomal data set along with the protein coding gene fragments with gaps coded as absence/presence

Table 2 Summary of the parsimony searches conducted separately on the ribosomal alignments constructed under different parameter combinations and the protein coding genes (*cox1*, *H3*), and combining the protein coding genes (cox1-H3) and each ribosomal gene with the protein coding genes (cox1-H3-16S, cox1-H3-18S, cox1-H3-28S).

								cox1		H3		cox1-H3		cox1-H3-16S					
GOP	GEP	TV:TS	#	μ	σ	T	L	T	L	T	L	T	L	T	L	MAX	ILD1	ILD2	RILD1	RILD2
16S																				
8	2	0.5	178	2.55	2.36	6	1571	4	1336	252	479	5	1857	72	3523	5285	0.0389	0.0270	0.0721	0.0171
8	4	0.5	151	2.58	2.55	40	1575	4	1336	252	479	5	1857	2	3522	5295	0.0375	0.0256	0.0693	0.0161
20	2	0.5	127	2.91	2.78	26	1610	4	1336	252	479	5	1857	6	3555	5303	0.0366	0.0248	0.0692	0.0159
24	**4**	**0.5**	**111**	**2.99**	**3**	**6**	**1666**	**4**	**1336**	**252**	**479**	**5**	**1857**	**6**	**3611**	**5422**	**0.0360**	**0.0244**	**0.0670**	**0.0157**
24	6	0.5	112	2.85	2.84	29	1667	4	1336	252	479	5	1857	2	3613	5455	0.0363	0.0246	0.0664	0.0158
18S																				
8	**2**	**0.5**	**26**	**15.62**	**34.96**	**> 10000**	**247**	**4**	**1336**	**252**	**479**	**5**	**1857**	**6**	**2123**	**3080**	**0.0287**	**0.0089**	**0.0599**	**0.0041**
8	4	0.5	40	1.6	1.1	> 10000	351	4	1336	252	479	5	1857	6	2230	3187	0.0287	0.0099	0.0627	0.0047
20	2	0.5	23	17.65	59.31	> 10000	248	4	1336	252	479	5	1857	6	2125	3082	0.0292	0.0094	0.0608	0.0043
24	4	0.5	22	18.5	81.19	> 10000	252	4	1336	252	479	5	1857	6	2130	3087	0.0296	0.0099	0.0618	0.0045
24	6	0.5	26	1.77	1.7	> 10000	374	4	1336	252	479	5	1857	6	2254	3211	0.0288	0.0102	0.0636	0.0049
28S																				
8	2	0.5	6	2.67	2.25	336	236	4	1336	252	479	5	1857	175	2159	3123	0.0500	0.0306	0.1007	0.0139
8	4	0.5	6	2.67	2.25															
20	**2**	**0.5**	**5**	**3.2**	**3.03**	**336**	**237**	**4**	**1336**	**252**	**479**	**5**	**1857**	**69**	**2159**	**3124**	**0.0496**	**0.0301**	**0.0998**	**0.0137**
24	4	0.5	5	3.2	3.03															
24	6	0.5	5	3.2	3.03															

GOP: gap opening penalty; GEP: gap extension penalty; TV : TS: transversion/transition ratio; #: number of gap absence/presence characters; μ: average length of gaps; σ: standard deviation of gap length; T: number of most parsimonious trees (MPTs); L: number of steps of the MPTs; MAX: maximum number of steps of the combined data matrices; ILD1: incongruence length difference among ribosomal, cox1 and H3; ILD2: incongruence length difference between ribosomal and protein coding genes combined: RILD1: rescaled incongruence length difference among ribosomal, cox1 and H3. RILD2: rescaled incongruence length difference between ribosomal and protein coding genes combined. Best parameter combination in bold. For the 28S, the five parameter combination assayed resulted in only two different alignments (8 : 2 = 8 : 4 and 20 : 2 = 24 : 4 = 24 : 6).

Table 3 Model of evolution and parameter values selected by ModelTest for each gene fragment and the concatenated data matrix.

		freqA	freqC	freqG	freqT	Ti/tv	[A-C]	[A-G]	[A-T]	[C-G]	[C-T]	[G-T]	I	Γshape
CO1	TVM + I + G	0.4008	0.0233	0.0685	0.5074	—	0	33.8474	0.9745	47.0798	33.8474	1.0	0.4079	0.2848
16S	GTR + I + G	0.4191	0.0631	0.0849	0.4329	—	11.4555	18.1272	24.7689	13.9460	77.9072	1.0	0.2531	0.6763
18S	K80 + I + G	0.25	0.25	0.25	0.25	1.5755	—	—	—	—	—	1.0	0.5751	0.8938
28S	TrN + I + G	0.2482	0.2345	0.3248	0. 1925		1.0	1.9093	1.0	1.0	6.1281	1.0	0.3997	0.5043
H3	GTR + I + G	0.3015	0.2151	0.2281	0.2554		4.4145	18.4007	6.0085	1.1428	25.0169	1.0	0.3707	0.4240
ALL	TVM + I + G	0.2972	0. 1725	0.2180	0.3123		1.3627	9.9510	12.8639	1.9008	9.9510	1.0	0.4971	0.5591

characters (collapsing rule minimum length = 0) yielded 2 trees of 4178 steps each (CI = 0.33, RI = 0.42) (Fig. 4).

Table 3 summarises the models selected by ModelTest for each gene fragment and the concatenated data. Maximum likelihood analyses resulted in 1 tree of –ln L = 21095.460811 (not shown). A majority rule consensus of 16 762 trees obtained after *burnin* from the Bayesian analyses of the concatenated data matrix along with maximum likelihood bootstrap support values is shown in Fig. 5.

Parsimony and model-based analyses of the static alignment support a split of the Hawaiian representatives into two different clades. The species *T. kauaiensis*, *T. grallator* and *T. posticatum* form a clade with high support (parsimony jackknife support, JS = 98; maximum likelihood bootstrap support, BS = 100, Bayesian posterior probability, PP = 1.0, hereafter referred as the *T. grallator*-clade), which all analyses identify as sister to the species *Exalbidion pallisterorum*, also with high support (JS: 98, BS: 96, PP: 1.0). The two remaining Hawaiian representatives join two *Rugathodes* species to form a clade that receives high support in all analyses (JS: 97, BS = 97, PP = 1.0), with the relationship between the unknown genus sp. and the *Rugathodes* species sister group being moderately supported (JS: 59, BS: 76, PP < 0.95).

The Theridiinae were recovered as monophyletic in all analyses (JS: 99, BS = 100, PP = 1.0). Although basal relationships among Theridiinae were poorly supported, several

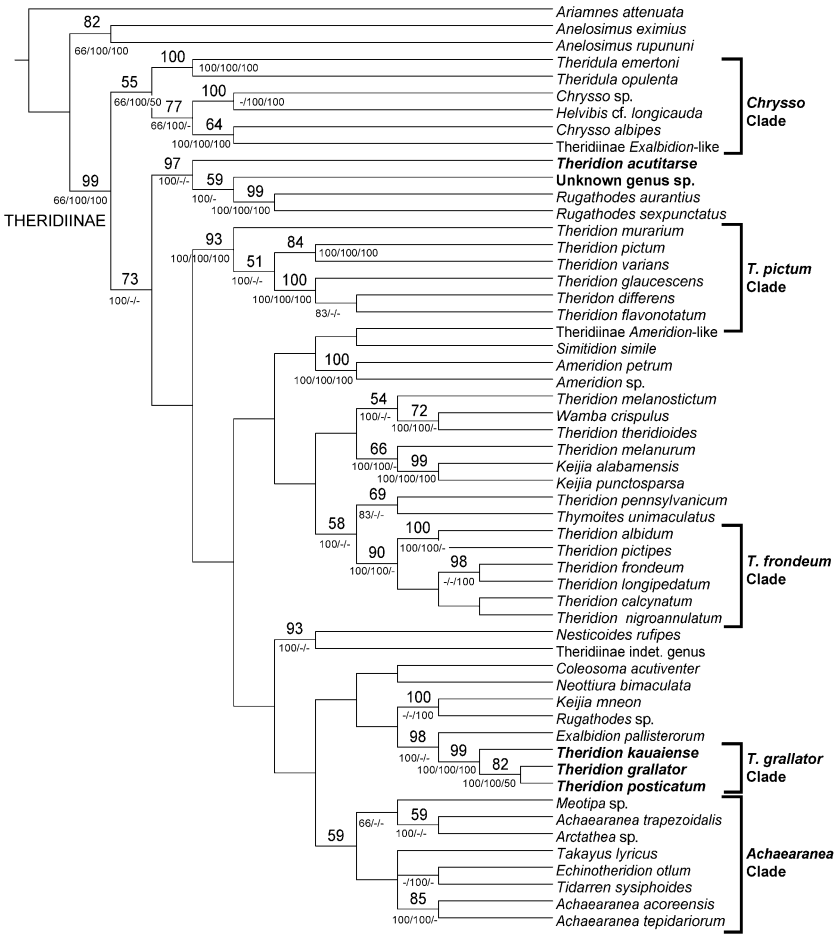

Fig. 4 Strict consensus of the two most parsimonious trees obtained from the analyses of the concatenated data matrix (see text). Number above branches jackknife support values (< 50% not shown). Numbers below branches, percentage of alignment parameter schemes that recovered the clade for 16S/18S/28S, respectively. –: Clade not supported or recovered by less than 50% of the alignment parameter schemes. Hawaiian species in bold.

clades appeared consistently with high support across the different analyses. The genera *Theridula*, *Chrysso*, *Helvibis* and the '*Exalbidion*-like' specimen form a clade poorly supported with parsimony (JS: 55) but with high support in model-based analyses (BS = 98, PP: 1.00, hereafter referred to as the *Chrysso*-clade), which is shown as the sister clade to the remaining Theridiinae (JS: 99, BS = 92, PP = 0.99). *Chrysso* itself is paraphyletic and includes *Helvibis* cf. *longicauda* and the *Exalbidion*-like specimen (JS: 77, BS = 94, PP = 1.0). Other well-supported clades include: (*T. murarium* + *T. pictum*, the type species of *Theridion*, + *T. varians* + *T. glaucescens* + *T. differens* + *T. flavonotatum*) (JS: 93, BS = 96, PP = 1.0, hereafter referred as *T. pictum*-clade); (*T. calcynatum*, *T. nigroannulatum*, T. longipedatum + *T. frondeum* + *T. albidum* + *T. pictipes*) (JS: 90, BS = 95, PP = 1.00, hereafter referred to as the *T. frondeum*-clade); and *Meotipa* sp. + *Arctathea* sp. + *Takayus lyricus* + *Echinotheridion otlum* + *Tidarren sysiphoides* + and the three *Achaearanea* species (JS: 59, BS = 92, PP = 0.99, hereafter referred to as the *Achaearanea*-clade); The remaining supported relationships involve species of the same nominal genus, or species pairs representing different genera, such as *Keija mneon* + *Rugathodes* sp. (JS: 100, BS = 100, PP = 1.0); *Nesticoides rufipes* + Theridinae indet. genus (JS:93, BS = 100, PP = 1.00); *T. pennsylvanicum* + *Thymoites unimaculatus* (JS: 69, BS = 92, PP = 0.98) and *T. theridioides* + *Wamba crispulus* (JS: 72, BS < 50, PP < 0.95).

Dynamic optimisation

Statistics of trees obtained from direct optimisation searches are summarised in Table 4. Analysis with gap opening and base transformations costs twice the cost of gap extension minimised the values of the ILD among gene fragments, and was used as a reference for further comparison among trees. The strict consensus of the 7 trees of 8044 steps found under the preferred parameter combination (Fig. 6) shows a very low level of resolution; most of jackknife support values above 50% concentrate towards the tips of the cladogram. Similarly, most of clades showed high sensitivity to changes in parameter costs, and were only supported by one or few parameter schemes. Relationships matched those found in static analyses, including the separation of Hawaiian representatives into two independent clades related to *Exalbidion*

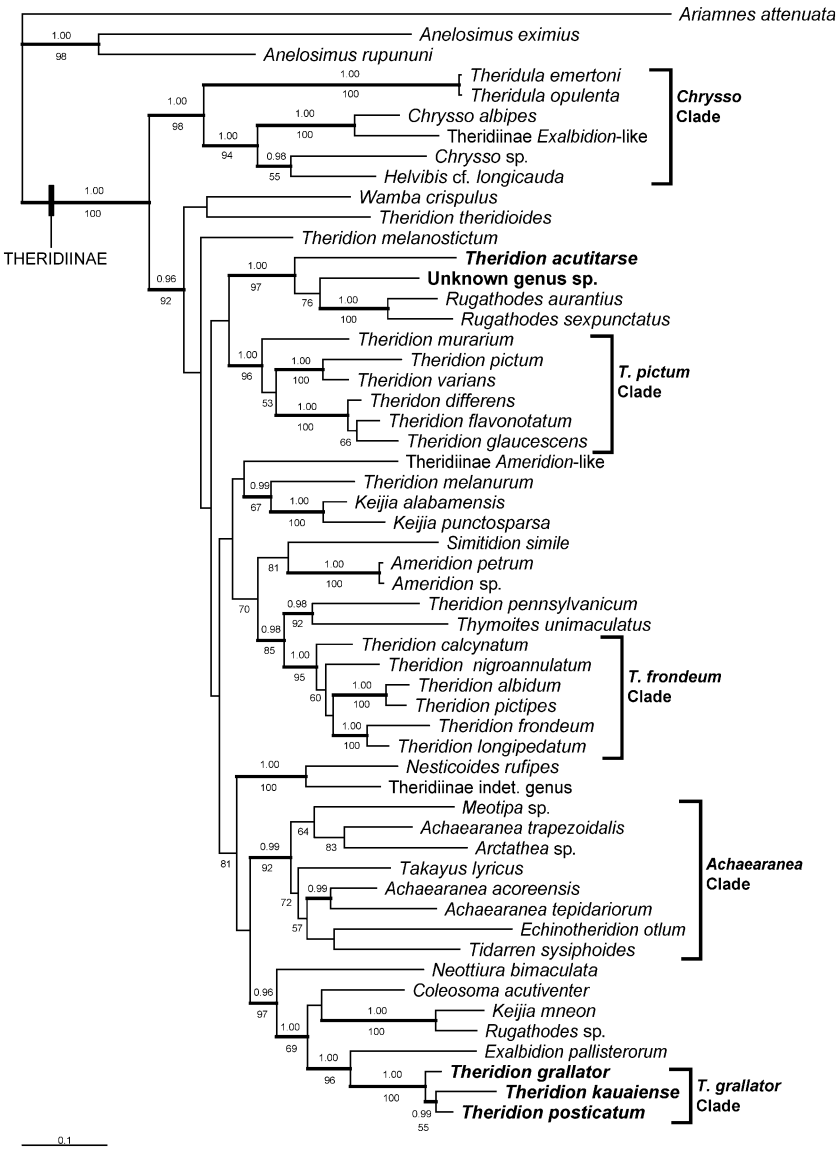

Fig. 5 A majority rule consensus of 16 762 trees obtained after *burnin* from the Bayesian analyses of the concatenated data matrix. Posterior probabilities (PP > 95%), above branches and maximum likelihood bootstrap support values (> 50%), below branches. Thick branches correspond to clades supported > 0.95 PP. Hawaiian species in bold.

Table 4 Summary of the direct optimisation searches conducted on the ribosomal genes separately (16S, 18S, 28S) and all combined with the protein-coding genes (ALL).

				ALL		16S		18S		28S		cox1		H3		
GOC	GEC	TV	TS	T	L	T	L	T	L	T	L	T	L	T	L	ILD
1	1	1	1	4	4225	4	1549	> 50	425	> 50	225	4	1336	252	479	0.0499
2	1	2	1	1	6437	1	2520	> 50	562	> 50	309	1	2112	31	619	0.0489
2	**1**	**2**	**2**	**6**	**8044**	**4**	**2910**	**> 50**	**695**	**> 50**	**442**	**4**	**2672**	**252**	**958**	**0.0456**
4	1	2	1	1	6921	3	2870	> 50	640	> 50	328	1	2112	31	619	0.0509
4	1	2	2	3	8560	1	3289	> 50	776	> 50	469	4	2672	252	958	0.0463
8	1	4	2	1	13350	1	5458	> 50	1111	> 50	647	1	4224	31	1238	0.0503

Separate analyses of protein coding genes were performed with static parsimony given that no length polymorphism was observed. GOC: gap opening cost; GEC: gap extension cost; TV: transversion cost; TS: transition cost; T: number of most parsimonious trees (MPTs); L: number of steps of the MPTs; ILD: incongruence length difference among ribosomal genes, cox1 and H3. Best parameter combination in bold.

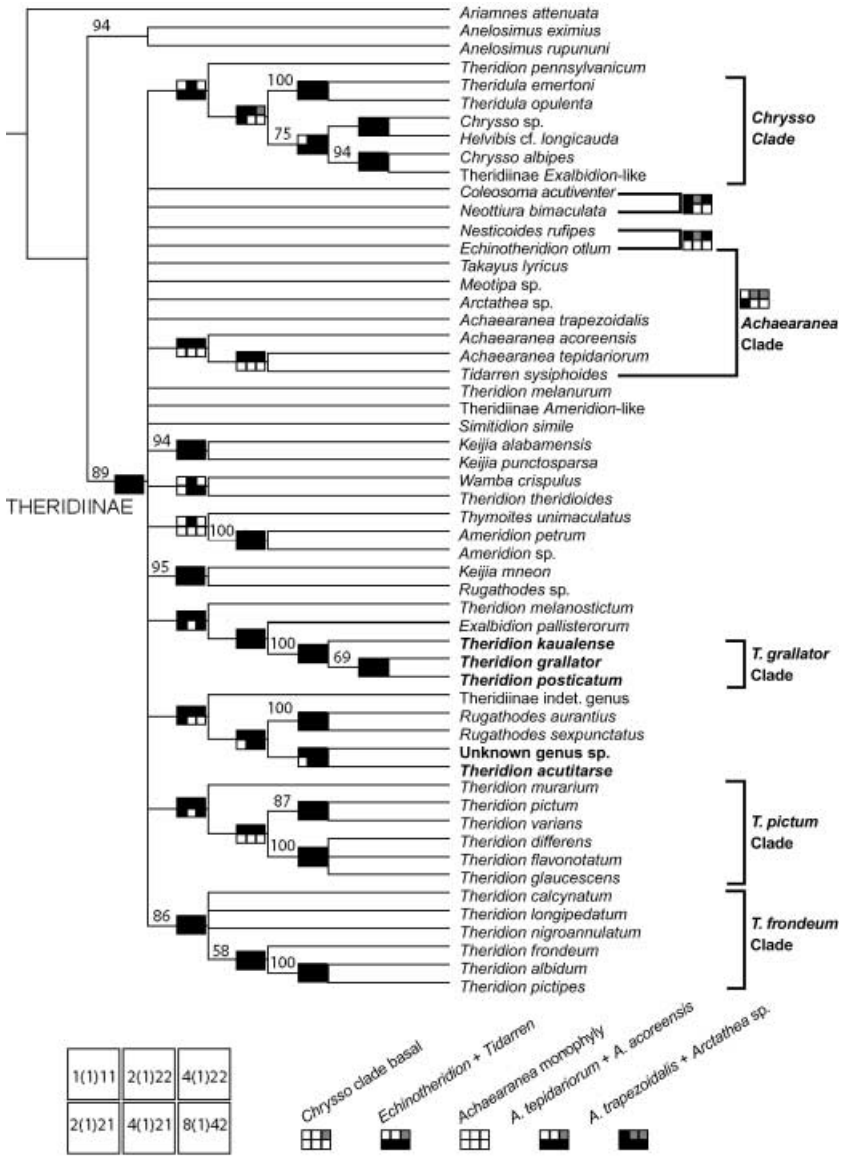

Fig. 6 Strict consensus of seven most parsimonious trees obtained by direct optimisation analysis of all gene fragments with gap opening cost 2, gap extension cost 1, transversion cost 2 and transition cost 2. Jackknife support values above branches. Those clades recovered in alternative parameter schemes in black squares or grey if only recovered in some of the trees. Alternative hypotheses not supported in the tree are listed below. Hawaiian species in bold.

and *Rugathodes*, respectively (JS < 50% but found in all parameter combinations), the monophyly of Theridiinae (JS = 89%, found in all parameter combinations), the *Chrysso*-clade (JS < 50% but found in four out of six parameter combinations) and paraphyly of *Chrysso* (JS < 75%, found in five out of six combinations), the *T. pictum*-clade (JS < 50% but found in five out of six parameter combinations), and the *T. frondeum*-clade (JS < 86%, found in all parameter combinations). The sister group relationship of the *Chrysso*-clade with the remaining Theridiinae was only observed with parameter combination gap opening 4 and base transformation twice the extension gaps. The *Achaearanea*-clade was recovered in three out of six parameter combinations.

Discussion

Source of colonists

The phylogenetic reconstructions give strong support for independent colonisation of the Hawaiian Islands by two different lineages within the Theridiinae. The *T. grallator*-clade appears to have colonised from the Americas, having as its sister the genus *Exalbidion*, which comprises five species from Central and South America; the examplar used in the current study, *E. pallisterorum*, is from Central America. However, an alternative source of colonists cannot be completely ruled out, given the number of lineages that were not sampled in the present study. Monophyly of the other two Hawaiian species analysed (*T. acutitarse* and the unknown genus sp.) is

supported in direct optimisation analyses, but model based analyses show them as paraphyletic with regards to *Rugathodes*, albeit in all cases with low support. A single origin of the Hawaiian *Rugathode*s-like lineages is a more plausible scenario than independent colonisations of such a remote archipelago by two very closely related lineages. The two Hawaiian *Rugathode*s-like species have as their sister the holarctic genus *Rugathodes*. The exemplars used in the current study, *R. aurantius* and *R. sexpunctatus* (type species of the genus), being palearctic in distribution. Interestingly, of the eight species currently placed in *Rugathodes*, four occur on oceanic islands, including the very isolated Azores, as well as Madeira and Japan.

Ongoing studies of arthropods suggest that perhaps the majority of the Hawaiian species are derived from the Americas. For example, the planthopper genus *Nesosydne* (Delphacidae) is distributed throughout the islands of the Pacific. In Hawaii there are a total of 82 species (Zimmerman 1948; Asche 1997), most being single island endemics. Recent morphological studies by Asche (1997) suggest that at least some of the Hawaiian delphacid taxa are of North American origin. Similarly, the cave dwelling *Oliarus* (Cixiidae) appear to have been derived from American ancestors (Hoch & Howarth 1999). A number of spider genera are represented by species radiations on the islands of Hawaii, Marquesas and Societies. However, at least in the genus *Tetragnatha*, these lineages are now known to be unrelated, with the Hawaiian *Tetragnatha* radiation being derived from an American source (Gillespie 2002). Endemic Hawaiian jumping spiders (*Havaika*, Salticidae) may also be of American origin, although this has not yet been confirmed (Arnedo & Gillespie 2006). Among crab spiders (Thomisidae), endemic lineages in Hawaii form a clade with those from the southern Polynesian islands of the Marquesas and Societies, with the entire clade apparently derived from a continental American source (Garb & Gillespie 2006).

Success of colonisation

Although the evidence presented suggests that two Theridiinae lineages have colonised and undergone local diversification in the Hawaiian Islands, they show a certain asymmetry in their relative diversification rates. According to genitalic patterns (Figs 2 and 3), the *T. grallator*-clade includes at least nine additional species distributed along the Hawaiian chain, while the *Rugathodes*-like lineage includes only *T. acutitarse* and the unknown genus sp. A similar phenomenon of independent colonisations of the islands by the same genus, and asymmetry in diversification rates, has been noted in the Hawaiian *Tetragnatha*, with much of the species diversity attributed to a single colonisation, while a second natural colonisation (*T. hawaiensis*) has resulted in little diversity (Gillespie *et al*. 1994). However, the pattern in the Hawaiian *Theridion* may be a taxonomic artefact. Ongoing research (Arnedo & Gillespie, unpublished data) has revealed that the species *T. acutitarse* may actually constitute a species-complex with as many as seven undescribed species found in Kauai, Oahu and Hawaii, that differ in size, abdominal dorsal marks and genitalia, though all are clearly closely related. The existence of two independent but related lineages that have diversified in parallel in the archipelago constitutes an ideal model for the comparative study of the effect of historical and ecological constraints on, among other, patterns of morphological change and community assembly.

Change subsequent to colonisation

Species of the *T. grallator* clade group with the genus *Exalbidion* in this study. This placement may seem surprising given that, in habitus, there is little resemblance between the two groups, for example, abdomens are wider than long in *Exalbidion* but the opposite seems to be the general trend in the Hawaiian forms. However, the difference in form may be attributed to change subsequent to colonisation in the Hawaiian species. On the other hand, *Exalbidion* species resemble Hawaiian species in displaying red, white and/or black dots on the abdomen (author's own observation) — *T. grallator* having the most dramatic and highly variable colouration of any known theridiid (Oxford & Gillespie 2001). Moreover, the relationship of the *T. grallator* clade with *Exalbidion* is not surprising in light of the genitalia; for example, the male palpal organ of *T. posticatum* and *E. rufipunctum* are quite similar, both species sharing a very prominent conductor. Between species in the *T. grallator*-clade, the most conspicuous somatic difference is size, which ranges from 7 to 10 mm in *T. kauaiense* to about 3–3.5 mm in *T. melinum* (Simon 1900). The species *T. grallator* shows the most divergent somatic morphology, characterised by very long spindly legs and an elongated abdomen (Fig. 1). This unusual morphology may well be related to an ecological or behavioural shift. The species is known to construct a flimsy web (Gillespie & Tabashnik 1994) that may suggest a particular predatory behaviour. Although little is known on the natural history of the remaining species, field observations suggest that they construct cobwebs on leaves and tree crevices, in a similar way to their continental relatives.

Theridion acutitarse and the unknown genus sp., together group sister to species of the genus *Rugathodes*. They closely resemble *Rugathodes* in possessing strongly developed chelicerae (Fig. 3), particularly in males, and in the general habitus (see, e.g., Knoflach 2004; fig. 36a,b). In addition, the palpal organ of the unknown genus sp. is similar to *Rugathodes pico* from the Azores (Merrett & Ashmole 1989), while the female genitalia resemble that of *R. sexpuctatus* (Levi 1957). These species may hence best be transferred to *Rugathodes*. As stated in the introduction, the peculiar somatic morphology of the unknown genus sp. characterised by slender long legs and a humpbacked elongated abdomen (Figs 1 and 3), made generic assignation difficult. Interestingly, the special somatic features

of this species resemble those of *T. grallator*, which suggest that the two species may have evolved parallel adaptations in response to similar selective pressures. Comparative data on the ecology of these species is required to explore this issue. In contrast, *T. acutitarse* combines a standard *Rugathodes*-like habitus with a dramatically different palpal organ compared to its Hawaiian relative, and all *Rugathodes* species. In this species the palp has undergone extreme simplification so that some sclerites (conductor and tegular apophysis) have been lost, others (median apophysis and embolus) modified. Further, the prominent lock system (a theridiid synapomorphy), whereby the median apophysis and the cymbium interact to lock the palpal bulb in the unexpanded palp and control its expansion during copulation, seems to have been lost. Similar loss has been documented in two other genera, *Theridula* (Agnarsson 2004; see fig. 81D; Levi & Levi 1962) and *Anelosimus* (Agnarsson 2006c; see fig. 55E), and illustrates a remarkable case of convergence. Such rapid and significant changes in island taxa are well documented, and have generally been attributed to genetic drift, the stochastic change in allele frequencies as a result of sampling. In small populations, such as those that might colonise islands, the effect is much more profound (founder effect) (Templeton 1980). Such illustration of convergence on islands has led to considerable confusion as to relationships of island taxa (Givnish 1997) and may be one reason for the recent proliferation of new *Theridion*-like genera (Wunderlich 1991, 1995a, 1995b; Yoshida 2001; Saaristo 2006) such as *Jamatidion* from Jamaica, *Sardinidion* from Sardinia and *Nipponidion* from Japan.

Phylogenetic structure of Theridiinae

This study offers the most detailed phylogenetic hypothesis of Theridiinae to date. Several findings concur with previous studies based on morphology (Agnarsson 2003, 2004), and molecular data (Arnedo *et al*. 2004; Agnarsson 2006b). The monophyly of Theridiinae is strongly supported in all studies, and under all methods and parameters in this study, confirming the value of the secondary loss of the colulus as a phylogenetic character (noteworthy as 'absences' in morphological data are often characterised as questionable evidence). Our findings confirm the para- or polyphyletic status of *Theridion*, *Achaearanea* and *Chrysso* which has been long suspected (e.g., Forster *et al*. 1990a; Agnarsson 2004). As expected, the genus *Theridion* is in a particularly poor taxonomic state, although adequately solving the taxonomy is a daunting task that will require species level revisions and phylogenetics of hundreds of species.

The current analysis may serve as a first guide to the task of assessing relationships among the assemblage of taxa within the Theridiinae, although the relative low numbers of type species sampled (7 out of 32) poses certain limitations. For example, it identifies two relatively large clades of *Theridion* species, the *T. pictum*-clade ('true' *Theridion*) and the *T. frondeum* clade, for which the name *Phylloneta* Archer, 1950 (type species *T. pictipes*) is available. Levi's species groups and taxonomical discussion may help allocate additional *Theridion* species to these clades. Our results also support to some recent efforts to split up *Theridion*, for example, the recently described or resurrected genera *Rugathodes* (Wunderlich 1987), *Ameridion* (Wunderlich 1995a) and *Takayus* (Yoshida 2001). Others, such as *Keijia* (Yoshida 2001); however, appear to be paraphyletic. The analysis also suggests some unexpected synonymies. For example, *T. theridioides* consistently groups with *Wamba crispulus* with high support and morphological examination reveals similarities in both habitus and genitalia of *T. theridioides* and *Wamba* species (I. Agnarsson, pers. obs.), suggesting they best be treated as congeners. However, *T. theridioides* is the type species of *Tobesoa* Keyserling, 1890 (currently in synonymy under *Theridion*) which may then be a senior synonym of *Wamba* O.P. Cambridge, 1896. Similarly, the results imply the paraphyly of *Achaearanea* (type *A. trapezoidalis*); if confirmed by future studies the name *Parasteatoda* (type *A. tepidariorum*) is available for *A. tepidariorum* and relatives.

The relatively basal placement of *Chrysso* has been found in all previous studies and is supported morphologically by the retention of prominent cheliceral teeth on both margins that are otherwise rare in the subfamily. Previous molecular analyses (Arnedo *et al*. 2004; Agnarsson 2006b) have also supported the *Chrysso*-clade and the *Achaearanea*-clade (in some form), all agreeing on the close relatedness between *Achaearanea*, *Tidarren* and *Echinotheridion*.

Further resolution of Theridiinae phylogenetic structure will require particularly dense sampling of non-monophyletic genera such as *Achaearanea*, *Chrysso* and *Theridion*, and inclusion of more genera. It may be useful to maximise representation of type species, both of currently recognised genera, as well as those currently in synonymy. Only with a robust phylogenetic assessment based on a dense taxon sample can the structure of the Theridiinae be understood.

Implication of the phylogeny for morphological and behavioural evolution

The phylogeny presented, although with limited resolution and hence limited predictive power, has some interesting implication for morphological and behavioural evolution within Theridiinae. First, as outlined above, the phylogenetic placement of *T. acutitarse* implies convergent simplification of the palpal organ within three genera of the 'lost colulus clade' with strikingly similar morphological results. The phylogeny also confirms convergent evolution of social behaviour within *Theridion* as the subsocial *T. pictum* (Ruttan 1990) and the social *T. nigroannulatum* (Avilés *et al*. 2006) belong to different species groups (or genera). This observation adds to the multiple origins of sociality already documented within Theridiidae

(Agnarsson 2006c; Agnarsson *et al*. 2006d). The results also continue to confirm high levels of convergence in web building behaviour and web structure within Theridiidae, whose study has been hindered by poor taxonomy and lack of phylogenetic hypotheses (Eberhard *et al*. in press).

The unusual sexual biology of *Tidarren* and *Echinotheridion* has been suggested as another example of behavioural convergence (Agnarsson 2006b). The two genera share unique sexual behaviour whereby the male amputates one of its palpi prior to mating and is then routinely cannibalised by the female as a part of the mating sequence (e.g., Knoflach & van Harten 2006). However, the current phylogeny implies that the behaviours are homologous, as the two taxa are sister in some of the analyses. However, their placement is poorly supported and more data is needed to adequately resolve this interesting issue.

Our results lend support to some previous relationships proposed based on morphological and behavioural evidence. For example, Knoflach (1998, 2004) found remarkable uniformity in the sexual behaviour of several *Theridion* species, which she referred to as the *T. varians* group (Knoflach 1998: 546), which largely overlaps our *T. pictum* ('true' *Theridion*) group. Knoflach (1998: 546) also pointed out that these species share a 'strikingly swollen dark epigaster in the male ...', a potential synapomorphy of 'true' *Theridion* that remains to be studied in most species. Clearly, advancing the phylogenetics of the Theridiinae is key to understanding behavioural and morphological evolution in this highly diverse group.

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